iCE3 System User Guide

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iCE3 System User Guide

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Chapter 1:

General Information

Chapter Overview

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Welcome

Congratulations on your purchase of the iCE3 system. ProteinSimple welcomes you as a new user. This user guide includes important safety information, site requirements, maintenance information, descriptions of the system and software as well as detailed operating instructions for setting up and running an assay on the iCE3 system.

Introduction to the iCE3 System

The iCE3 system combines the separation fidelity of gel-based IEF with the automation and quantitation of capillary column sample injection and detection. This combination provides a robust system that allows rapid method development and use of generic methods.

Capillary isoelectric focusing is a powerful technique for the quantitative analysis of proteins separated by isoelectric point. The iCE3 system performs free solution isoelectric focusing in a capillary column and detects focused protein zones using a whole column UV absorption detector. This unique technology incorporates the resolution of traditional gel IEF with the advantages of quantitation and automation found in column-based separation while eliminating the need for a mobilization step.

iCE systems come complete with iCE CFR control software, Chrom Perfect analysis software, computer and one of two autosampler options. The PrinCE Next autosampler allows for low volume sample injections, and the Alcott 720NV autosampler offers on-board mixing and extended throughput capability.

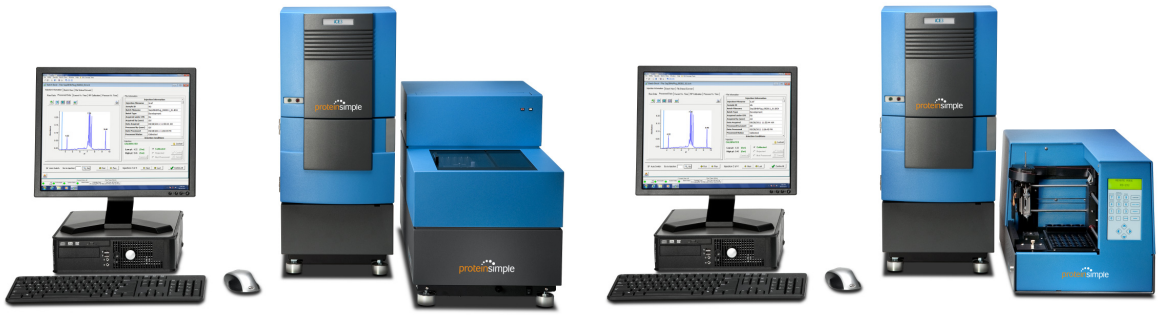


Figure 1-1: *iCE system with PrinCE Next autosampler (left) and with Alcott 720NV autosampler (right).*

Safety

User Attention Notifications

Several user attention phrases are used throughout this manual. Each phrase should draw the following level of attention from the user:

NOTE	Points out useful information.
IMPORTANT	Indicates information necessary for proper instrument operation.
CAUTION	Cautions users regarding potentially hazardous situations in regard to user injury or damage to the instrument if the information is not heeded.
!WARNING!	Warns users that serious physical injury can result if warning precautions are not heeded.

Chemical Hazards

!WARNING! CHEMICAL HAZARD.
Some chemicals used can be potentially hazardous, and can cause injury or illness.

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials.
- Minimize contact with and inhalation of chemicals. Wear appropriate personal protective equipment when handling chemicals (e.g., safety glasses, gloves, or clothing). For additional safety guidelines consult the SDS.
- Do not leave chemical containers open.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended on the SDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

Chemical Waste Hazards

!WARNING! BIOHAZARD

Samples and waste contents should be handled by procedures recommended in the CDC/NIH manual: Biosafety in Microbiological and Biomedical Laboratories (BMBL). The manual is available from the U.S. Government Printing Office or online at http://www.cdc.gov/biosafety/publications/bmbl5/.

Depending on the samples used, waste contents may constitute a biohazard. Use precaution when emptying waste. Dispose of waste contents in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

- Read and understand the Safety Data Sheets (SDSs) provided by the manufacturers of the chemicals in the waste before you store, handle, or dispose of chemical waste.
- Minimize contact with chemical waste. Wear appropriate personal protective equipment when handling chemicals (e.g., safety glasses, gloves, or clothing).
- Use precaution when emptying the waste vial.
- Dispose of waste vial and bottle contents in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

Waste Production and Disposal

The iCE3 system produces approximately 20 µL of waste per sample analysis and will contain the following:

- Sample
- Methyl cellulose (~0.5%)
- Carrier ampholytes
- pI markers
- Sample additives

Additionally, the Anolyte and Catholyte used in the cIEF cartridge will also need to be discarded and replaced after each batch and before short and long term storage:

- Catholyte - 100 mM sodium hydroxide in 0.1% methyl cellulose, 1.5 mL
- Anolyte - 80 mM phosphoric acid in 0.1% methyl cellulose, 1.5 mL

Waste should be disposed of in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

Safety Data Sheets

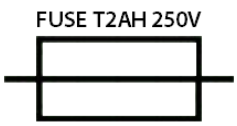
Some chemicals used with the iCE3 system may be listed as hazardous. Warnings are displayed on the labels of all chemicals when hazards exist.

SDSs provide users with safety information needed to store, handle, transport and dispose of the chemicals safely. ProteinSimple recommends updating laboratory SDS records periodically.

Safety Data Sheets for ProteinSimple reagents are available online at http://www.proteinsimple.com/technical_library.html or by calling (888) 607-9692. Otherwise call the chemical manufacturer directly or visit their web site.

Instrument Safety Labels

The following safety labels are located on the iCE3 system. Each label will display a safety alert symbol indicating a potential safety hazard.

Symbol	Description
	Ultraviolet Light
	Risk of Electric Shock.
	Refer to the iCE3 System User Guide before proceeding.
	Danger of hazardous waste. Use caution in these areas. This warning only applies if using hazardous material. The iCE3 system reagents are not considered hazardous waste. If you are using hazardous materials, please contact your field service representative to place labels in the appropriate locations.
	Fuse Symbol. 2A, 250 V fuse. T - time lag, H - high capacity.

!WARNING!
If the unit is not used as specified by ProteinSimple, the overall safety will be impaired.

!WARNING!
If the unit is damaged and does not function properly, stop the unit safely and contact ProteinSimple Technical Support immediately.

!WARNING!
No user replaceable/serviceable parts except for system tubing and the fuse located in the power entry module.

CAUTION
Do not remove system covers. Doing so will expose users to hazardous voltages and UV radiation.

Interlock Sensor

An interlock sensor detects when the door is open and closed. The high voltage is automatically turned off and the UV light is shuttered when the door is open.

!WARNING!
Do not override the interlock sensor. The sensor protects users from exposure to UV light and high voltage.

UV Safety

CAUTION
The iCE system contains a UV lamp. The system enclosure confines the radiation within the system and shields the user from exposure. Exposure to UV radiation can cause permanent damage to the eyes and skin.

Prior to System Operation

Ensure that all users of the iCE3 system have:

- Received instruction in general safety practices for laboratories
- Received instruction in specific safety practices for the instrument
- Received instruction on handling of biohazards if biohazardous materials are to be used on the system
- Read and understood all related SDSs
- For Prince Next users: Reviewed the Prince Next User Manual

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CAUTION

Avoid using the iCE3 instrument in a manner not specified by ProteinSimple. While the system has been designed to protect the user, this protection may be impaired if the instrument is used improperly.

Customer Service

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Legal Notices

NOTE: Read the Legal Notices carefully before using your iCE3 system.

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Chapter 2:

Fundamentals of IEF and cIEF

Chapter Overview

- Fundamentals of cIEF
- Whole-Column Detection cIEF

Fundamentals of cIEF

Isoelectric focusing, or IEF, is a mode of electrophoresis that separates amphoteric molecules in solutions. Capillary isoelectric focusing, or cIEF, is simply performing IEF in a capillary rather than in a traditional gel-based system.

Amphoteric Molecules

In aqueous solutions, acids are hydrogen (H+) donors and bases are H+ acceptors. An amphoteric molecule can either donate or accept a H+. Therefore, they can act either as an acid or a base. Amino acids, peptides and proteins are three examples of amphoteric molecules. In a low pH (acidic) environment, an amphoteric molecule is forced to accept a H+ to become a positively charged molecule. Applying the same principle, when this molecule is in a high pH (basic) environment, it has to release a H+, becoming a negatively charged molecule. However, there is a pH environment that is neither high pH nor low pH for this amphoteric molecule. At this pH, the molecule's net charge is zero. This pH value is the pI value of this molecule. This property of amphoteric molecules is the core principle of IEF.

IEF Principles

Imagine placing an amphoteric molecule in a pH gradient with voltage applied across the pH gradient in the direction of low pH to high pH. The pH gradient is sandwiched between an acid (anolyte, HA) and a base (catholyte, BOH). As shown in Figure 2-1 (top), if this molecule happens to be in a region where the pH value is higher than its pI, it will be negatively charged and driven to the lower pH regions by the applied voltage. Once this molecule arrives at the region where the pH value is lower than its pI value, it will be positively charged and driven back by the applied voltage to the high pH regions. The final result: the molecule stops at a place in the pH gradient where the pH value equals its pI value since at this spot, the molecule's charge is zero and can't be driven in any direction by the applied voltage. Different proteins have different pIs, so they can be separated and focused in the pH gradient (Figure 2-1 bottom).This is the principle of IEF separation.

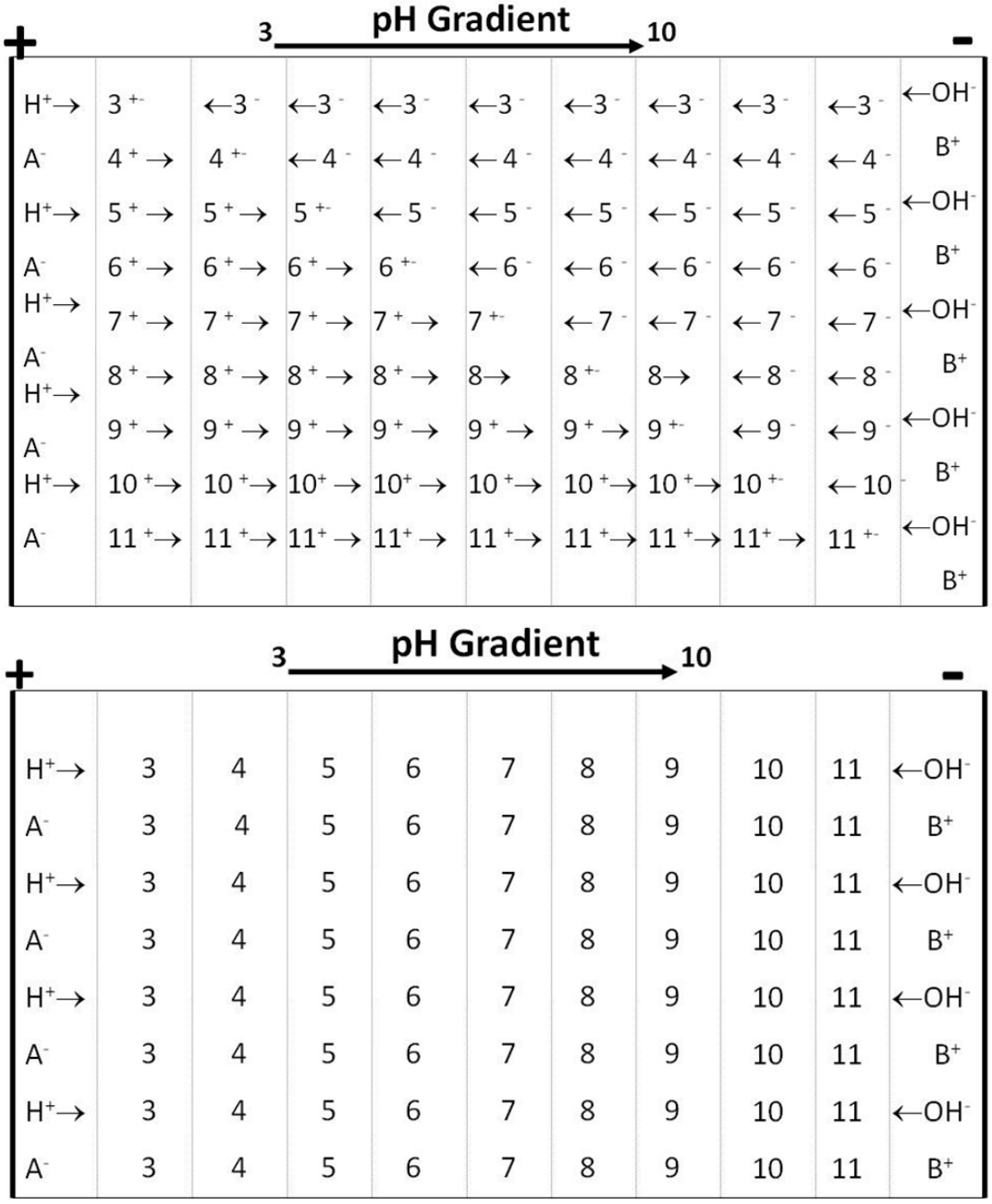

Figure 2-1: *Molecules with different pI values are placed in a pH gradient under an applied voltage (top). The arrows indicate the direction of their mobility. Final result after the molecules reach the place where their pIs are equal to pH values (bottom).*

The IEF Technique is Unique

IEF is a mode of electrophoresis for amphoteric molecules, especially peptides and proteins. It separates molecules purely based on their pI values, in contrast to other electrophoresis techniques which typically utilize some form of size-based separation. IEF is also a concentrating technique since molecules having the same pIs are focused into narrow zones in the pH gradient. At the end of the separation, all these zones are stationary. IEF is also an equilibrium separation technique, and provides the highest resolution of all charge-based separation techniques for proteins.

Traditional Slab Gel IEF

Traditional IEF is performed in polyacrylamide slab gels. First, a pH gradient is created on the slab gel by commercial carrier ampholytes under a separation voltage. Then, protein samples are loaded into the gel to start the separation. Although slab gel IEF has high resolution for protein separation, it tends to be slow, labor- intensive, and not quantitative. It was recognized among scientists for a long time that if high-resolution IEF could be performed in a column format, significant advantages over slab gel IEF in terms of automation, separation speed and quantitation could be realized. This was why exploration of cIEF began in 1985.

Conventional cIEF

Despite the appeal of cIEF since it was proposed in 1985, widespread acceptance as a replacement for slab gel IEF did not occur. The main factor for the slow acceptance of cIEF was the difficulty in performing cIEF using commercial, general-purpose capillary electrophoresis (CE) instruments (now referred to as conventional cIEF). Figure 2-2 shows the steps of how a commercial CE instrument is used to run conventional cIEF.

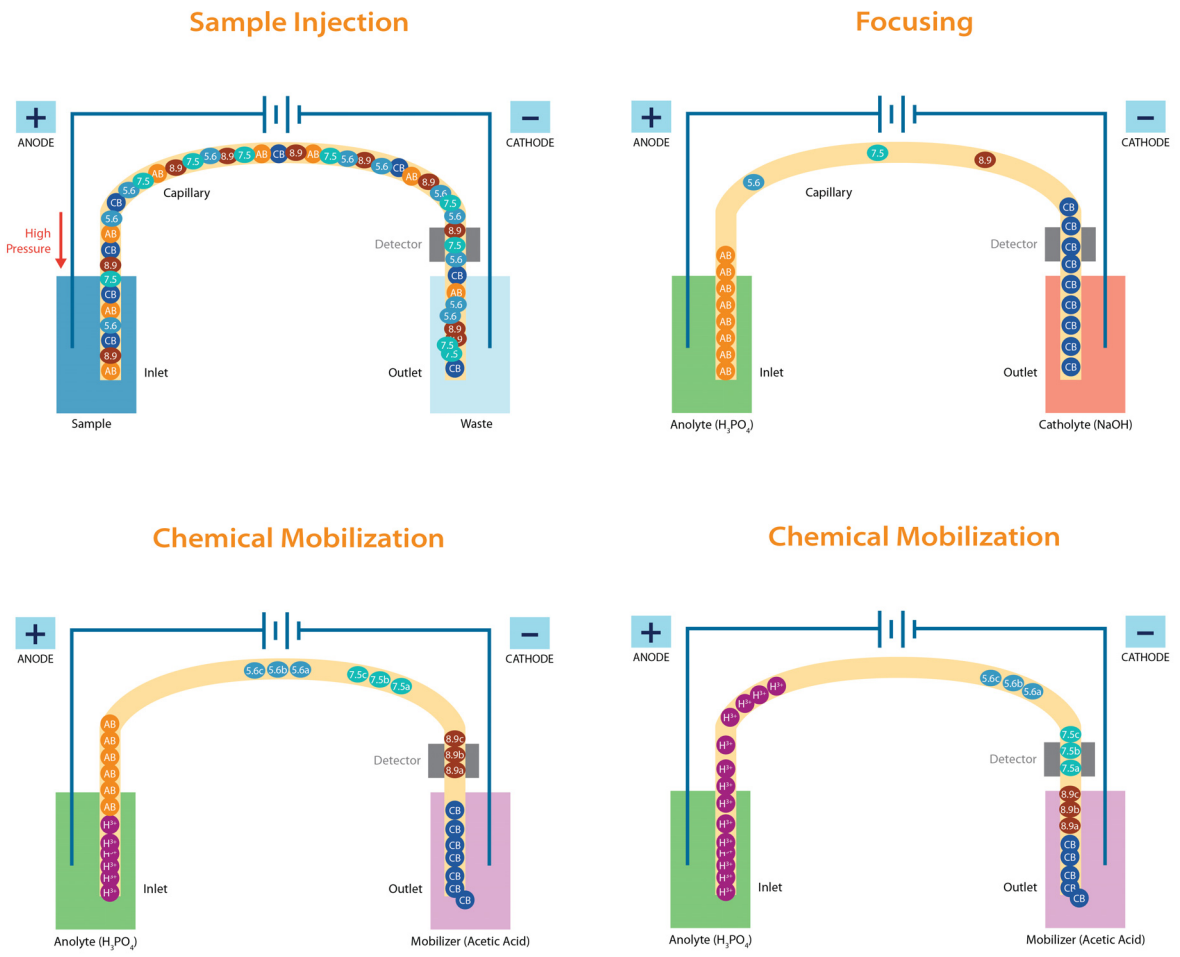

Figure 2-2: *Conventional cIEF sample injection (top left), focusing (top right), and mobilization (bottom, left to right).*

Protein samples are first premixed with carrier ampholytes, additives and pI markers. The mixture is injected to fill the entire column. Then, the ends of the column are dipped into anolyte (acid) and catholyte (base). A separation voltage is applied across the column. Under the voltage, a pH gradient is created within the column from the anodic end to the cathodic end of the column. Proteins are separated and focused along the pH gradient. When IEF is complete, all the proteins are focused into narrow zones and stop at their pI point. Now the question is: how to detect these zones?

These commercial CE instruments are equipped with a single point, on-column detector. In conventional cIEF, after the isoelectric focusing process, a mobilization process is necessary to move all the focused protein zones past the detection point of the on-column detector in order to detect these zones. The mobilization process introduces many problems such as poor resolution, poor reproducibility and long sample

analysis times (less than 2 samples/hour). The mobilization step utilizes pressure or chemical mobilization. Pressure mobilization compromises resolution, and chemical mobilization can change the pH gradient. Maintaining the pH gradient during chemical mobilization requires all ampholytes have the same "mobility" in the electric field. Unfortunately, basic ampholytes have different mobilities than acidic and neutral ampholytes. This results in a mixed-mode separation which is no longer just pI-based. The profile with conventional cIEF becomes more complex (as shown in Figure 2-2) and is not always reproducible. In conventional cIEF, the dynamic process of IEF within the separation column is not monitored, which makes it difficult to optimize focusing time, one of the most important parameters in cIEF. It is also impossible to find problems in the IEF process within the separation column, such as sample aggregation and precipitation.

Whole-Column Detection cIEF

ProteinSimple (formerly Convergent Bioscience) was the first to commercialize the whole-column detection cIEF technique in the iCE IEF Analyzer instrument. The iCE instrument revolutionized cIEF technology. Figure 2-3 shows the principle of performing cIEF using ProteinSimple's iCE3 system.

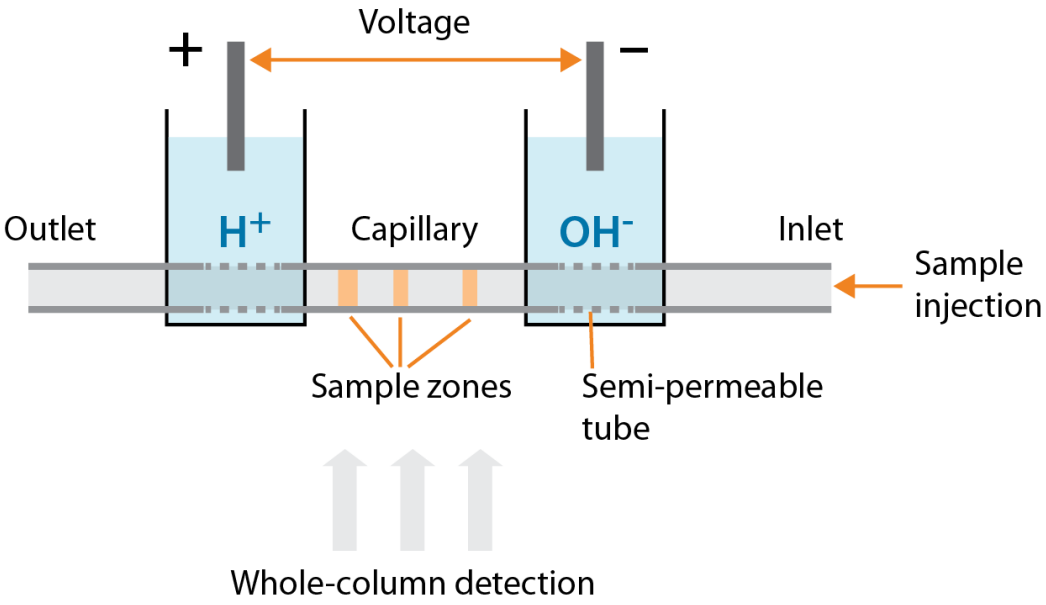

Figure 2-3: *Principle of cIEF on the iCE3 system.*

In performing cIEF on the iCE instrument, protein samples are first premixed with carrier ampholytes, additives and pI markers. The mixture is injected to fill the entire capillary column. A separation voltage is applied to the anolyte and catholyte tanks. Under the voltage, a pH gradient is created within the column. Proteins are separated and focused along the capillary column. The whole-column detector monitors the IEF process

in an on-line fashion within the separation column, and the focusing time can be optimized in a single sample run. At the end of the focusing process, all the focused protein zones within the column are recorded by the detector without disturbing the separation resolution. Finally, the column is washed and ready for the next sample injection. Any sample precipitation and aggregation during focusing can be observed. Different additives are easily selected to improve reproducibility when issues are identified. The biggest advantage of whole-column detection cIEF is its fast and easy method development because of the ability to monitor the IEF process within the separation column.

Chapter 3:

Site Preparation and Installation

Chapter Overview

- Introduction
- Space Requirements
- Environmental Requirements
- Electrical Requirements
- Software and Computer Requirements
- Chemical and Consumable Requirements

Introduction

This chapter will help you prepare your laboratory for the installation of the iCE3 system with either a PrinCE Next or Alcott 720NV autosampler. Please complete the space, electrical and environmental requirements prior to scheduling installation and training.

NOTE: Please wait for an authorized ProteinSimple representative to unpack and install the iCE system. Do not attempt to do this as improper handling of the instrument and computer can result in personal injury or damage to the system.

Space Requirements

Please provide adequate space to accommodate the system and computer, with sufficient clearance for heat ventilation and service accessibility. The iCE3 system is a bench-top instrument and requires a sturdy, level laboratory bench or table.

IMPORTANT

Please ensure that the bench or table used for the iCE3 system is horizontally level at all times for proper operation.

---

iCE3 System with PrinCE Next Autosampler

System dimensions without the computer are as follows:

Dimension	Centimeters
Width	61
Depth	66
Height	66

Table 3-1: *Dimensions of iCE3 system with PrinCE Next autosampler.*

System components are typically installed as shown in Figure 3-1, however the computer can be placed on either side of the system.

NOTES:
Please ensure that adequate bench space is available next to the system for the computer.

If a local printer will be used, please allow appropriate bench space next to the computer. An additional power socket and printer cable will also be required.

Figure 3-1: *iCE3 instrument with PrinCE Next autosampler bench-top configuration.*

Allow at least 16 cm of clearance at the back of the system for ventilation and cable access. Space requirements for the iCE3 system with the PrinCE Next autosampler and a computer are as follows:

Dimension	Centimeters
Width	116
Depth	82
Height	66

Table 3-2: *Space requirements for iCE3 system with PrinCE Next autosampler and computer.*

iCE3 System with Alcott 720NV Autosampler

System dimensions without the computer are as follows:

Dimension	Centimeters
Width	65
Depth	55
Height	66

Table 3-3: *Dimensions of iCE3 system with Alcott 720NV autosampler.*

System components are typically installed as shown in Figure 3-2, however the computer can be placed on either side of the system.

NOTES:
Please ensure that adequate bench space is available next to the system for the computer.

If a local printer will be used, please allow appropriate bench space next to the computer. An additional power socket and printer cable will also be required.

Figure 3-2: *iCE3 instrument with Alcott 720NV autosampler bench-top configuration.*

Allow at least 15 cm of clearance at the back of the system for ventilation and cable access. Space requirements for the iCE3 system with the Alcott 720NV autosampler and a computer are as follows:

Dimension	Centimeters
Width	116
Depth	70
Height	66

Table 3-4: *Space requirements for iCE3 system with Alcott 720NV autosampler and computer.*

Environmental Requirements

Please provide appropriate heating and cooling to maintain a constant laboratory temperature. For optimal system performance, the laboratory environment must meet the following criteria:

Requirement	Specification
Operating temperature range	18 - 25 °C
Operating humidity range	40-80%, non-condensing

Table 3-5: *iCE3 system environmental requirements.*

Electrical Requirements

Installation of the system requires a dedicated, grounded circuit capable of delivering the appropriate current and voltage for your country. The general electrical requirements for the instrument are 110-120 V AC/60Hz or 220-240 V AC/50Hz with a power consumption of 210 W.

- The power requirements for select countries are listed below.

Region	Volts (AC)	Frequency (Hz)	Amps
US and Canada	120	60	2
Europe	230	50	1
UK	230	50	1

Table 3-6: *iCE3 system power requirements.*

NOTE: For proper operation in Japan at 100 VAC (50 Hz), the iCE3 system must be used with a step-up transformer. This is included with the iCE3 system shipment.

The system label (Figure 3-3) displays the system power specifications:

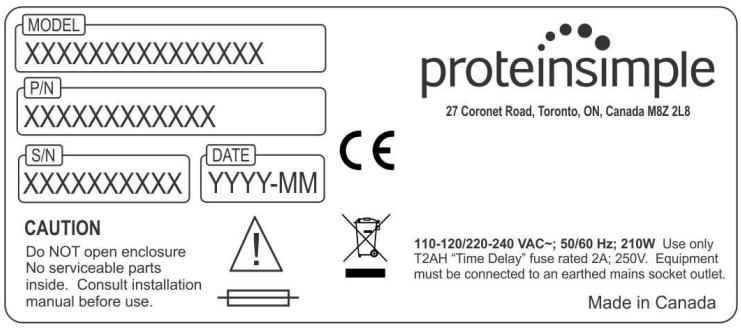

Figure 3-3: *iCE3 system label.*

In addition to the electrical power requirements listed above, ProteinSimple requires the grounded circuits terminate at the receptacles, and the receptacles must be located within 10 ft (3 m) of the instrument, and a power strip with a minimum of five sockets also be available.

NOTE: The instrument is provided with a grounded plug. In order to assist with the safety of the product, please verify that the plug is inserted into a grounded receptacle.

Main Power Configuration

The iCE3 system may configured for either 120 V AC/60 Hz or 230 V AC/50 Hz operation. The instrument is shipped with the fuse removed. The service rep will install the correct fuse during the installation. To install a fuse:

1. Locate the fuse box in the power entry module on the rear panel of the instrument (Figure 3-4).
2. Make sure the power switch is off.

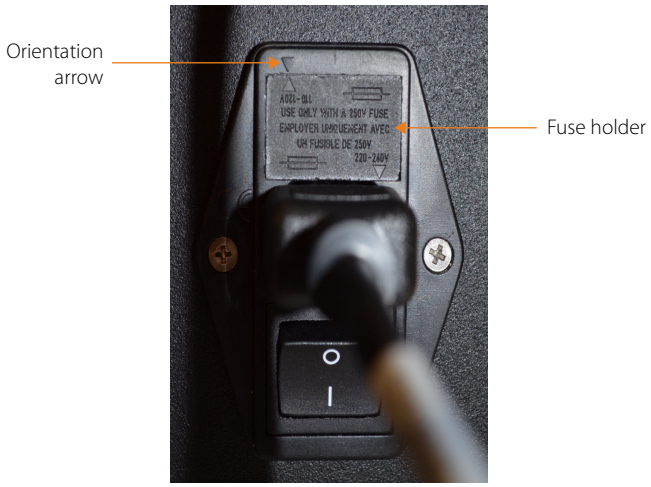

Figure 3-4: *Fuse holder in power entry module.*

3. Use a flat-head screwdriver to gently pry the fuse holder open. Remove the fuse holder.
4. There are two fuse positions in the fuse box. Insert a 2A time lag high capacity 250 VAC fuse (P/N 011-800), making sure to insert it in the correct position for your laboratory voltage as shown in Figure 3-5.

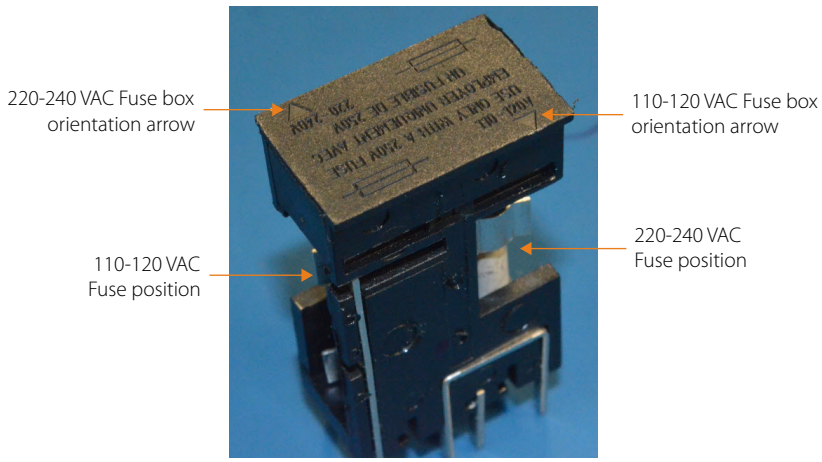

Figure 3-5: *Fuse position in fuse box.*

5. Reinsert the fuse holder, making sure to align the arrow on the fuse box associated with the correct voltage for your laboratory (110-120 VAC or 220-240 VAC, shown in Figure 3-5) with the orientation arrow on the power entry module (Figure 3-4).

6. Plug the instrument power cord into the system and turn the power on.

PrinCE Next Autosampler Main Power Configuration

The PrinCE Next autosampler will be delivered configured for the correct electrical service.

1. Locate the Power Switch (front of unit, bottom left side) and ensure that the rocker switch is pressed down.
2. Connect the power cord.

Alcott 720NV Autosampler Main Power Configuration

The Alcott Autosampler should be delivered configured for the correct electrical service.

- Locate the Power Switch (rear of unit, bottom left side) and ensure that the rocker switch is in the OFF position.
- Connect the power cord.

Software and Computer Requirements

ProteinSimple provides a computer to run the iCE3 system and analyze resulting data using iCE CFR Software. The software will be installed during system setup by a service engineer, and this procedure is also part of IQ/OQ. The computer must interface properly with the iCE3 system. ProteinSimple has extensively tested, approved, and strongly recommends using the computer provided with the system.

If you will be providing the computer, it must meet the recommended or minimum requirements listed below. As computer specifications change, please contact ProteinSimple Technical support for the most current requirements prior to selecting your computer.

RBC System Configuration

- **Operating System:** Windows 10 Professional 64-bit English Edition
- **Computer/Processor:** Intel CORE i5-7400 3 GHz 6 MB 4-Core S1151 Processor
- **Memory:** 8 GB RAM
- **Hard Disk:** 500 GB 7200RPM hard drive
- **Network Interface:** 10/100/1000 Ethernet
- **Required options:**
  - Second Serial Port Adapter, Low Profile (P/N 331-1957) or Second Serial Port PCIe, Low Profile (P/N 430-4203)

- PCIe 10/100/1000 Network Card, Low Profile (P/N 430-3948) or PCIe 10/10/1000 Network Card, Low Profile (P/N 430-4205) for the second network port

Minimum PC Requirements

- **Operating System:** Windows 7 Professional 64-bit English Edition SP1 or Windows 10 Professional
- **Computer/Processor:** 1 GHz 64-bit (x64)
- **Memory:** 6 GB RAM
- **Hard Disk:** 40 GB with 650 MB free space [NTFS format]
- **Display:** 17" monitor capable of supporting 1280x768 resolution
- **Drives:** DVD-ROM Drive
- **Video Card:** Any card capable of the display output listed above
- **Keyboard/Mouse:** Required
- **Ports:** Two 10/100/1000 Ethernet Two RS-232 Serial [9-pin D-connectors]

NOTE: Communication problems between the computer and autosampler may be experienced when using a computer that is not approved by ProteinSimple. If a non-approved computer must be used, please contact ProteinSimple well in advance of the system installation to ensure compatibility.

Chemical and Consumable Requirements

Table 3-7 provides a list of routinely used chemicals required during normal operation of the iCE3 system. Please visit www.proteinsimple.com/consumables_ice.html for a complete list of available iCE Kits and reagents.

Item	Information	Part Number
FC cIEF Cartridge Chemical Test Kit	This kit provides all the reagents needed to verify operation of the iCE3 system. One kit is supplied with each new system.	ProteinSimple P/N 101801
FC cIEF Cartridge (FC-coated)	One package containing two cIEF cartridges is supplied with each new system.	ProteinSimple P/N 101701
HT cIEF Cartridge Chemical Test Kit	This kit provides all the reagents needed to verify operation of the iCE3 system with the HT cartridge. This kit is not shipped with the system but is available for purchase separately.	ProteinSimple P/N P-0000033-00
HT cIEF Cartridge	This kit is not shipped with the system but is available for purchase separately.	ProteinSimple P/N P-0000035-00
iCE Method Development Kit	This kit provides all the reagents you need to analyze proteins on iCE systems. The kit includes a test mix, anolyte, catholyte, Methyl Cellulose, five types of ampholytes (Pharmalyte pH ranges 3–10, 2.5–5, 5–8 and 8–10.5 and Servalyte pH range 2–9), eight pI markers (3.21, 5.12, 6.14, 7.05, 7.65, 8.18, 9.46 and 10.10) and additives (urea and arginine). You can use this kit with either the FC or the HT cIEF Cartridge.	ProteinSimple P/N 042-848
Carrier ampholytes	For example, Pharmalyte 3-10.	Available from various vendors
pI Markers	A full suite of cIEF-compatible pI markers are available from ProteinSimple. For the full list of pI markers, visit www.proteinsimple.com/consumables_ice.html	See the ProteinSimple website
Deionized water	Use 0.22-micron filtered deionized water (HPLC grade or better). For example, Milli-Q water.	N/A
Catholyte	100 mM sodium hydroxide in 0.1% methyl cellulose, 100 mL bottle.	ProteinSimple P/N 102506 Electrolyte kit
Anolyte	80 mM phosphoric acid in 0.1% methyl cellulose, 100 mL bottle.	

Item	Information	Part Number
Methyl Cellulose Stock Solution	1% methyl cellulose solution, 100 mL bottle.	ProteinSimple P/N 101876
Cartridge Rinse Solution	0.5% methyl cellulose solution, 200 mL (qty of 2 100 mL bottles).	ProteinSimple P/N 102505

Table 3-7: *Routinely used chemicals.*

Table 3-8 provides a list of routinely used consumables required during normal operation of the iCE3 system with either the PrinCE Next autosampler or the Alcott 720NV autosampler.

Item	Information	Part Number
PrinCE Next Autosampler		
Vials, 2 mL glass	Vial Pack, 12 mm, 2 mL glass (qty 100)	ProteinSimple P/N 045-132
Vial inserts, 300 µL	Vial Insert Pack, 300 µL, PP (qty 100)	ProteinSimple P/N 045-135
Vial septa	Septa Pack, iCE3 (qty 100)	ProteinSimple P/N 102031
96-well plates	Required 96-well plates for use with PrinCE Next: • Flat bottom, clear (PS) • Sterile flat bottom clear (PS)	Grenier P/Ns: • 655101 • 655161
Sealing film	Required for use on 96-well plates.	Excel Scientific P/N XP-100
Alcott 720NV Autosampler		
Vials, 300 µL PP	Vial Pack, 12 mm PP with 300 µL insert (qty 100)	ProteinSimple P/N 045-133
Vials, 2 mL glass	Vial Pack, 12 mm, 2 mL glass (qty 100)	ProteinSimple P/N 045-132
Vial inserts, 300 µL	Vial Insert Pack, 300 µL, PP (qty 100)	ProteinSimple P/N 045-135
Vial septa (12 mm vials)	Septa Pack, iCE3 (qty 100)	ProteinSimple P/N 045-134
Vials, 10 mL	Vial Pack, 10 mL, glass, amber (qty 10)	ProteinSimple P/N 045-139
96-well plates	The Alcott 720NV can be used with any 96-well plate.	N/A
Sealing film	Required for minimizing sample evaporation.	Excel Scientific P/N XP-100

Table 3-8: *Routinely used consumables.*

Chapter 4:

iCE3 Systems Overview

Chapter Overview

- Overview of iCE3 Instrument
- Overview of iCE3 System with PrinCE Next Autosampler
- Overview of iCE3 System with Alcott 720NVNV Autosampler
- System Specifications

Overview of iCE3 Instrument

The iCE3 instrument (Figure 4-1) is a capillary-based separation platform that provides complete assay automation and quantitative analysis of protein samples. The individual hardware components of the iCE3 system are described in this section.

External Components

Figure 4-1: *iCE3 instrument external components.*

CAUTION

Do not remove system covers. Doing so will expose users to hazardous voltages and UV radiation.

System Door

The iCE3 system door provides access to the inside of the instrument for cartridge installation. To open the door, pull the handle forward.

Interlock Sensor

An interlock sensor detects when the door is open and closed. The high voltage is automatically turned off and the UV light is shuttered when the door is open.

!WARNING!

Do not override the interlock sensor. The sensor protects users from exposure to UV light and high voltage.

NOTES:

The iCE3 system door must be closed prior to starting a run.

Opening the iCE3 system door during a run will abort the batch in progress.

Status Lights

Two LEDs are located on the front panel to indicate system status:

- **Green**:
  - A solid green light indicates the instrument connection to the computer is established and the system is ready for use.
  - A flashing green light indicates the instrument connection to the computer has been lost or that the system door is open.
- **Red**: Indicates the UV lamp is on.

Internal Components

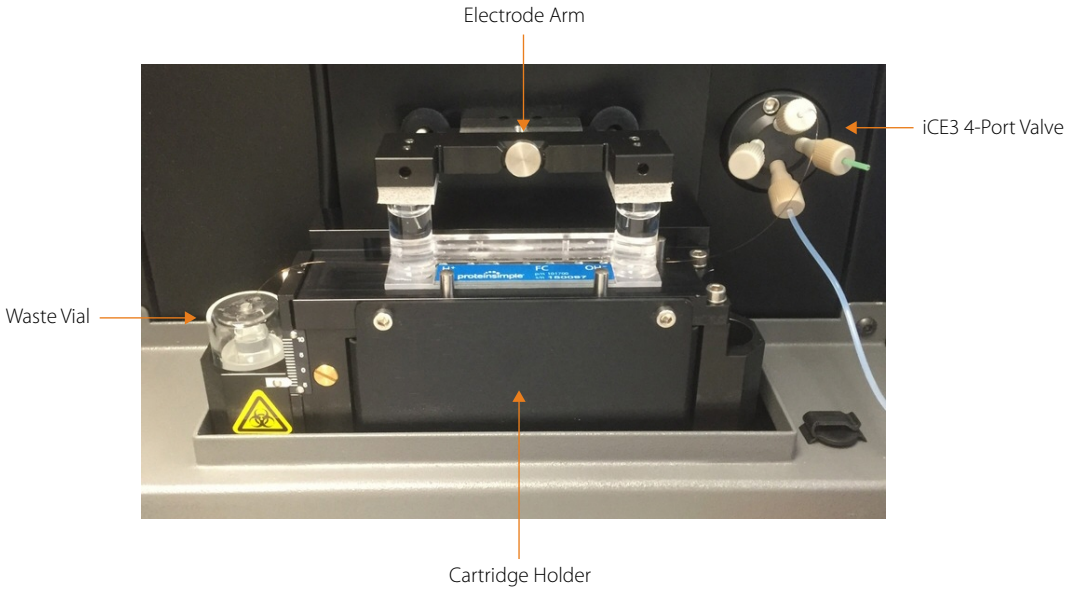

Figure 4-2: *iCE3 internal components.*

Cartridge Holder

The cIEF Cartridge is held in place by the cartridge holder. Three alignment pins and a spring pin ensure proper cartridge installation and positioning (Figure 4-3).

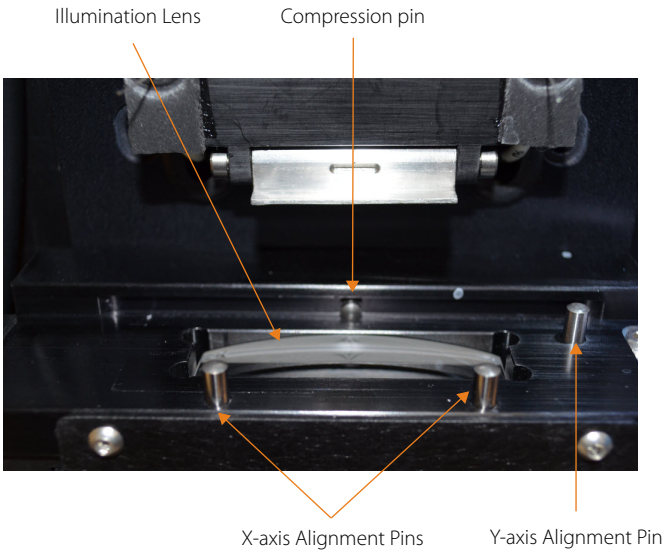

Figure 4-3: *Cartridge holder.*

Illumination Lens

UV light (280 nm) for whole-column detection is focused through the illumination lens (Figure 4-3). During focusing, UV light passing through the cartridge from the illumination lens is detected.

CAUTION

The iCE system contains a UV lamp. The system enclosure confines the radiation within the system and shields the user from exposure. Exposure to UV radiation can cause permanent damage to the eyes and skin.

CAUTION

The surface of the UV lamp enclosure may be hot. Do not open the cover to the UV lamp.

Electrode Arm

A hinged electrode arm containing an anode and cathode is located above the cartridge holder (Figure 4-4). Depending on the date the iCE3 system was purchased, the electrode arm may be different. The original electrode arm is shown in Figure 4 (top). The updated electrode arm is shown in Figure 4 (bottom). The updated electrode arm includes thicker closed foam pads and a locking mechanism that reduce the effect

of carbon dioxide on the catholyte. The updated electrode arm allows you to run 100 samples in a single batch. If your system has the older style foam pads without a locking mechanism, you will be limited to 30 - 40 runs before the anolyte and catholyte need to be replaced.

Prior to starting a run, the electrode arm needs to be seated firmly on the cIEF Cartridge.

- **Original electrode arm:** Simply pull down the arm until it seats firmly on the cIEF cartridge tanks.
- **Updated electrode arm:** Use the plunger to seat the arm on the cIEF cartridge tanks. To flip the handle between the up and down positions, pull the plunger forward, move the handle and release so that the plunger locks into place. In the down position, the plunger face should be fully flush with the handle if seated correctly, and the foam should fully seal the top of the cartridge tanks.

This places each of the electrodes directly into the electrolyte (anolyte and catholyte) tanks on the cartridge. When the run begins, voltage is applied between the electrodes to create a separation gradient in the capillary. To remove the cIEF Cartridge, pull the electrode arm up to its fully open position.

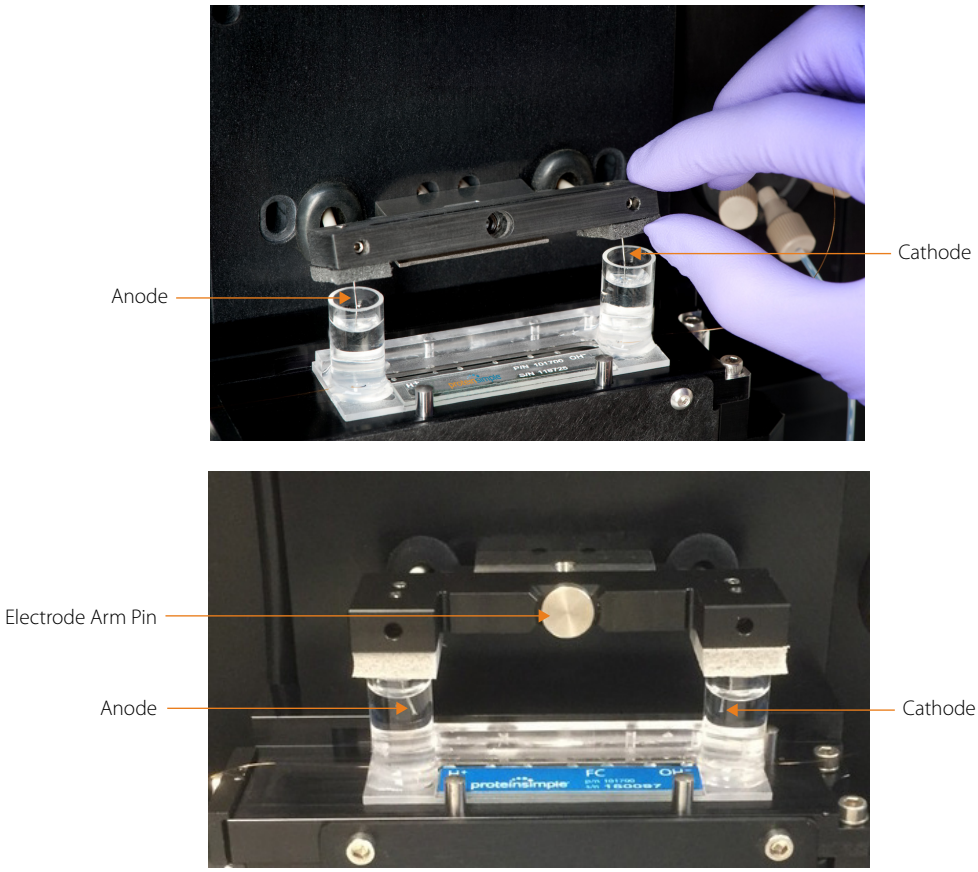

Figure 4-4: Original electrode arm (top) and updated electrode arm (bottom).

iCE Valve

The iCE valve (Figure 4-5) provides fluidic control for sample loading from the autosampler into the cIEF cartridge, and when flushing fluidic pathways between injections.

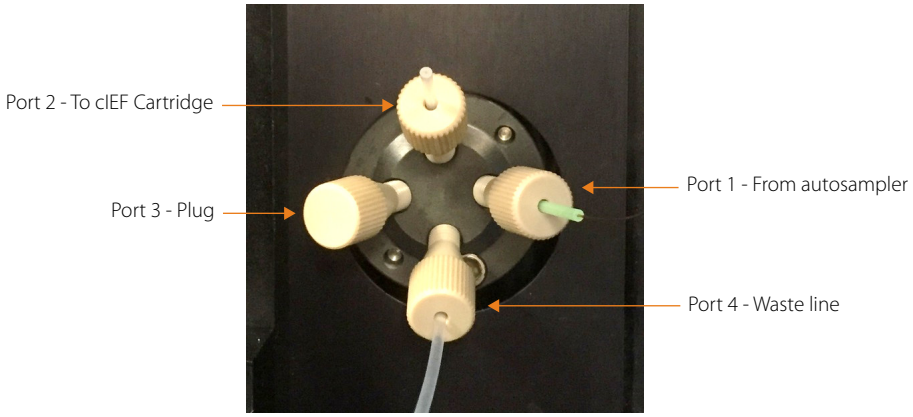

Figure 4-5: *iCE valve (plumbing for PrinCE Next autosampler shown).*

The iCE valve has four ports that are plumbed a follows:

- **Port 1** - From autosampler
- **Port 2** - To ciEF Cartridge
- **Port 3** - Plugged
- **Port 4** - To waste

The iCE valve is controlled automatically using the protocol defined in the batch file. When the iCE valve is in the load position (Figure 4-6 top), sample, buffer, water or air from the autosampler enters port 1 and is flushed through port 4 to waste. When the iCE valve is in the inject position (Figure 4-6 bottom), fluid from the autosampler enters port 1 and travels through port 2 directly into the cIEF cartridge.

CAUTION

Do not exceed the maximum pressure of 2500 psi. The valve should only be used with water and methylcellulose as per the iCE protocols.

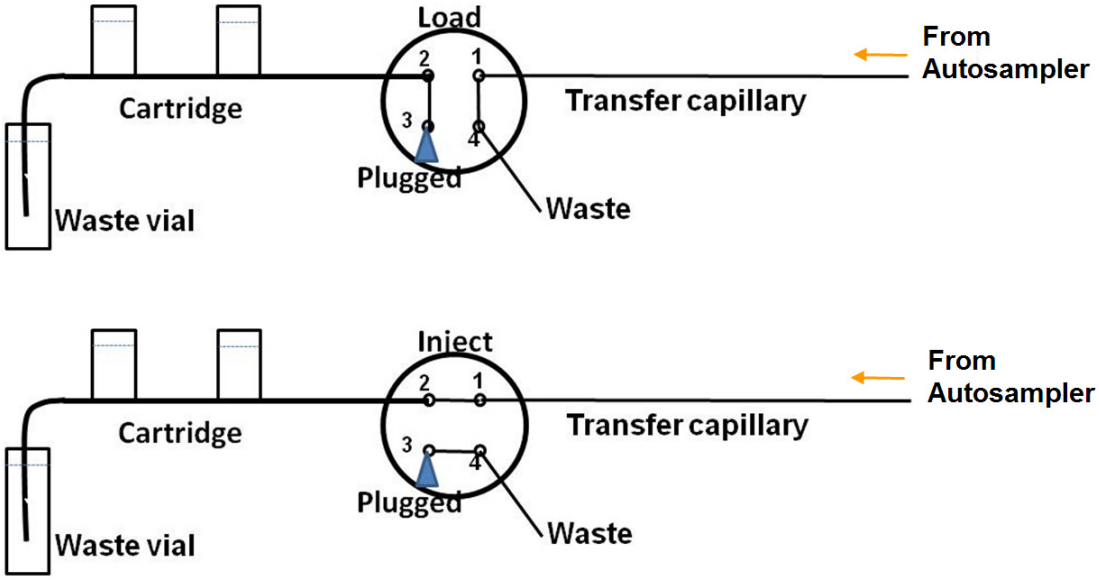

Figure 4-6: iCE valve in load position (top) and inject position (bottom).

Waste Containers

!WARNING! BIOHAZARD

Samples and waste contents should be handled by procedures recommended in the CDC/NIH manual: Biosafety in Microbiological and Biomedical Laboratories (BMBL). The manual is available from the U.S. Government Printing Office or online at http://www.cdc.gov/biosafety/publications/bmbl5/.

Depending on the samples used, waste contents may constitute a biohazard. Use precaution when emptying waste. Dispose of waste contents in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

Cartridge Waste Vial

A glass vial (Figure 4-7) collects waste that exits the cIEF cartridge, and should be filled with water at all times. The cover can be removed and replaced to empty or refill the vial, and contains a small hole so the cartridge capillary outlet can be routed into the waste vial.

Figure 4-7: *Cartridge waste vial and cover.*

Waste Bottle

The system waste bottle collects sample overflow as well as water and buffer flush waste from port 4 of the iCE valve. The waste bottle should be emptied as needed.

Rear Panel

Figure 4-8: Rear panel.

- **Fans:** The fans keep the internal components of the instrument at or near ambient temperature.

NOTE: In order to maintain proper instrument operation, ensure the fan exhaust is not blocked.

- **Air Filters:** The filters prevent dust from entering the instrument.
- **System Power:** The power components consist of the main power switch and power entry.

NOTE: Ensure there is at least 15 cm of clearance behind the instrument to allow the power cord to be removed if the instrument must be immediately powered off in the event of a hazard.

!WARNING!
Only use the power supply cord provided with the instrument. If the power supply cord is damaged, please contact ProteinSimple Technical Support.

!WARNING!
No user replaceable/serviceable parts except for system tubing and the fuse located in the power entry module.

!WARNING! SHOCK HAZARD
Disconnect the power cord from the instrument power input to disconnect power to the instrument.

- **Fuse box:** Houses one 2A time lag high capacity 250 V AC fuse.

!WARNING! FIRE HAZARD
Replace fuse only with same type and rated certified fuse. Fuse rating: T2AH 250V

- **Serial Port:** The RS232 connector is used to connect to the iCE3 instrument to the computer.

Instrument Label

An instrument label (Figure 4-9) is located above the serial port. It includes the following information: the ProteinSimple location, model, power requirements, serial number and certification markings.

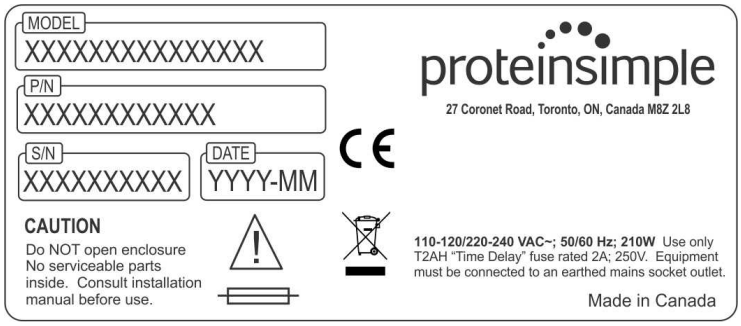

Figure 4-9: *iCE3 instrument label.*

NOTE: The instrument serial number is also located on the inside the system door.

Safety Certifications and Compliances

The iCE3 instrument complies with:

- **All CE mark requirements**

Safety Requirements for Electrical Equipment for Measurement, Control and Laboratory Use

- Certified to IEC/EN/CSA/UL 61010-1 2nd Edition and 61010-2-081:04

EMC requirements – Electrical equipment for measurement, control and laboratory use

Type tested and found to comply with the limits for a Class A digital device, pursuant to:

- EN 61326-1:2005
- FCC 47 CFR Part 15 (Class A – office use only)
- ICES-003 Issue 4 February 2004 (Class A – office use only)

This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to Part 15 of the FCC Rules.

FCC Notice (U.S. Only)

This equipment has been tested and found to comply with the limits for a Class A digital device pursuant to Part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the manufacturer's instruction manual, may cause harmful interference with radio communications. Operation of this equipment in a residential area is likely to cause harmful interference, in which case you will be required to correct the interference at your own expense.

Operation is subject to the following two conditions:

- This device may not cause harmful interference.
- This device must accept any interference received, including interference that may cause undesired operation.

Electromagnetic Interference (EMI) is any signal or emission, radiated in free space or conducted along power or signal leads, that endangers the functioning of radio navigation or other safety service or seriously degrades, obstructs, or repeatedly interrupts a licensed radio communications service. Radio communications services include but are not limited to AM/FM commercial broadcast, television, cellular services, radar, air-traffic control, pager, and Personal Communication Services (PCS). These licensed services, along with unintentional radiators such as digital devices, including laboratory equipment, contribute to the electromagnetic environment.

Electromagnetic Compatibility (EMC) is the ability of items of electronic equipment to function properly together in the electronic environment. While this laboratory equipment has been designed and determined to be compliant with regulatory agency limits for EMI, there is no guarantee that interference will not occur in a particular installation. If this equipment does cause interference with radio communications services, which can be determined by turning the equipment off and on, you are encouraged to try to correct the interference by one or more of the following measures:

- Reorient the receiving antenna.
- Relocate the equipment with respect to the receiver.
- Move the instrument away from the receiver.
- Plug the instrument into a different outlet so that the instrument and the receiver are on different branch circuits.

If necessary, consult a ProteinSimple Technical Support representative or an experienced radio/television technician for additional suggestions.

ICES-003 Notice (Canada only)

This Class A digital apparatus complies with Canadian ICES-003.

Cet appareil numérique de la classe A est conforme à la norme NMB-003 du Canada.

Safety Guidelines

!WARNING!
If the unit is not used as specified by the manufacturer the overall safety will be impaired.

!WARNING!
If the unit is damaged and does not function properly, stop the unit safely and contact ProteinSimple Technical Support immediately.

!WARNING!
No user replaceable/serviceable parts except for system tubing and the fuse located in the power entry module.

!WARNING! FIRE HAZARD
Replace fuse only with same type and rated certified fuse. Fuse rating: T2AH 250V.

CAUTION
Avoid using the iCE3 instrument in a manner not specified by ProteinSimple. While the system has been designed to protect the user, this protection may be impaired if the instrument is used improperly.

CAUTION
Do not remove system covers. Doing so will expose users to hazardous voltages and UV radiation.

Chemical Hazard

!WARNING! BIOHAZARD

Samples and waste contents should be handled by procedures recommended in the CDC/NIH manual: Biosafety in Microbiological and Biomedical Laboratories (BMBL). The manual is available from the U.S. Government Printing Office or online at http://www.cdc.gov/biosafety/publications/bmbl5/.

Depending on the samples used, waste contents may constitute a biohazard. Use precaution when emptying waste. Dispose of waste contents in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

Interlock Sensor

An interlock sensor detects when the door is open and closed. The high voltage is automatically turned off and the UV light is shuttered when the door is open.

!WARNING!

Do not override the interlock sensor. The sensor protects users from exposure to UV light and high voltage.

UV Safety

CAUTION

The iCE system contains a UV lamp. The system enclosure confines the radiation within the system and shields the user from exposure. Exposure to UV radiation can cause permanent damage to the eyes and skin.

General Guidelines and Information

System Operation: Notification of Intended Use

NOTE: The iCE3 instrument is for research use only. Not for use in diagnostic procedures.

Lifting and Moving the System: Use Proper Lifting Precautions

IMPORTANT

Use caution when lifting or moving the iCE3 instrument. The system weight is 20 kg. ProteinSimple recommends two people lift the system onto the laboratory bench. Proper lifting safety precautions must be taken.

The iCE3 system should never be moved after it is installed. If it must be moved, please contact a ProteinSimple Service Representative.

Overview of iCE3 System with PrinCE Next Autosampler

The iCE3 system with PrinCE Next autosampler (Figure 4-10) provides automated injection of samples into the cIEF cartridge. The autosampler can accommodate either 11 mm vials or 96-well plates, and provides temperature control between 4-40 °C.

Figure 4-10: *iCE3 with PrinCE Next autosampler.*

PrinCE Next Autosampler Components

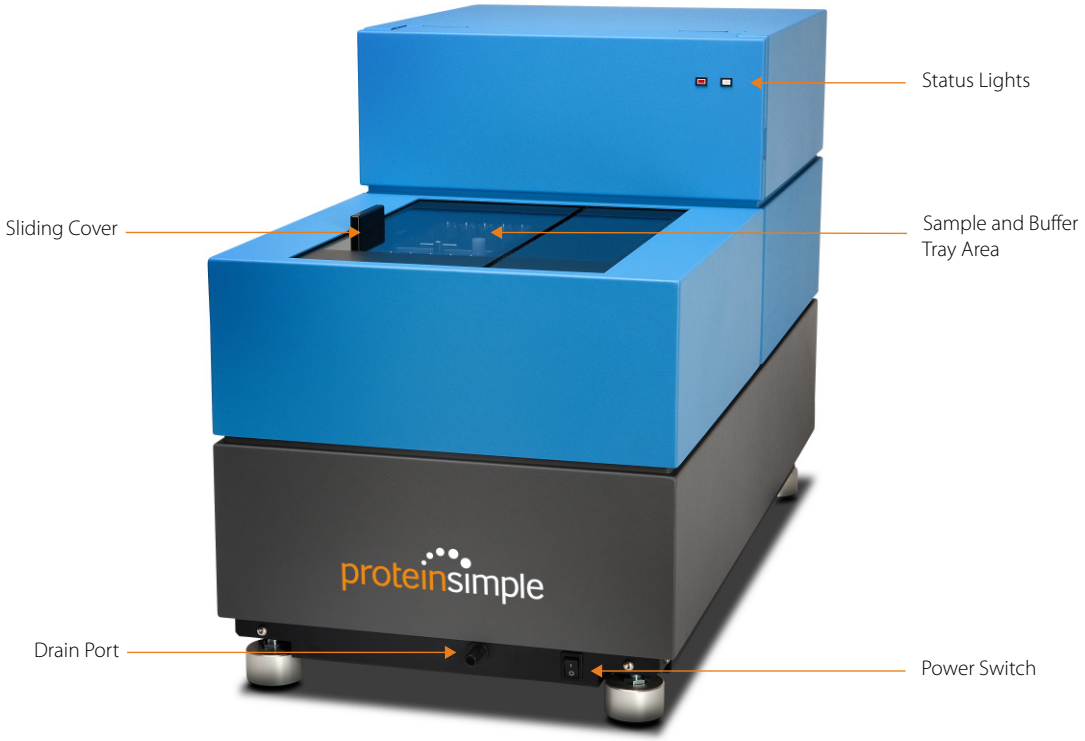

Figure 4-11: *PrinCE Next autosampler components.*

Status Lights

Two LEDs are located on the front panel to indicate system status:

- **Green**:
  - A solid green light indicates the instrument is powered on and the connection to the computer is established.
  - A flashing green light indicates the unit is powered on but the connection to the computer has been lost.
- **Red**: Indicates the sliding cover is open.

Sample and Buffer Tray Area

The PrinCE Next autosampler has two tray positions (Figure 4-12). Trays can be accessed by sliding the cover to the right.

- **Buffer: Z Tray (back position)** - Used for assay reagents such as water, TTM solution, buffers and the drying vial. This position can accommodate an 11 mm vial adapter only.
- **Sample: S Tray (front position)** - Used for samples only. This position can accommodate an 11 mm vial tray or a 96-well plate adapter.

NOTE: Each 11 mm vial tray can accommodate a maximum of 50 vials.

The tray area temperature is controlled through iCE CFR Software and can be set between 4-40 °C. The cover should be closed during a batch so the set temperature can be maintained properly.

Figure 4-12: *Sample and buffer trays: sample vial tray (left) and 96-well plate tray (right).*

Drain Port

The drain port allows condensation generated during sample and buffer tray cooling to be routed through the drain tube to waste.

Power Switch

The power switch is located on the front of the unit on the lower right.

Rear Panel

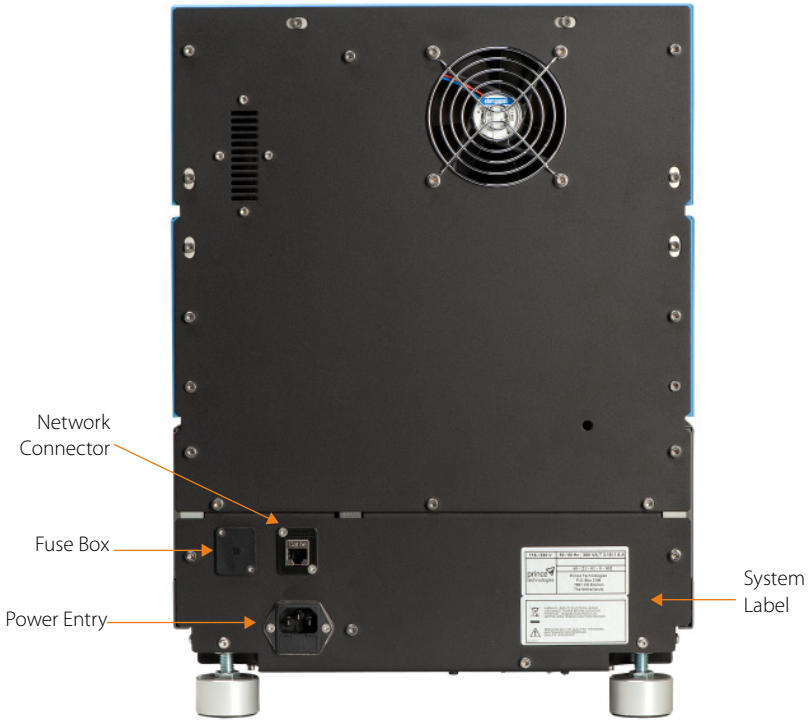

Figure 4-13: Rear panel.

- **System Power:** The power components consist of the main power switch and power entry.

!WARNING!

Only use the power supply cord provided with the instrument. If the power supply cord is damaged, please contact ProteinSimple Technical Support.

!WARNING!
No user replaceable parts except the fuses in the power entry module.

!WARNING! SHOCK HAZARD
Disconnect power cord from instrument power inlet to disconnect power to the instrument.

- **Fuse box:** Houses either one 3.5 amp,115V fuse or one 1.6 amp, 230V fuse depending on voltage requirements.
- **Network Connection:** The 10/100/BASE-T Ethernet (RJ-45 connector) is used to connect to the autosampler to the computer.

Installing Sample and Buffer Tray Adapters

Before using the autosampler, adapters must be installed in the sample and buffer trays. To do this:

1. Slide open the cover door.
2. Make sure the adapter screw (center) is in the unlocked position (Figure 4-14).

The white pin to the right of the adapter screw is the capillary height adjustment plunger.

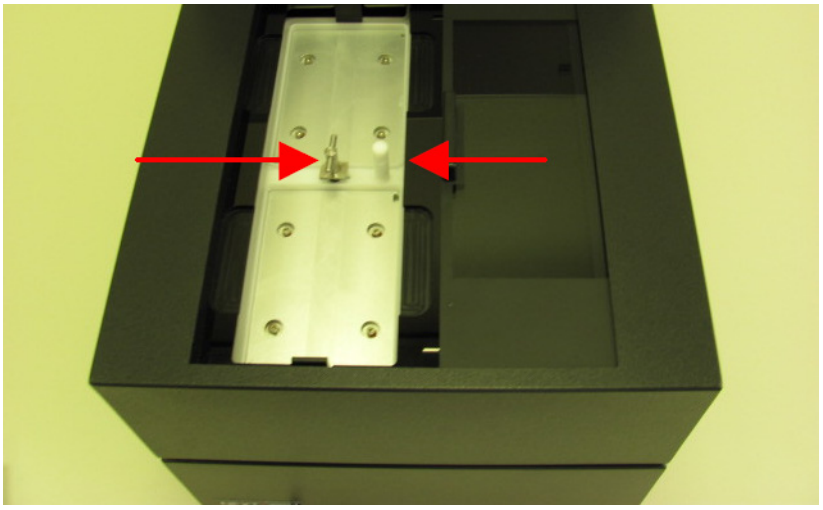

Figure 4-14: *Adapter screw (center) and capillary height adjustment plunger (right).*

3. Place a slotted vial adapter in the rear buffer tray (Figure 4-15 left) and either a slotted vial adapter or a 96-well plate adapter in the front sample tray (Figure 4-15 right). Press down on the adapter until the tabs snap in place.

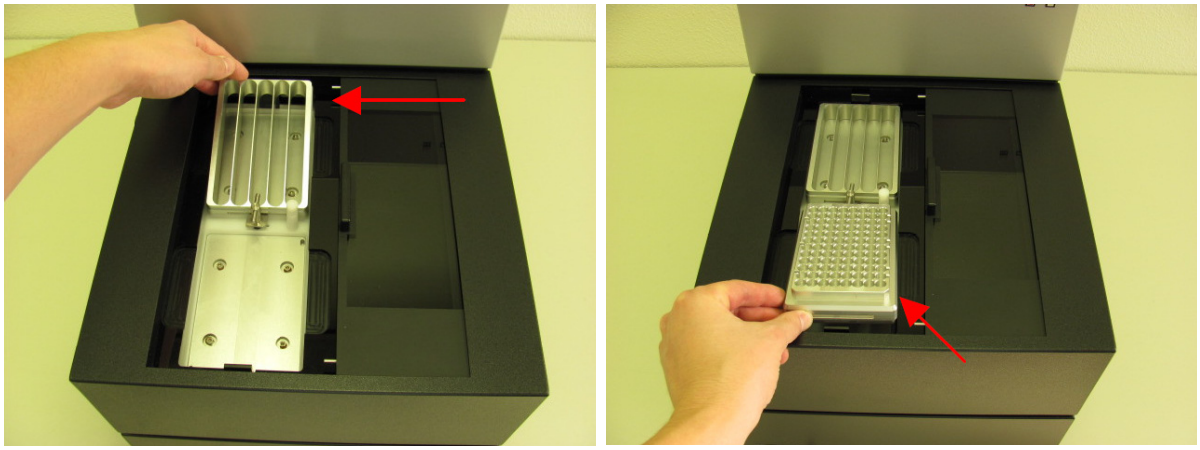

Figure 4-15: *Inserting vial adapter in buffer tray (left) and adapter in sample tray (right) - 96-well plate adapter shown.*

4. Turn the adapter screw to its locked position (Figure 4-16).

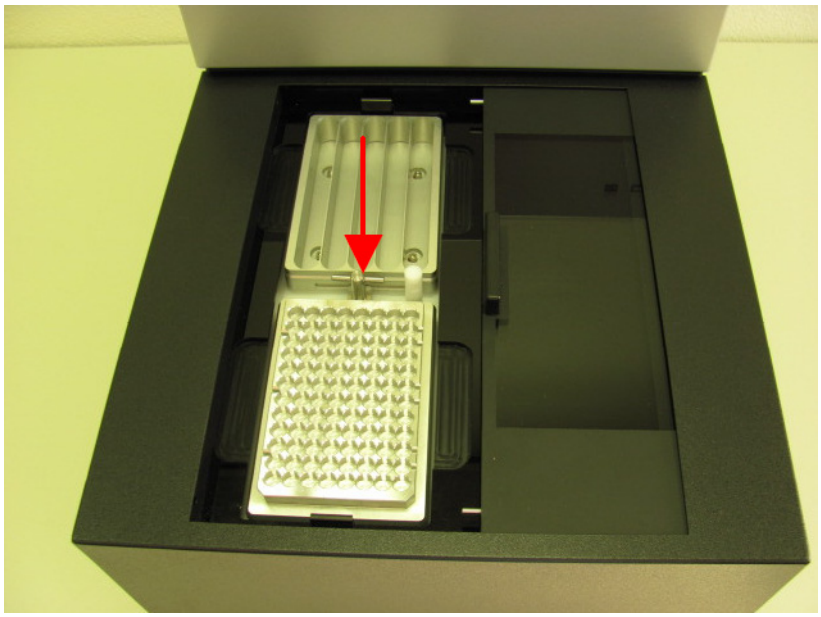

Figure 4-16: *Locking the adapter screw.*

5. Vial and plate holders can now be placed on the adapters (Figure 4-17).

NOTE: Vial holders should be installed so that the vial position labels are on the left.

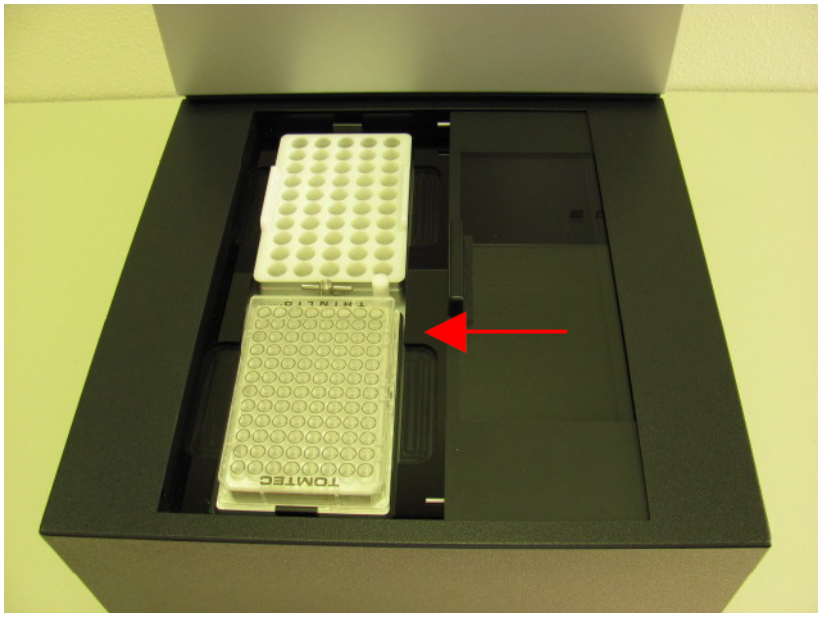

Figure 4-17: *Vial holder installed in buffer tray (back) and plate holder installed in sample tray (front).*

Tray Detection

After vial and/or plate holders are inserted into the adapters and the autosampler cover door is closed, the XYZ stage moves the tray area to the tray detect position (Figure 4-18). The autosampler then detects what tray adapters are being used (11 mm vials or 96-well plate) automatically.

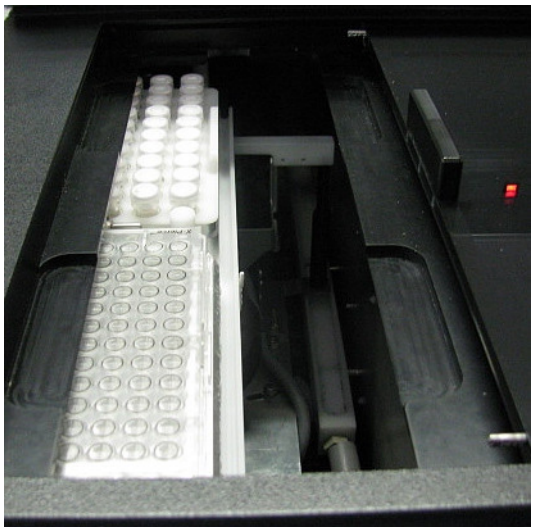

Figure 4-18: *Tray recognition position.*

Door Detection

When the PrinCE Next autosampler door is opened, the XYZ stage will stop immediately. When the door is closed, the tray area will move to the Sample Load position. If the door is opened and closed a second time, the tray area will move to the Tray Detection position. The autosampler will alternate between these two positions whenever the door is opened and closed. Use the Manual Control module in iCE CFR Software to move the tray back to the correct vial position.

IMPORTANT

After opening and closing the door it is important to move the transfer line back to a water vial to prevent clogging. If the transfer line is left exposed to air after a short-term shutdown, the methyl cellulose can dry out and clog the transfer line.

NOTE: Do not open the autosampler door when a batch is running, this will abort the batch.

Making Fluid Connections

Plumbing the iCE Valve

The ports on the iCE valve are connected as follows when the iCE3 instrument will be used with a PrinCE Next autosampler:

- **Port 1** - Transfer capillary from autosampler
- **Port 2** - To ciEF cartridge
- **Port 3** - Plugged
- **Port 4** - To waste

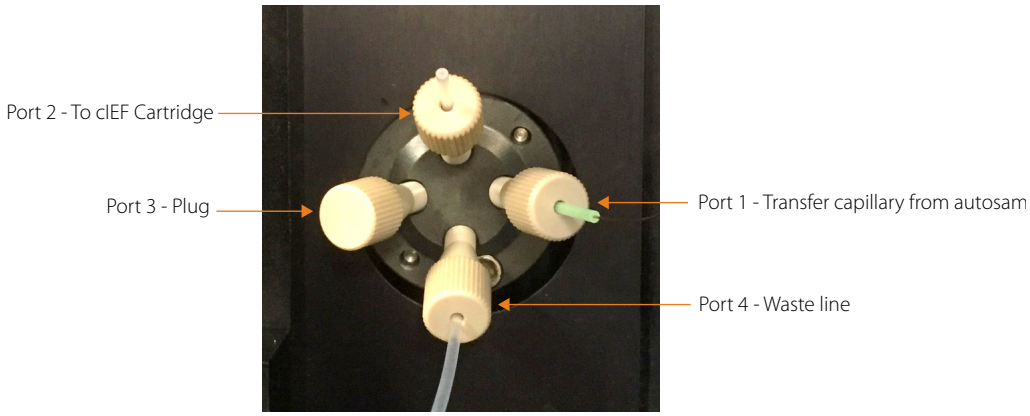

Figure 4-19: *iCE valve connections.*

1. Insert the fitting side of the waste line (P/N 045-089) into port 4 of the iCE valve (Figure 4-19) and tighten finger-tight.
2. Route the waste line through the tubing guide just below the valve. This allows the waste line to be routed out of the internal compartment.
3. Place the other end of the waste line in the waste bottle.

Figure 4-20: *Waste line routed through tubing guide.*

4. Insert a plug (P/N 102677) into port 3 and tighten finger-tight.
5. Insert a cartridge inlet sleeve fitting (P/N 045-070, clear sleeve) into port 2, but do not tighten the fitting until you are instructed to during the cartridge installation procedure.
6. Insert the transfer line sleeve fitting (green sleeve) from the transfer capillary kit (P/N 045-074) into port 1, but do not tighten the fitting until you are ready to install the transfer capillary.

Installing the Transfer Capillary

A transfer capillary is used to connect the PrinCE Next autosampler to the iCE valve.

To install the transfer capillary:

1. In iCE CFR Software, select **Utility** from the main menu, click **Maintenance** and then **Manual Control.** The manual instrument control screen will display (Figure 4-21):

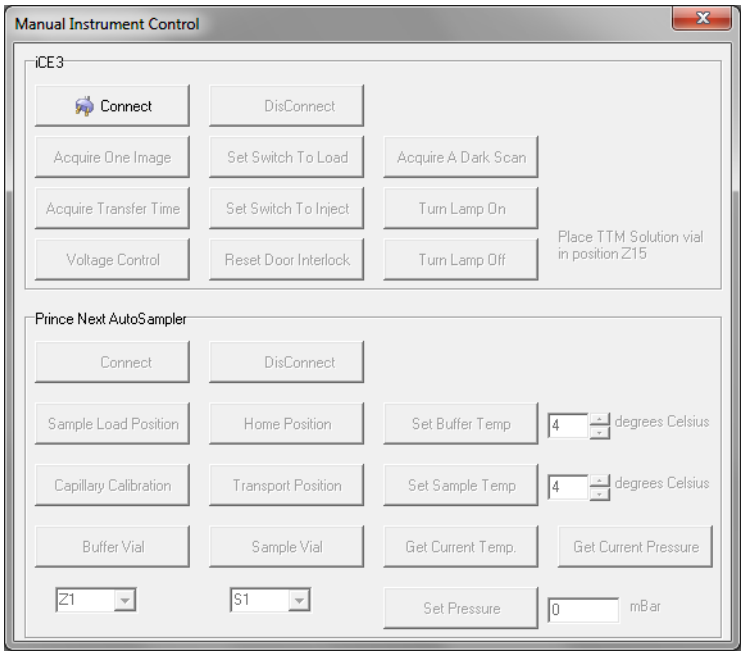

Figure 4-21: *Manual instrument control screen.*

2. In the iCE3 box, click **Connect**. Wait for the software to connect to the iCE3. When the connection is made, the buttons in the box will become active (Figure 4-22).

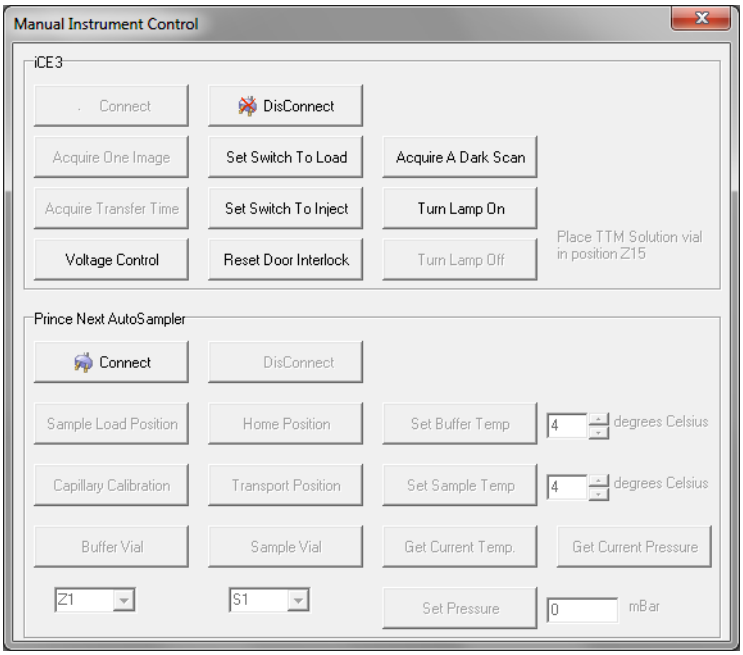

Figure 4-22: *iCE3 connected.*

3. In the PrinCE Next autosampler box, click **Connect**. Wait for the software to connect to the autosampler. When the connection is made, the buttons will become active (Figure 4-23).

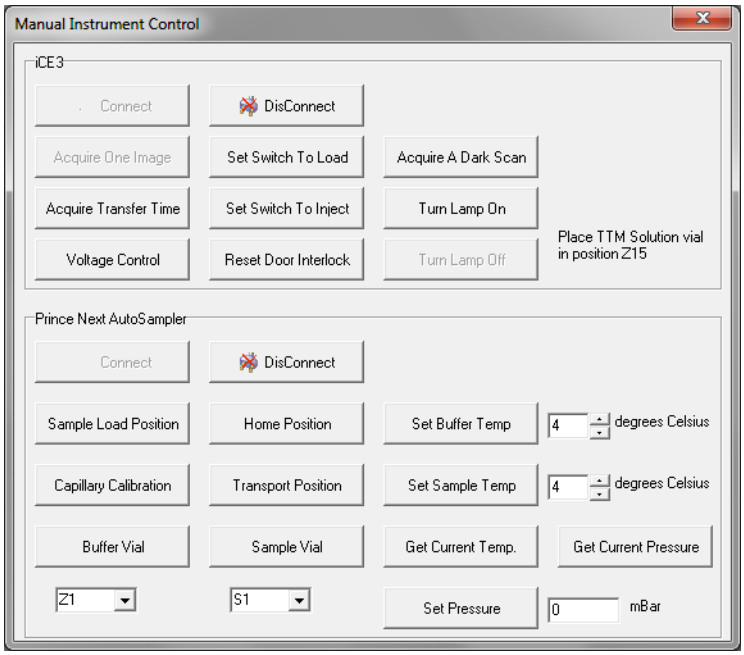

Figure 4-23: *PrinCE Next autosampler connected.*

4. Click **Capillary Calibration** in the PrinCE Next autosampler box.
5. Open the iCE3 system door and slide open the autosampler cover.
6. Thread the transfer capillary through the capillary guide (Figure 4-24).

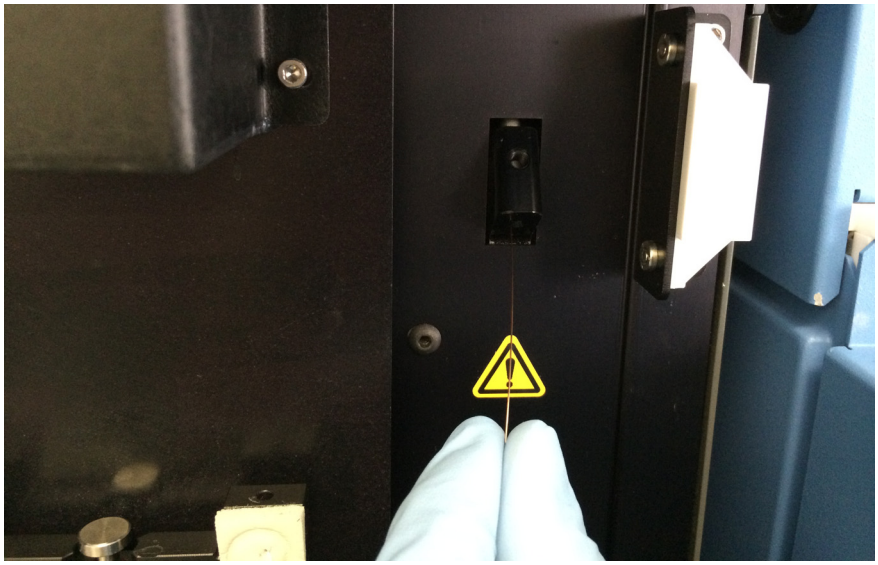

Figure 4-24: *Inserting the transfer capillary into the capillary guide.*

7. Align the iCE3 system and autosampler capillary opening. Ensure the gap from wall to wall between the autosampler and the iCE3 is approximately 14 mm (Figure 4-25).
8. Continue threading until the capillary comes out the right-side port of the iCE3 and goes into the left-side port of the autosampler (Figure 4-25).

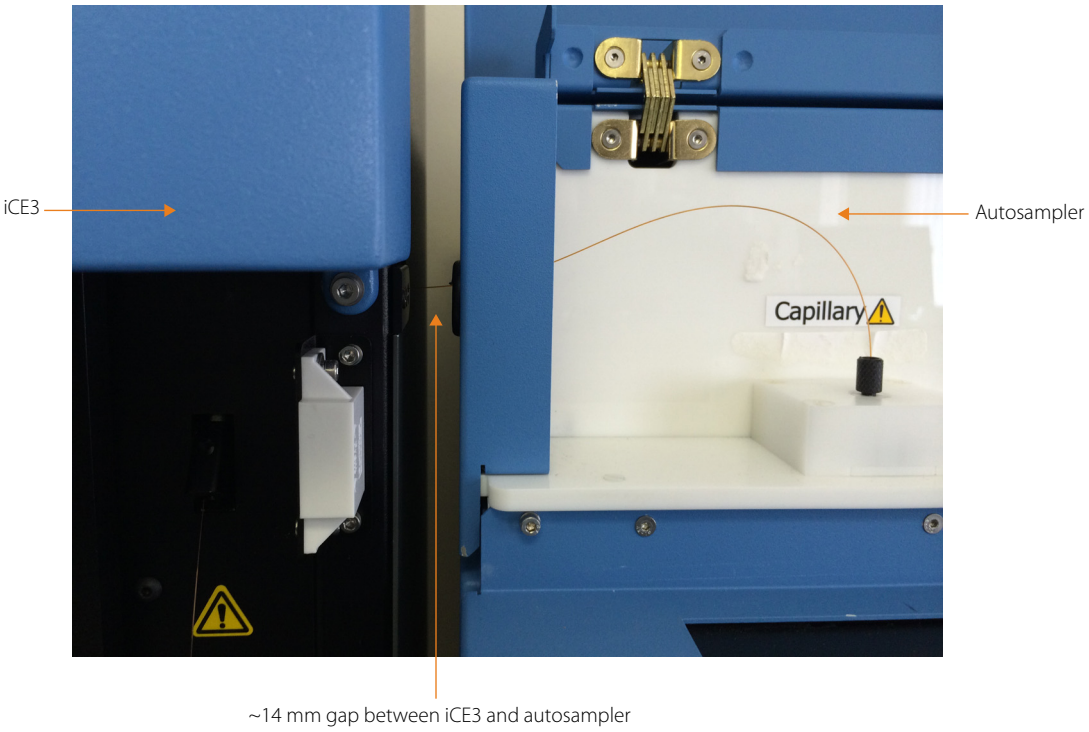

Figure 4-25: *Transfer capillary entering autosampler left-side port.*

9. Loosen the black clamping nut inside the autosampler chamber. Thread the capillary through the fitting (Figure 4-26) until it reaches a hard stop. Gently wiggle and slide the capillary up and down a few times to make sure it has completely reached the hard stop.

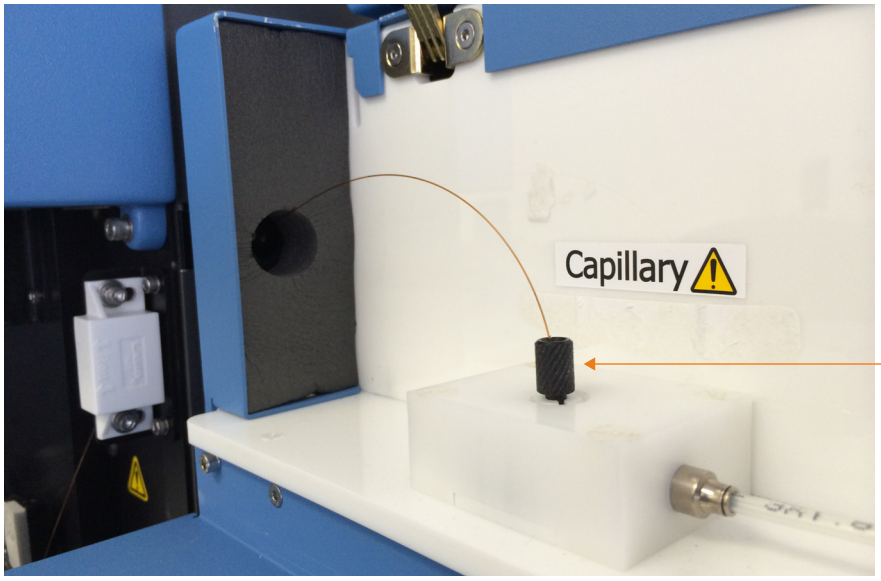

Figure 4-26: Threading the transfer capillary through autosampler fitting.

10. While holding the capillary at the stop position, tighten the fitting finger-tight plus approximately 1/4 turn. Gently tug on the capillary to make sure it's secured in place.
11. Click **Home Position** in the control screen.
12. Close the sample tray door and slide the main autosampler cover closed.
13. Thread the transfer capillary through the transfer line sleeve fitting (green sleeve) into port 1 of the iCE valve until it reaches a hard stop (Figure 4-27).
14. Gently wiggle and slide the capillary up and down a few times to make sure it has completely reached the hard stop.
15. While holding the capillary at the stop position, tighten the fitting to finger-tight plus 1/8 turn. Gently tug on the capillary to make sure it's secured in place.

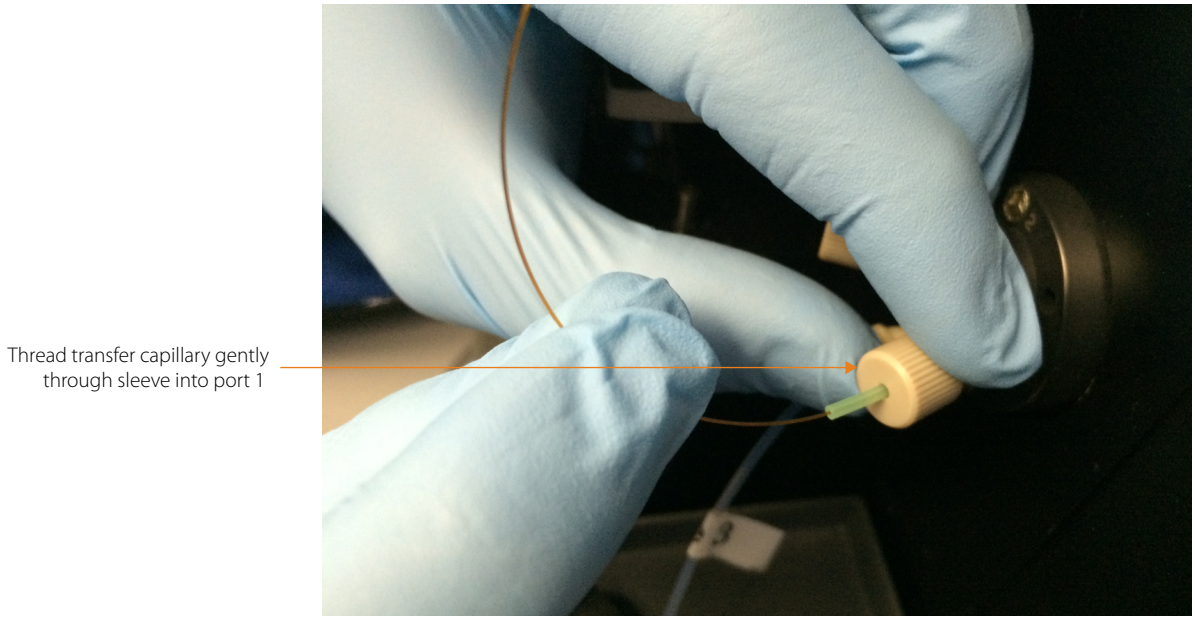

Figure 4-27: *Transfer capillary installation.*

16. In the control screen, click **Disconnect** in the PrinCE Next autosampler box, then click **Disconnect** in the iCE3 box. Close the control screen.

Overview of iCE3 System with Alcott 720NVNV Autosampler

The iCE3 system with Alcott 720NVNV autosampler (Figure 4-28) allows injection of samples into the cIEF cartridge using a positive displacement syringe pump system. The autosampler can accommodate either 11 mm vials or a 96-well microtiter plate, and provides temperature control between 4-40 °C.

Figure 4-28: *iCE3 with Alcott 720NVNV autosampler.*

Alcott 720NV Autosampler Components

Figure 4-29: *Alcott 720NV autosampler components.*

Tray Area

The Alcott 720NV autosampler is designed for use with two trays:

- **48/4 Tray:** Accommodates 48 (11 mm) vials plus 4 (10 mL) vials.
- **96/4 Tray:** Accommodates 48 a single 96-well microtiter plate plus 4 (10 mL) vials.

The tray area temperature is controlled through iCE CFR Software and can be set between 4-40 °C.

Figure 4-30: *Alcott 720NV autosampler with 48/4 tray (left) and 96/4 tray (right).*

Rear Panel

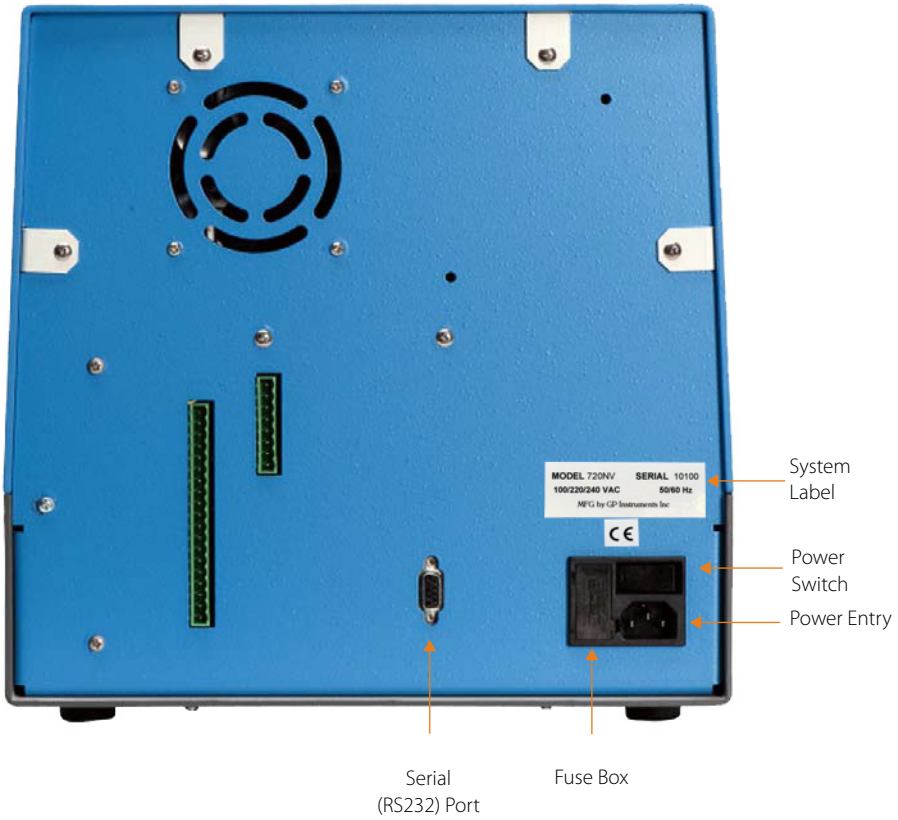

Figure 4-31: Rear panel.

- **System Power:** The power components consist of the main power switch and power entry.

!WARNING!

Only use the power supply cord provided with the instrument. If the power supply cord is damaged, please contact ProteinSimple Technical Support.

!WARNING!

No user replaceable parts except the fuses in the power entry module.

!WARNING! SHOCK HAZARD

Disconnect power cord from instrument power inlet to disconnect power to the instrument.

- **Fuse box:** Houses one 3AG 2 amp slow blow 250 V AC fuse.
- **Serial Port:** The RS232 connector is used to connect to the autosampler to the computer.

On-Board Mixing

The Alcott 720NV autosampler has an on-board mixing feature for automated sample preparation. Samples can be mixed with up to three different cIEF reagents just prior to injection, eliminating manual preparation and potential pipetting errors as well as sample stability issues.

For a standard assay, only the protein sample is added to sample vials or plate wells. Assay reagents such as a master mix, water, ampholytes or methyl cellulose are placed in positions A-C of the sample tray (Figure 4-32). Sample preparation and on-board mixing is then programmed in the batch and executed right before a sample is injected. For programming details, see "Using the On-Board Sample Mixing Feature" on page 153.

Figure 4-32: On-board mixing reagents and sample vial positions (48/4 tray shown).

Making Fluid Connections

Plumbing the iCE Valve

The ports on the iCE valve are connected as follows when the iCE3 instrument will be used with an Alcott 720NV autosampler:

- **Port 1** - Transfer tubing from autosampler
- **Port 2** - To ciEF Cartridge
- **Port 3** - Plugged

- **Port 4** - To waste

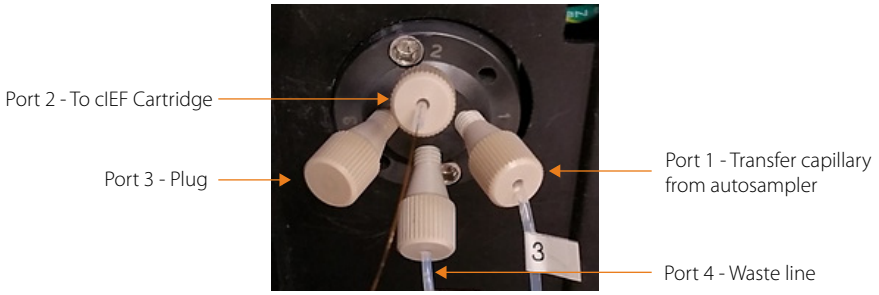

Figure 4-33: *iCE valve connections*

1. Insert the fitting side of the waste line (P/N 045-089) into port 4 of the iCE3 valve (Figure 4-19) and tighten finger-tight.
2. Route the waste line through the tubing guide just below the valve. This allows the waste line to be routed out of the internal compartment.
3. Place the other end of the waste line in the waste bottle.

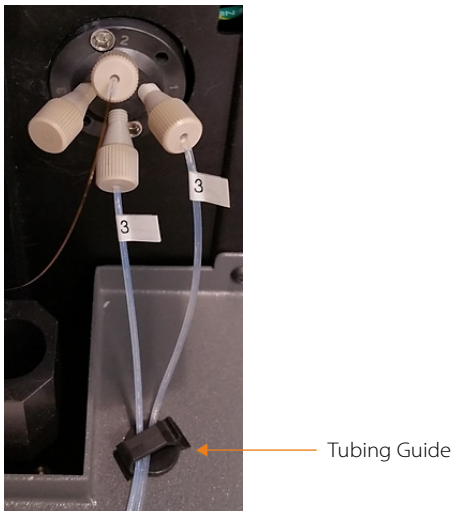

Figure 4-34: *Waste line routed through tubing guide.*

4. Insert a plug (P/N 102677) into port 3 and tighten finger-tight.
5. Insert a cartridge inlet sleeve fitting (P/N 045-070, clear sleeve) into port 2, but do not tighten the fitting until you are instructed to during the cartridge installation procedure.
6. Insert one end the transfer line (P/N 045-072) into port 1 and tighten finger-tight.

7. Use the Sleeve Tool (P/N 045-285) to tighten the fitting a quarter-turn clockwise.
8. Remove the Sleeve Tool. Gently pull the capillary to confirm it's properly secured. The capillary should not slide out of the fitting.
9. Insert the other end of the transfer line into the outlet port on the left side of the autosampler and tighten finger-tight (Figure 4-35).

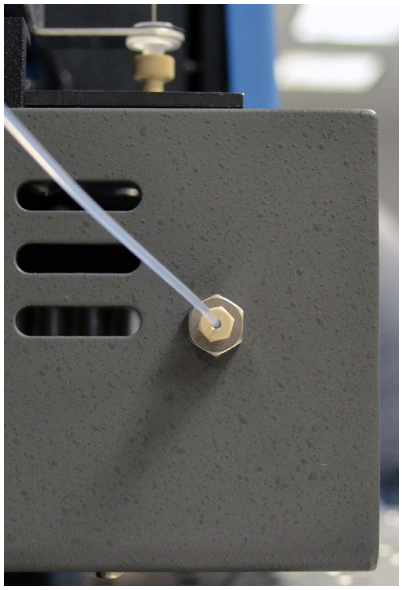

Figure 4-35: *Alcott 720NV outlet port.*

Plumbing the DI Water Supply Bottle

1. Fill the wash bottle with 150 mL of HPLC-grade deionized water.
2. Screw the lid on the waste bottle. Make sure the inlet filter is fully submerged.
3. Connect the water bottle line to the left side of the syringe pump head (Figure 4-36).

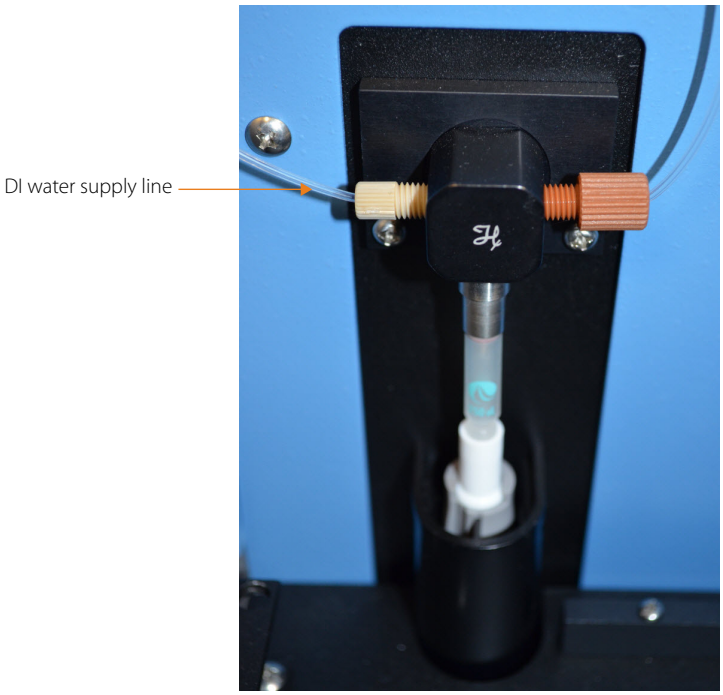

Figure 4-36: *Alcott 720NV syringe pump.*

System Specifications

Description	Specification
iCE3 Instrument	
Capillary	Fluorocarbon coated
Capillary Dimensions	5 cm x 100 µm
Detection	Whole column light adsorption, 280 nm
Detection Linear Range	> 2 orders of magnitude
Focusing Voltage	600 V/cm
Dimensions	60.5 cm H x 28.25 cm W x 31 cm D
Weight	20 kg
Power Requirements	110-120 / 220-240VAC +/- 10% ~, 60/50Hz
Power Consumption	210 W
Operating Humidity Range	40-80%, non-condensing
Operating Temperature Range	15-25 °C
Pollution Degree	Pollution Degree 2
PrinCE Next Autosampler	
Tray Capacity	Buffer Tray: 50 vials (11 mm) Sample Tray: 50 vials (11 mm) or single 96-well microtiter plate
Typical Sample Volume	14 µL
Sample Cooling	4-40 °C
Dimensions	34 cm H x 66 cm W x 66 cm D
Weight	28 kg
Power Requirements	100-240 VAC~, 50/60 Hz
Power Consumption	350 W

Description	Specification
Alcott 720NV Autosampler	
Tray Capacity	48/4 Tray: 48 (11 mm) vials plus 4 (10 mL) vials 96/4 Tray: 96-well microtiter plate plus 4 (10 mL) vials
Typical Sample Volume	25 µL
Sample Cooling	4-40 °C
Dimensions	66 cm H x 65 cm W x 55 cm D
Weight	16 kg
Power Requirements	115-240 VAC~ ,50/60 Hz
Power Consumption	80 W

Table 4-1: *iCE3 system physical specifications.*

For indoor use only. Use up to altitudes of 1524 meters (5000 feet).

Chapter 5:

Running the iCE System with the PrinCE Next Autosampler

Chapter Overview

- System Power Up
- PrinCE Next Autosampler Vial and Plate Guidelines
- Setting the Sample Tray Type
- Using the HT Cartridge
- Installing the Transfer Capillary
- Installing a cIEF Cartridge
- Preparing the System to Run a Batch
- Setting up a Batch
- Starting a Batch
- Updating a Running Batch (Batch On-the-Fly)
- End of Day Shutdown

System Power Up

1. Power up the system computer.
2. Login to Windows and wait for the program to initialize.
3. Power up the iCE3 instrument and autosampler.
4. Wait for the system to initialize. After initialization, the autosampler trays will move to the load position.
5. Double-click on the iCE CFR Software icon on the computer desktop to open the application.

PrinCE Next Autosampler Vial and Plate Guidelines

ProteinSimple recommends using only the vials and snap caps or 96-well plates shown in Figure 5-1. For a complete list of vial, plate and sealing film part numbers, please see Table 3-8 on page 30.

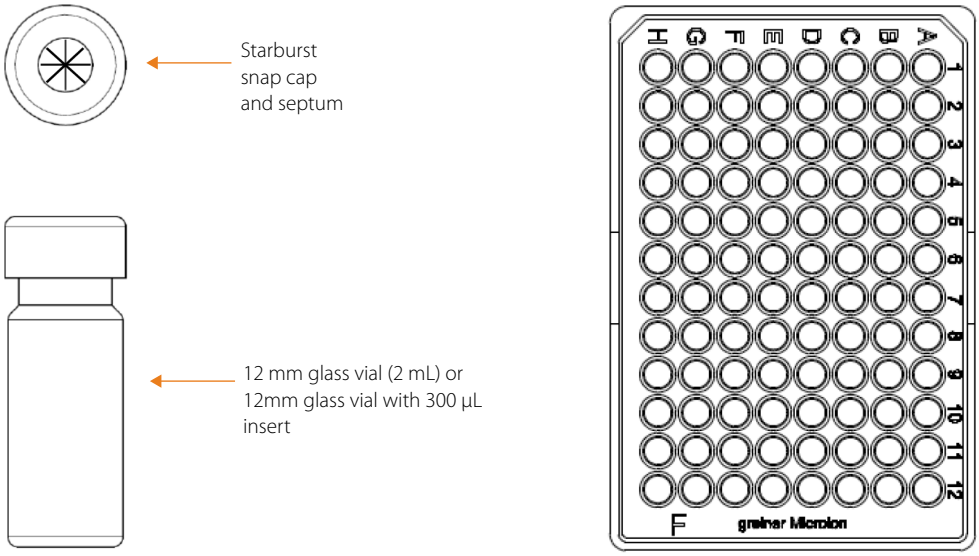

Figure 5-1: *11 mm vials with snap cap and septa (left) and 96-well plate (right).*

Vial Handling Guidelines

- 12 mm (2 mL) glass vials should be used for buffer or water.
- 12 mm glass vials with 300 µL inserts should be used for TTM solution and samples.
- Clean vials prior use. This removes particles that could cause capillary blockages.
- Vials should always be capped before being placed in the autosampler.

- Do not reuse snap caps.
- Do not fill vials more than 80% full.
- A minimum volume of 50 µL is required for vials with 300 µL inserts. Do not under-fill vials or let the liquid level get too low, this can lead to poor protein focusing.

96-Well Plate Handling Guidelines

- 96-well plates should only be used for samples.
- 96- well sample plates must be sealed before being placed in the autosampler.
- Do not reuse sealing film.

Setting the Sample Tray Type

Before using the system, you must first select the sample tray type that will be used. To do this:

NOTE: Only administrators can change system configuration settings.

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1. In iCE CFR Software, click **Utility** in the main menu and select **System Configuration**. The following screen will display:

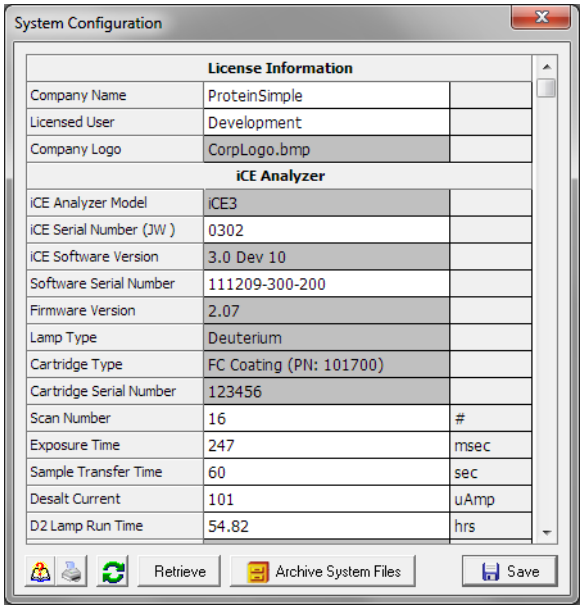


Figure 5-2: System configuration screen.

2. Scroll to the end of the configuration file using the scroll bar.
3. Click in the **Tray Type** box and select the tray you want to use:

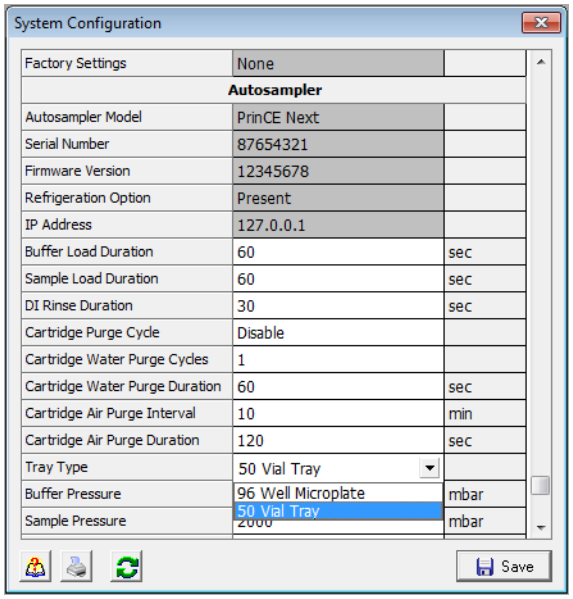


Figure 5-3: Selecting a tray type.

4. Click **Save**.

Using the HT Cartridge

Due to the lower viscosity of the methyl cellulose-free samples that can be separated on the HT Cartridge, sample loading and injection times can be modified to reduce the total time of analysis and increase efficiency of sample volume usage when using the HT cIEF Cartridges.

If using iCE CFR Software version 4.1 and higher:

When installing the HT Cartridge on a system with iCE CFR Software version 4.1 and higher, the system will automatically set the default sample load duration, it doesn't need to be set manually. If you wish to confirm the sample load duration you can do so using the procedure that follows.

If using iCE CFR Software version 3.0 and 4.0x:

As changing the **Sample Load Duration** in the **System Configuration** settings will require a cartridge installation procedure be completed prior to system use, perform this task prior to installing a HT cIEF cartridge. Then do the following:

*NOTE: When FC cIEF cartridges and methyl cellulose containing samples will be used again in the future, ProteinSimple recommends changing the **Sample Load Duration** back to the default setting of 60 seconds prior to installing a FC cIEF Cartridge with iCE CFR software versions 3.0 and 4.0x.*

1. Click **Utility** in the main menu and select **System Configuration**. The following screen will display:

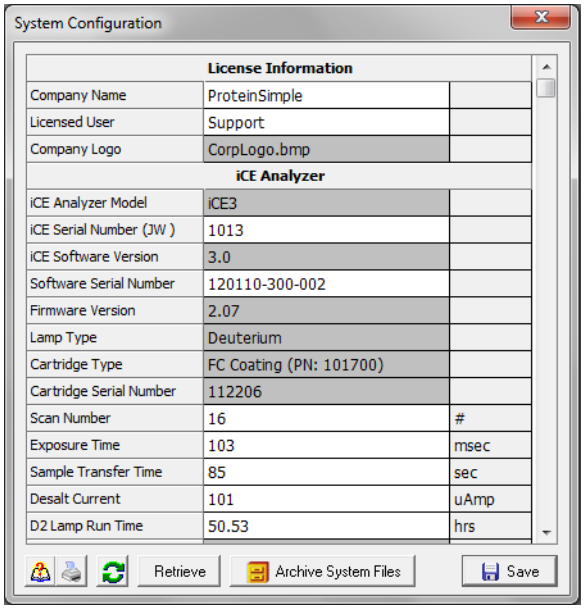


Figure 5-4: *System Configuration window.*

2. Scroll down until you reach the **Sample Load Duration** parameter, then click on the parameter setting field and change the value from 60 to 30:

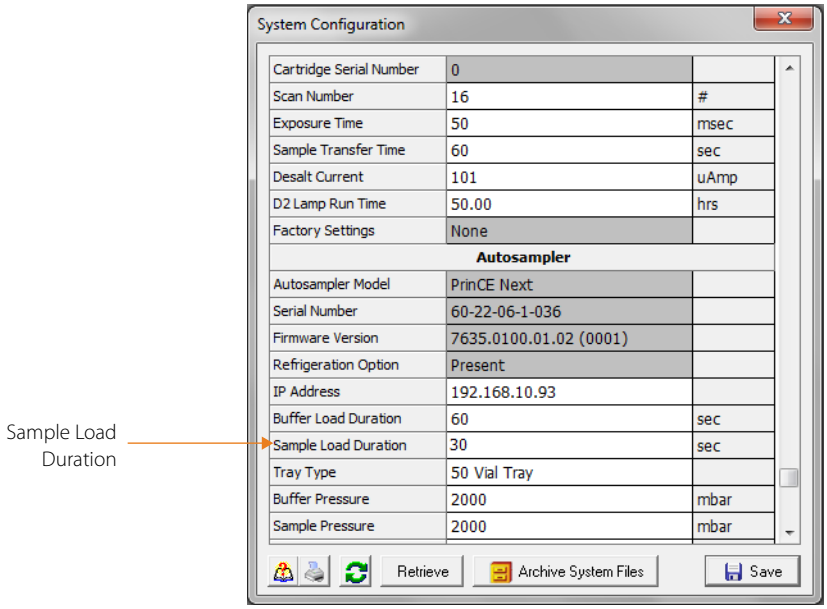


Figure 5-5: Changing Sample Load Duration Setting for HT cIEF Cartridge.

3. Click **Save** and then click **Yes** in the Confirm window.

Installing the Transfer Capillary

The transfer capillary must be installed prior to running the system. Please see "Making Fluid Connections" on page 55 for instructions on installing the transfer capillary on the iCE3 with a PrinCE Next autosampler.

Installing a cIEF Cartridge

NOTE: If a cartridge is already installed, you can skip to the quick startup procedure.

Step 1 - Prepare Reagents

1. Prepare the following reagent vials:

NOTE: Do not fill vials past 80% full. The minimum volume for 2 mL vials is 0.5 mL, and the minimum volume for vials with 300 µL inserts is 50 µL.

If using iCE CFR Software version 3.0:

- 5 vials of 0.5% methyl cellulose buffer
- 1 vial of HPLC-grade deionized water
- 1 vial of TTM solution, 200 µL
- 1 empty vial (used as a drying vial)

If using iCE CFR Software version 4.0 and higher:

- 5 vials of 0.5% methyl cellulose buffer
- 5 vials of HPLC-grade deionized water
- 1 vial of TTM solution, 200 µL
- 1 empty vial (used as a drying vial)

NOTES:
Use the appropriate TTM solution for cartridge you will be using. For an FC cIEF Cartridge, use the original FC cIEF Cartridge TTM solution (contains methyl cellulose) P/N 102672. For an HT cIEF Cartridge, use the HT cIEF Cartridge TTM solution (does not contain methyl cellulose) P/N P-0000037-00.

Use fresh 0.5% methyl cellulose solution every day. Bacteria can grow in the solution when it is stored at room temperature.

2. Centrifuge the TTM solution at 10,000 RPM for 3 minutes. Aspirate approximately 75% of the clean supernatant from the top without disturbing the separated layer on the bottom. Then transfer the solution to a sample vial.

Step 2 - Fill the Cartridge Waste Vial

3. Remove the cover on the cartridge waste vial.
4. Take the cartridge waste vial out of the iCE3.
5. Fill the vial with HPLC-grade deionized water until it overflows.
6. Reinstall the vial in the iCE3.
7. Replace the cover.

Step 3 - Insert the cIEF Cartridge in the iCE3

8. Either install a new or previously used cartridge as follows:

Installing a new cartridge:

- Remove the new cartridge from its packaging. Wash both electrolyte tanks three times with water and then gently shake the excess water from the cartridge.
- Clean the cartridge lens with residue-free, oil free condensed air:
  - Place the can's nozzle, or tube opening, 10 to 12 inches away from the top surface of the cIEF Cartridge.
  - Depress the aerosol actuator down halfway to generate a gentle flow of air.
  - Sweep the air flow across the entire space between the electrolyte tanks.
  - Turn the cIEF Cartridge over. Sweep the air flow across the back surface between the two electrolyte tanks.
  - Turn the cIEF Cartridge over again, and gently clean the top surface again.

Installing a previously used cartridge:

- Rinse the top and bottom of the cIEF Cartridge with HPLC-grade deionized water and allow the cartridge to air dry.
- Wash both electrolyte tanks three times with water, then gently shake the excess water from the cartridge.
- Clean the lens on the cartridge holder using residue-free, oil-free condensed air per the instructions above.

IMPORTANT: Do not touch the metal optical slit on the cIEF cartridge or the area beneath it.

---

9. Open the front door of the iCE3 instrument to access the cartridge holder (Figure 5-6).

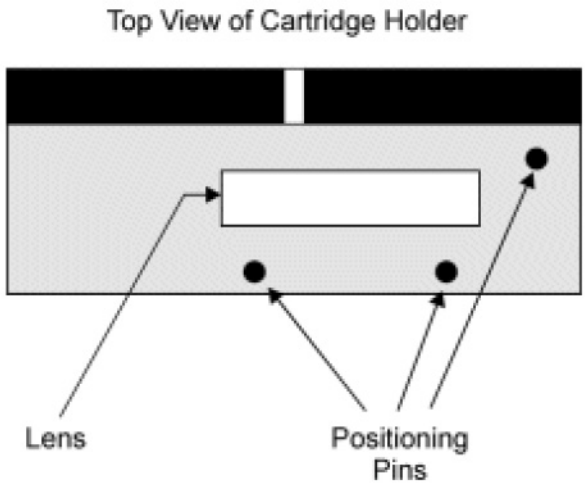


Figure 5-6: *Cartridge holder top view.*

10. Hold the cartridge above the front positioning pins and about 1-2 mm away from the pin on the right (Figure 5-7).

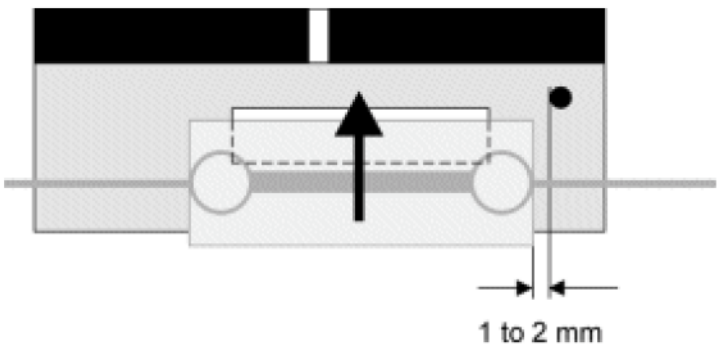


Figure 5-7: *Positioning the cartridge for installation.*

11. Tilt the cartridge at a 30-degree angle so it can be pushed towards the plunger at the back of the holder and still clear the front pins (Figure 5-8).

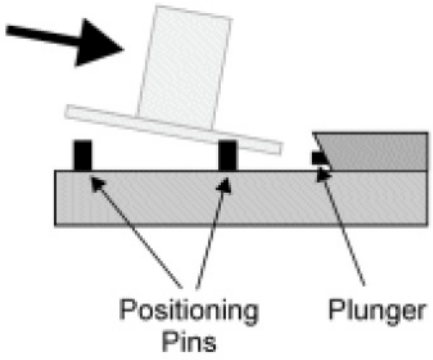


Figure 5-8: *Tilting the cartridge for installation.*

12. Push the cartridge against the plunger and then set it down gently on the lens so its positioned between the plunger and the two front pins (Figure 5-9).

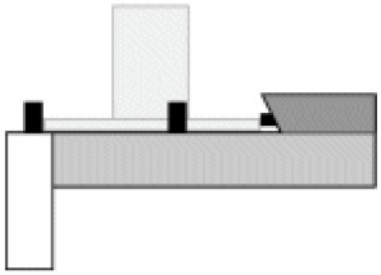


Figure 5-9: *Cartridge inserted between plunger and front pins.*

13. Gently slide the cartridge to the right until it stops at the right positioning pin (Figure 5-10).

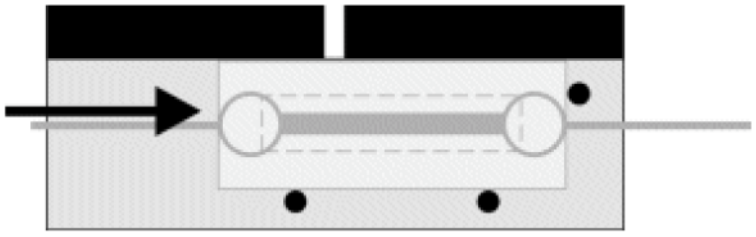


Figure 5-10: *Slide cartridge against the right pin.*

14. Make sure the cartridge is sitting firmly on the glass surface of the lens in the cartridge holder.

Step 4 - Run the Cartridge Installation Procedure

15. In iCE CFR Software, select **Utility** from the main menu, click **Maintenance** and then **Install/Replace Cartridge**. The following page displays:

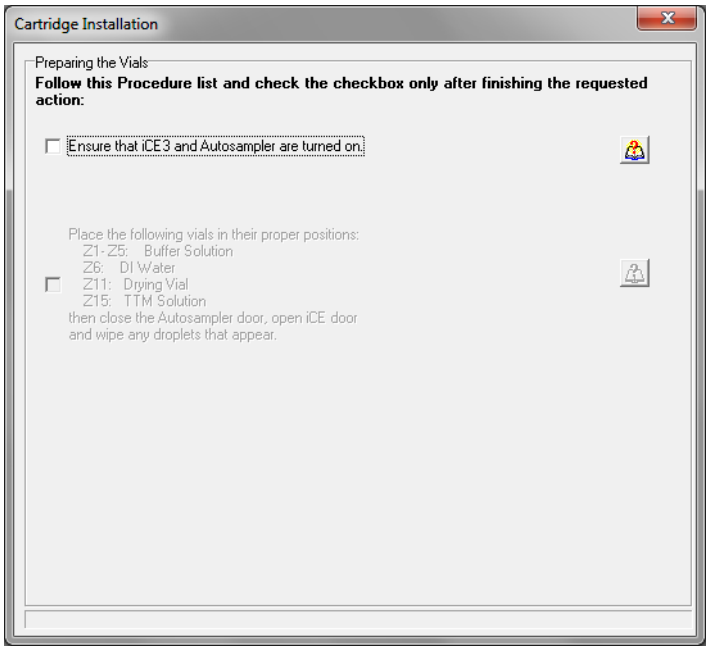


Figure 5-11: *Cartridge installation page 1.*

NOTE: The software will step you through the procedure. Click the checkbox as you complete each step to proceed to the next step.

---

16. Make sure the iCE3 and autosampler are turned on.
17. Place the prepared reagent vials in the buffer tray (Z tray, back) of the autosampler, making sure that row 1 is towards the back. Load the reagent vials in a vial holder as follows. The holder will be placed in the autosampler's buffer area (Z-tray) in an upcoming step.

    If using iCE CFR Software version 3.0:

    - **Z1 - Z5** - 0.5% methyl cellulose buffer
    - **Z6** - DI water

- **Z11** - Drying vial
- **Z15** - TTM solution

If using iCE CFR Software version 4.0 and higher:

- **Z1 - Z5** - 0.5% methyl cellulose buffer
- **Z6 - Z10** - DI water
- **Z11** - Drying vial
- **Z15** - TTM solution

Close the autosampler cover.

18. Wait for the system to perform the protocol and monitor the following as it progresses:
    - Droplets should be seen exiting the main waste line (from port 4 of the iCE valve) during the load step. If no droplets are seen:
        - **Check fluid connections.** A fitting that is either overtightened or too loose at port 1 on the iCE valve can result in no droplets at the waste line. Check port 1 for leaks. In both cases, simply remove the fitting and transfer line sleeve and reinsert as instructed in "Making Fluid Connections" on page 55.
        - **Check vials.** Confirm all vials are in their correct location and filled adequately.
        - **Check pressure and ensure it's at 2000 mBar.** If the system isn't holding pressure, confirm all vials are in the correct position. If this does not resolve the issue, please contact Technical Support.

      If none of these resolve the issue, exit the procedure and refer "Troubleshooting Fluid Path and Flow Issues" on page 243.
    - Droplets should be seen exiting the cartridge inlet sleeve fitting (port 2) during the inject step. If droplets are not seen during this step please see "Troubleshooting Fluid Path and Flow Issues" on page 243.
    - If using iCE CFR Software v4.1 and higher, the air drying step has been removed from the installation procedure. Skip to the next step. If using iCE CFR Software v3.0 and 4.0, air bubbles should be observed exiting the main waste line (port 4) and cartridge inlet sleeve fitting (port 2) during the drying steps.
19. Once you've completed the above steps, page 2 of the installation wizard will display. Select the correct cartridge from the drop down menu (FC Cartridge or HT Cartridge). Enter the cartridge serial number and click **OK**:

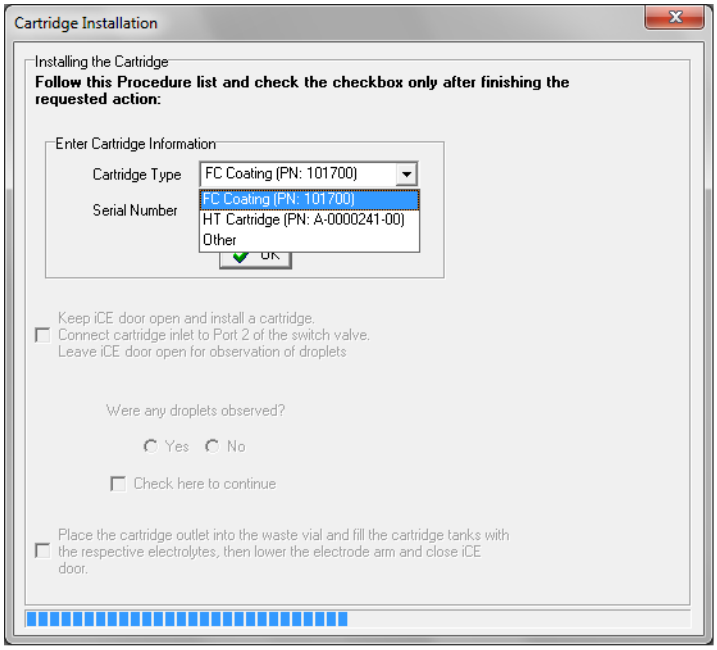


Figure 5-12: *Cartridge installation page 2.*

20. Remove the cartridge inlet sleeve and finger nut fitting from port 2 of the iCE valve.
21. Hold the cartridge inlet sleeve with two fingers of one hand, and hold the cartridge inlet capillary with two fingers of your other hand (Figure 5-13).

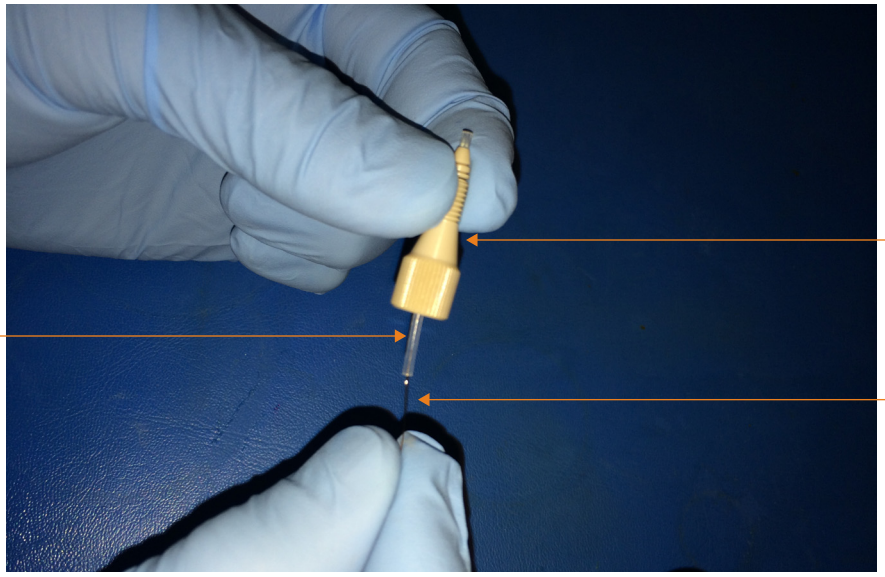


Figure 5-13: *Holding the cartridge inlet sleeve and cartridge inlet capillary.*

22. Insert the cartridge inlet capillary into the opening at the bottom of the tubing (Figure 5-13).
23. Gently push the cartridge inlet capillary through the tubing until the end of the capillary protrudes approximately 2.0 mm past the cartridge inlet sleeve (Figure 5-14).

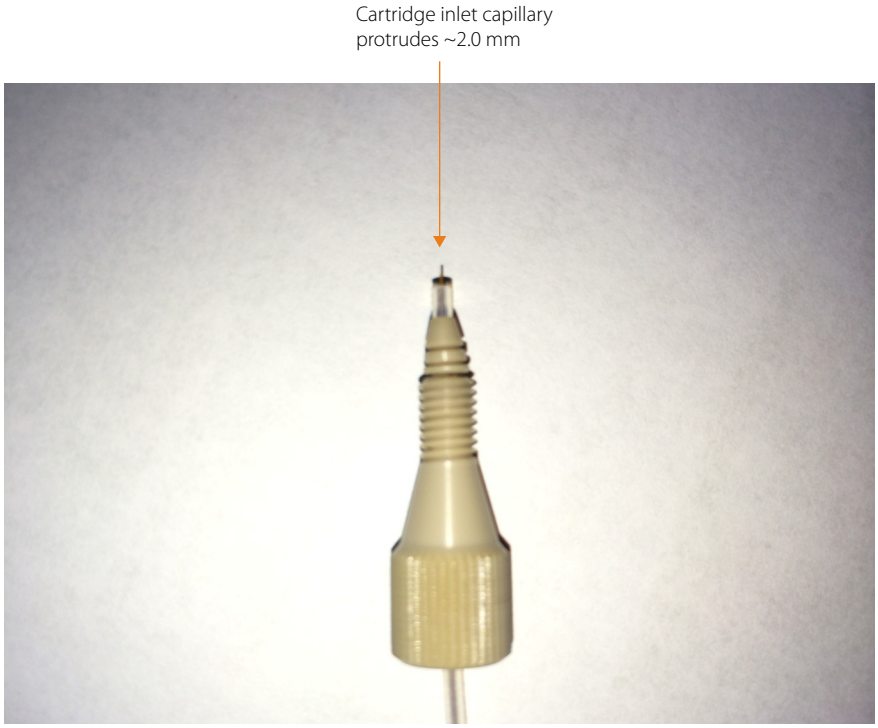


Figure 5-14: *Cartridge inlet capillary approximately 2.0 mm past the cartridge inlet sleeve.*

24. Hold the cartridge inlet sleeve and the cartridge inlet capillary and gently push inward while gently threading the cartridge inlet sleeve fitting into port 2 (Figure 3) until it reaches a hard stop.

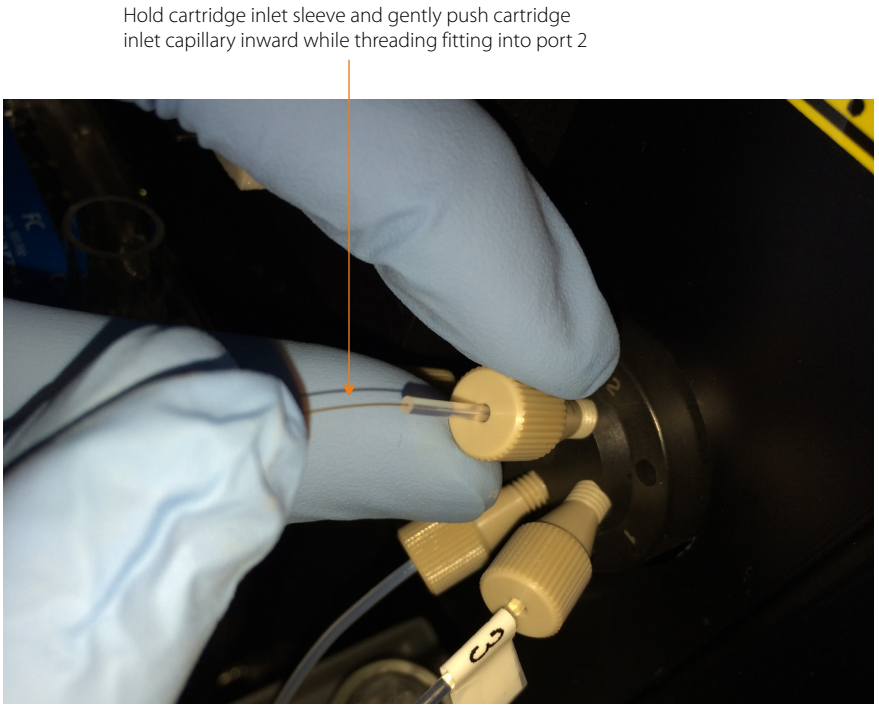


Figure 5-15: *Cartridge inlet installation.*

25. Tighten the fitting finger-tight plus approximately a 1/8 turn.
26. Once the cartridge is installed, check the box indicated in the cartridge installation screen. Wait for the system to perform the protocol and monitor the following as it progresses:
    - Droplets should be seen exiting the main waste line (port 4) during the load step.
    - Droplets should be seen exiting the cartridge outlet capillary (it will take a few minutes for this to occur).

NOTE: If droplets are not observed exiting the cartridge outlet capillary, remove the cartridge from the system and wash the cartridge using the Cartridge Wash Procedure to determine if the cartridge is clogged. If the capillary cartridge is clogged, a new cartridge should be installed.

---

27. Insert the cartridge outlet capillary into the cartridge waste vial, making sure that the end is completely below the liquid level in the vial.
28. Add anolyte to the left cartridge tank, and catholyte to the right tank. Fill each until the level is approximately 2 mm from the top of the tank.

29. Lower the electrode arm.
30. Close the iCE door.

Cartridge Light Intensity Calibration

31. The system will perform the cartridge UV light intensity calibration. The following screen will display:

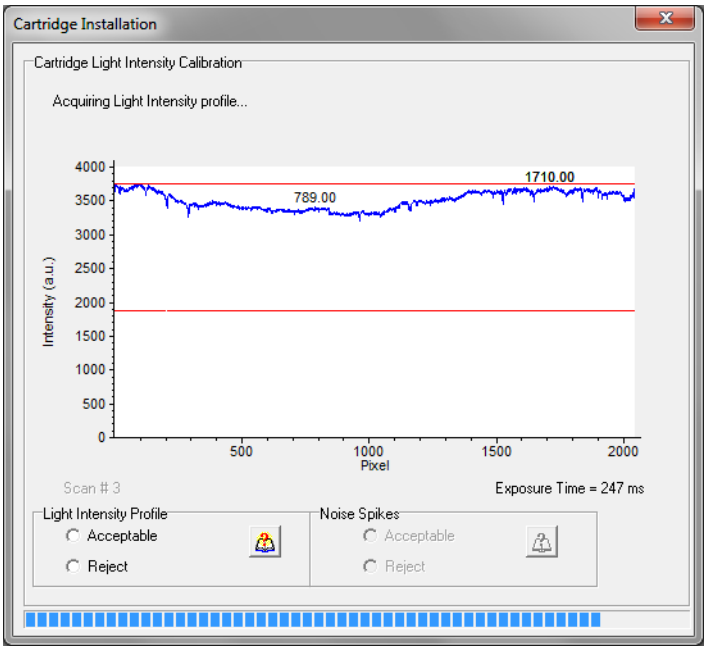


Figure 5-16: *Cartridge UV light intensity calibration screen.*

A good cartridge light intensity calibration profile will have no or very few spikes, and will maintain a signal between the red lines, as shown in Figure 5-16.

32. Click **Acceptable** to accept the calibration, or **Reject** to rerun the calibration.
33. Click **Acceptable** to accept the number of noise spikes, or **Reject** to rerun the calibration.

Sample Transfer Time Measurement

34. The software will now display the sample time transfer measurement screen. The purpose of the transfer time measurement is to ensure the sample completely fills the cartridge during sample loading.

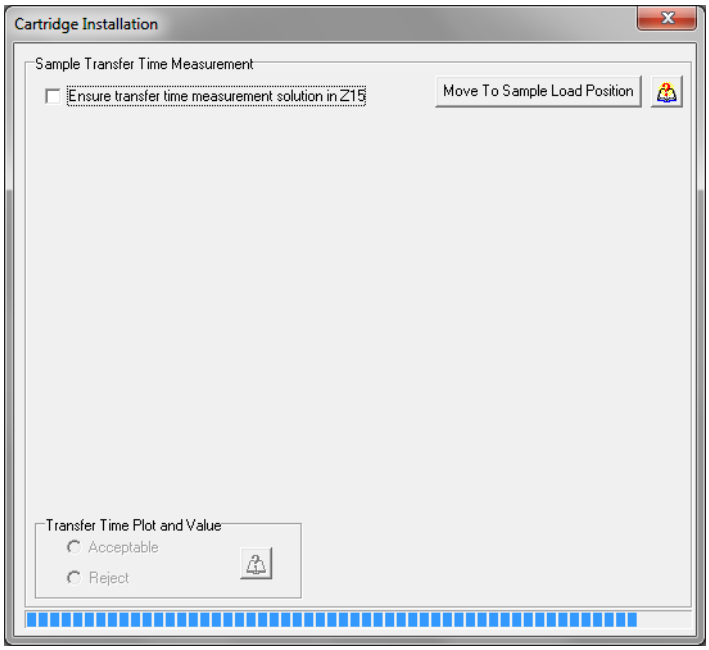


Figure 5-17: *Sample transfer time measurement screen.*

Wait for the measurement to complete, or click **Stop TTM** when an acceptable plot has been obtained:

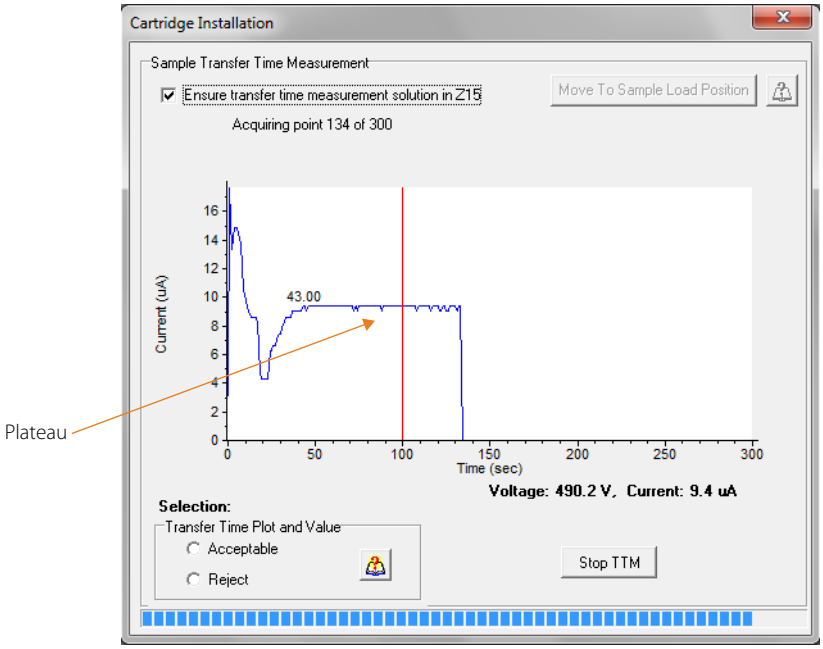


Figure 5-18: Example transfer time plot.

Accepting the Transfer Time Value and Plot

The transfer time value is indicated by the red vertical line. The transfer time value should be on the plateau section but not close to the start of the plateau to ensure complete sample loading. You can adjust the transfer line by right clicking on the plateau and selecting **Set Transfer Time**. If the plot meets the requirements below, click **Accept**. If it does not meet these requirements, click **Reject** to rerun the measurement.

- **2000 mBar injection pressures (default setting)** - An acceptable transfer time plot has the following characteristics:
  - The plateau begins prior to 60 seconds for TTM solutions containing methyl cellulose and prior to 30 seconds for methyl cellulose-free TTM solutions.
  - The plateau height is greater than 5 µA (when the ProteinSimple TTM sample is used).
- **1000 mBar injection pressures** - An acceptable transfer time plot has the following characteristics as shown in Figure 5-18:
  - The plateau begins prior to 120 seconds for TTM solutions containing methyl cellulose and just prior to 60 seconds for methyl cellulose-free TTM solutions.
  - The plateau height is greater than 5 µA (when the ProteinSimple TTM sample is used).

Preparing the System to Run a Batch

After a short-term shutdown:

Step 1 - Prepare Your Samples

NOTE: Do not fill vials past 80% full.

1. Prepare samples and centrifuge at 10,000 RPM for 3 minutes. Aspirate approximately 75% of the clean supernatant from the top without disturbing the separated layer on the bottom. Then transfer this solution to a sample vial or plate well.
   - **12 mm vials (300 µL insert)** - Use a minimum volume of 50 µL per vial
   - **96-well plate** - Use a minimum volume of 50 µL per well
2. The vials or 96-well plate will be placed in the autosampler's sample area (S-tray) in an upcoming step:
   - **12 mm vials** - Load sample vials into a vial holder starting at position **S1**.
   - **96-well plate** - Place the plate directly in the plate holder.

NOTE: Don't load samples until requested (just prior to running the batch) as this can leave the transfer line exposed to air and result in a blockage. If you do open and close the autosampler door to load samples prior to programming the batch, go to Manual Control and manually move the transfer line back to a water vial. Please note this may require you to reinstall the cartridge.

Step 2 - Prepare Assay Reagents

3. If you've just completed the installation of a new cartridge, the reagents are already in the autosampler and you can move on to "Setting up a Batch" on page 100. If you are starting the system after a short term shutdown, prepare the following reagents vials but do not load them into the autosampler until instructed to do so during the start up procedure.

NOTE: Do not fill vials past 80% full. The minimum volume for 2 mL vials is 0.5 mL, and the minimum volume for vials with 300 µL inserts is 50 µL.

If using iCE CFR software version 3.0:

- 5 vials of 0.5% methyl cellulose buffer
- 1 vial of HPLC-grade deionized water

- 1 vial of TTM solution, 200 µL
- 1 empty vial (used as a drying vial)

If using iCE CFR software version 4.0 and higher:

- 5 vials of 0.5% methyl cellulose buffer
- 5 vials of HPLC-grade deionized water
- 1 vial of TTM solution, 200 µL
- 1 empty vial (used as a drying vial)

NOTES:
Prepare a TTM sample only if you want to run a sample time transfer measurement before starting a batch.

Use the appropriate TTM solution for cartridge you will be using. For an FC cIEF Cartridge, use the original FC cIEF Cartridge TTM solution (contains methyl cellulose) P/N 102672. For an HT cIEF Cartridge, use the HT cIEF Cartridge TTM solution (does not contain methyl cellulose) P/N P-0000037-00.

Step 3 - Run the Startup Procedure

4. If you've just completed the installation of a new cartridge, you can skip the startup procedure and move on to "Setting up a Batch" on page 100. If you are starting the system after a short term shutdown, proceed to the next step.

NOTE: If you are using iCE CFR Software version 4.0 or higher, you can run either the startup procedure or the quick startup procedure. The quick startup eliminates the three-minute rinse with methyl cellulose. The quick startup is not available with iCE CFR Software version 3.0, so the startup procedure must be used.

---

5. In iCE CFR Software, select **Operate** from the main menu and click **Startup**. The following screen will display:

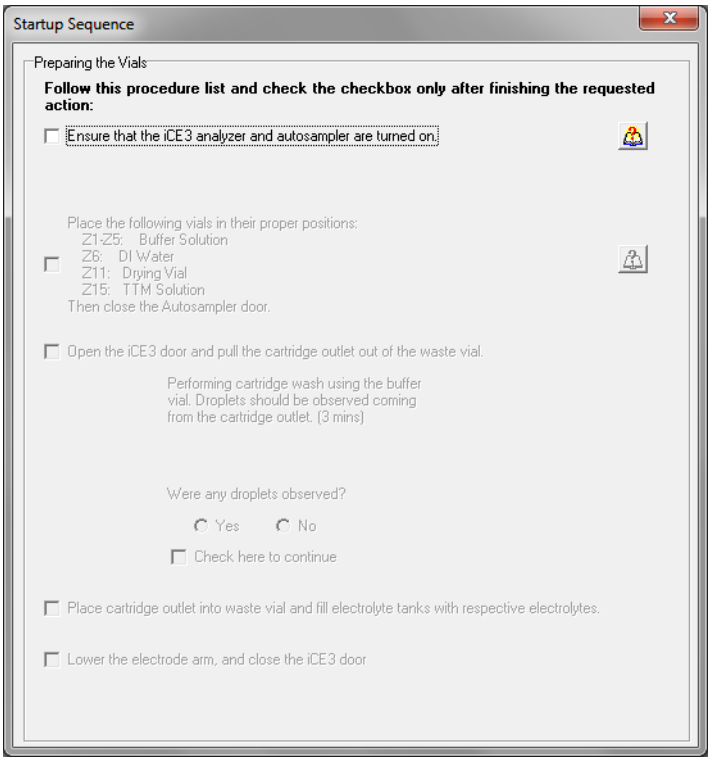


Figure 5-19: *Startup sequence screen.*

NOTE: The software will step you through the procedure. Click the checkbox as you complete each step to proceed to the next step.

---

6. Place the prepared reagents in the buffer area (Z tray, back) of the autosampler, making sure that row 1 is towards the back. Close the autosampler cover.
7. Open the iCE3 system door and remove the cartridge outlet capillary from the cartridge waste vial (if it is not already).
8. Wait for the system to perform the cartridge wash protocol. Droplets should be seen exiting the cartridge outlet capillary during the wash.
9. Insert the outlet capillary into the cartridge waste vial, making sure that the end is completely below the liquid level in the vial.
10. Add anolyte to the left cartridge tank, and catholyte to the right tank. Fill each until the level is approximately 2mm from the top of the tank.

11. Lower the electrode arm.
12. Close the iCE3 door.
13. Optional. The software will allow you to run a new cartridge light intensity profile. To skip and move on to the next step, select **Don't acquire Light Intensity Profile**. To proceed select **Acquire Light Intensity Profile**.

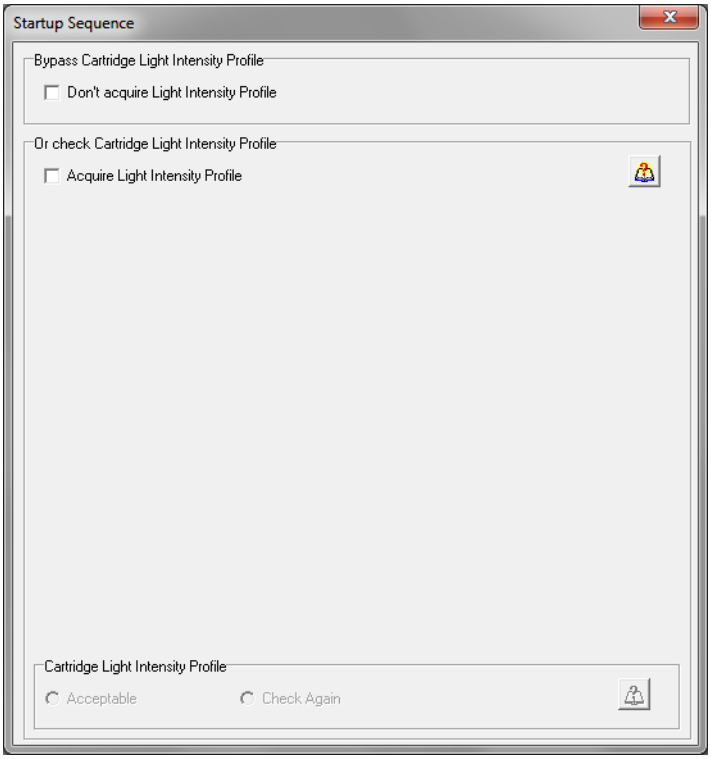


Figure 5-20: *Cartridge light intensity profile option in startup sequence.*

14. Optional. The software will allow you to run a new time transfer measurement. To skip this step and complete the startup sequence, select **Accept current measurement and complete startup**. To proceed click **Place time transfer measurement solution vial in position Z15.**

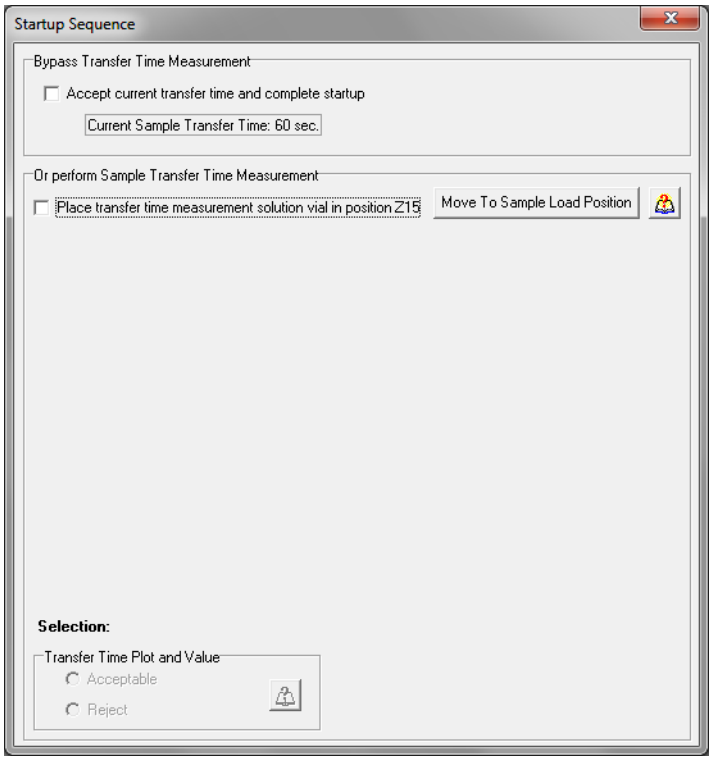


Figure 5-21: *Time transfer measurement option in startup sequence.*

Setting up a Batch

Step 1 - Create a Batch File

1. Select **Batch/Data** from the main menu and click **Development**. The following screen will display:

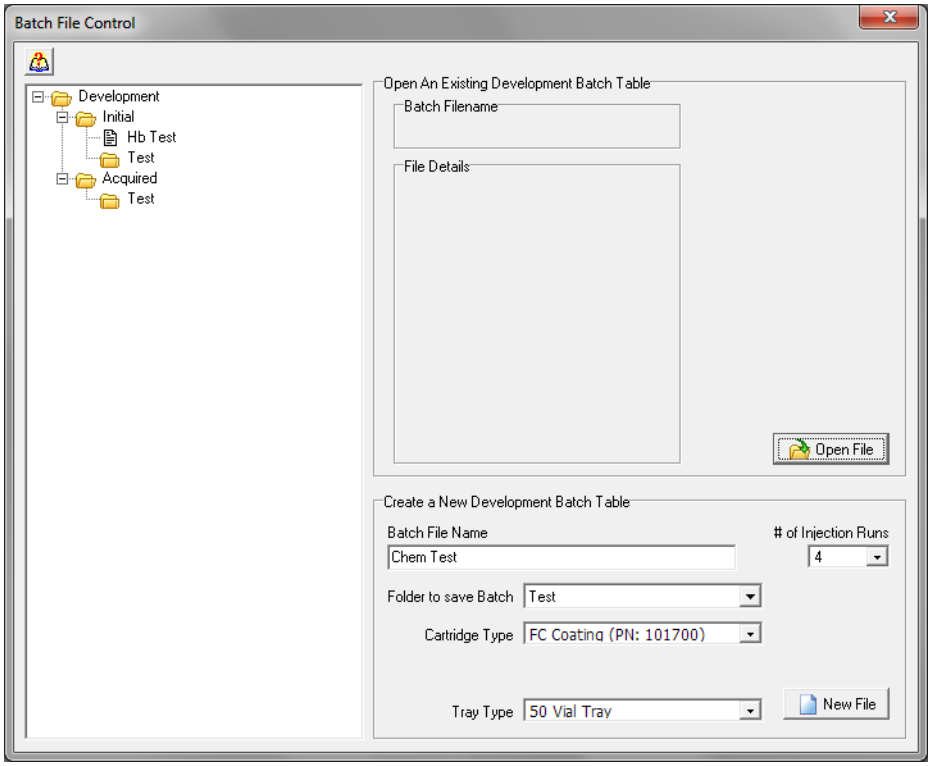


Figure 5-22: *Batch file control screen.*

2. To use a previously created batch file, expand the directory tree on the right, select the batch file and click **Open File**. Otherwise proceed to the next step.
3. Enter a name for the batch in the Batch File Name box.

NOTE: The batch name including its file path cannot exceed 255 characters.

---

4. Select a folder to save the batch file to. If the Folder to Save Batch box is left empty or NONE is selected, the batch file will automatically save to the Initial folder. To save the batch in a different folder or create a new batch folder, click the arrow. Select a folder or click **Create new folder** and enter a new name.

5. Set the number of injections. Click the arrow in the # of Injection Runs box and select the number of injections for the batch.

NOTES:
Multiple injections can be made from the same sample vial.

If needed, more injections can be added in the iCE Parameters table as described in the next section.

---

6. Select the sample tray type. Click the arrow in the Tray Type box and select **50 vial tray** or **96-well plate**.
7. Click **New File**.

Step 2 - Set iCE Parameters

The iCE Parameters tab will display (Figure 5-23). The table contains one row per injection, based on the number of injections selected previously. Each injection is saved as an individual file in the batch.

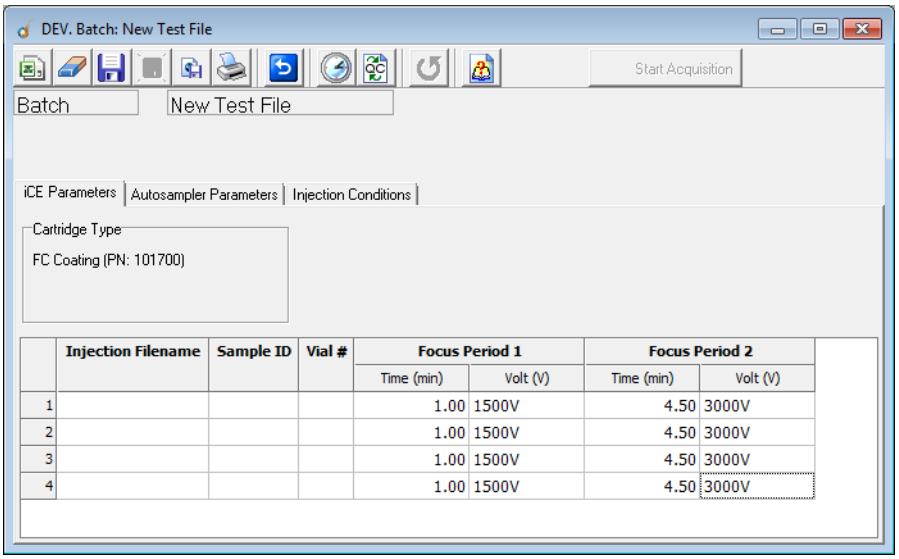


Figure 5-23: *iCE parameters.*

8. Enter a file name for the first injection. Click the row 1 cell under Injection Filename and type a name.
9. Enter a sample ID for the first injection. Click the row 1 cell under Sample ID and type a name.
10. Select the vial/well number for the first injection. Double-click the first cell under Vial # and select a vial number or plate well.

11. Set focus period 1 values for the first injection. ProteinSimple recommends using the default settings. To change these values, click in the row 1 cell under Time and enter a new value, then do the same for the Volt setting.
12. Set focus period 2 values for the first injection. ProteinSimple recommends using the default settings. To change these values, click in the row 1 cell under Time and enter a new value, then do the same for the Volt setting.

Filling the Table

13. You can now enter information for the remaining injections manually, or use the table's right-click menu to auto-fill rows.
    a. **To copy a row and use it to fill other rows** - Click the first cell of the row you want to fill from. Hold the mouse button down and select the rest of the cells in the row. Right click and select a fill option (Figure 5-24 top).
    b. **To copy a cell and use it to fill other cells in the column** - Click the cell you want to fill from. Hold the mouse button down and select the other cells in the column you want to fill. Right click and select a fill option (Figure 5-24 bottom).

Filling the Table: Copy/Paste

a. **Copy/paste for file name, sample ID and vial number** - The first three columns can be filled in from a spreadsheet using copy and paste.
b. **Review imported values** - Review imported values to ensure they are correct.

Filling the Table: CSV Import

a. **Click the CSV button in Development Mode** - This fills the cells with the same information in the selected .csv template. (Figure 5-25).
b. **Use the navigation window to select your updated template** - Modify the ProteinSimple template to your method and sample names.
c. **Review imported values** - Review imported values to ensure they are correct.

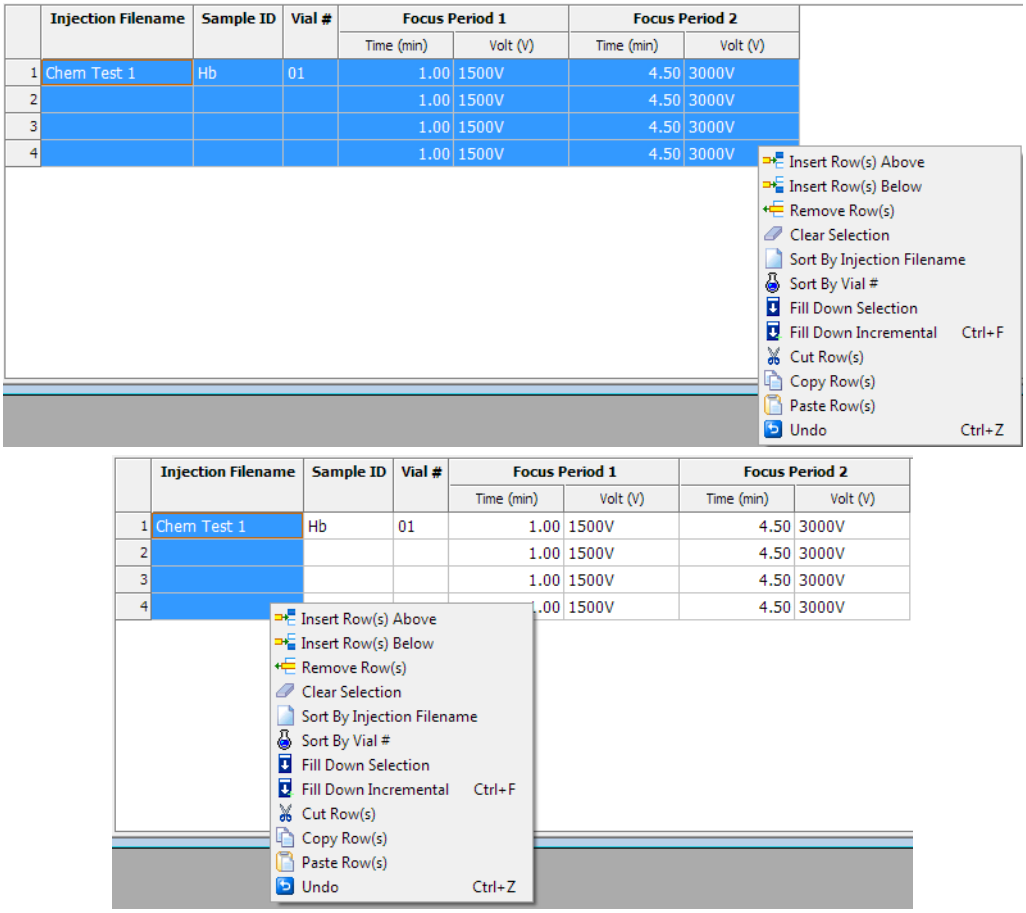


Figure 5-24: *Selecting rows and cells to auto-fill the table.*

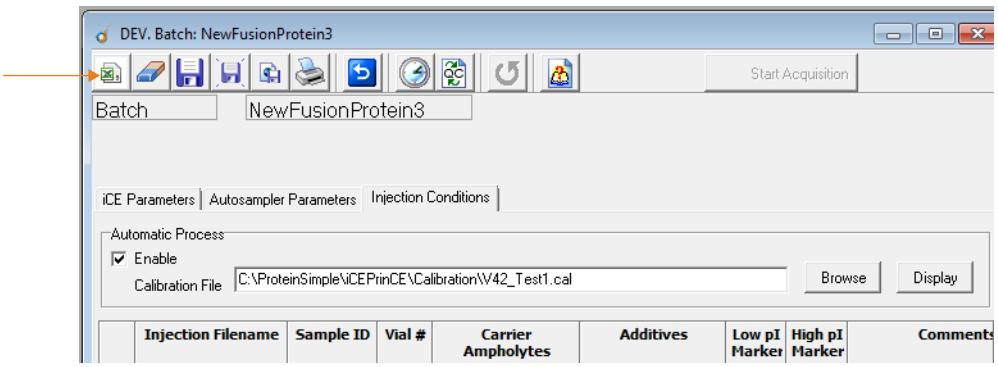
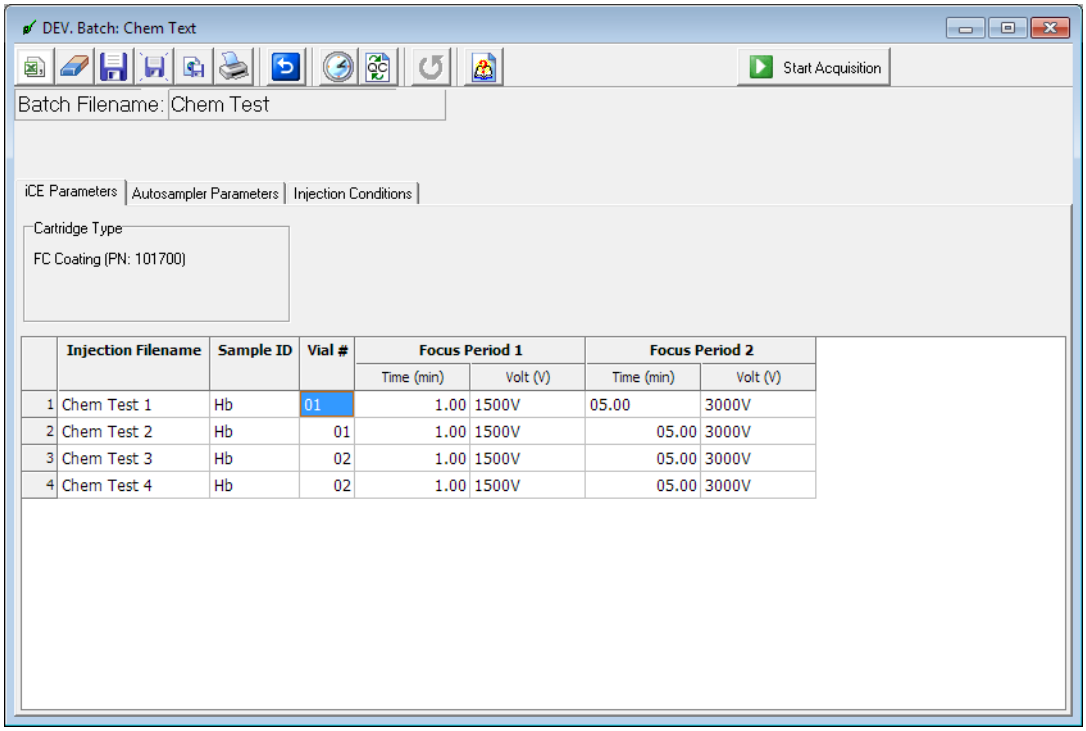


Figure 5-25: *Filling table with CSV button.*

The following fill and other right click table menu options are available:

- **Fill Down Selection** - Fills the selected cells with the same information.
- **Fill Down Incremental** - Fills the selected cells with the same information, but adds numbered increments in the Injection Filename, Sample ID and Vial # rows. If a number is entered as the last value in the cell to fill from, the remaining cells will increment from that value.
- **Insert Row(s) above** - Inserts a user-entered number of rows above the current selection. The new rows will populate using the selected row's parameters.
- **Insert Row(s) below** - Inserts a user-entered number of rows below the current selection. The new rows will populate using the selected row's parameters.
- **Remove Row(s)** - Removes the selected rows. Only one cell in the row needs to be selected.
- **Clear Selection** - Clears the information in the selected cells.
- **Sort by Injection Filename** - Sorts the table so rows with the same injection file names are grouped together.
- **Sort by Vial #** - Sorts the table so rows using the same vial or well number are grouped together.
- **Cut Row(s)** - Removes the currently selected row. Only one cell in the row needs to be selected.
- **Copy Row(s)** - Copies the currently selected row. Only one cell in the row needs to be selected.
- **Paste Row(s)** - Pastes the copied row into the selected row. Only one cell in the row needs to be selected.
- **Undo** - Reverts the last row-related action. You can continue to click undo to revert multiple actions.

14. When all the information has been entered, you can move on to the next tab. An example of a completed iCE Parameters table is shown in Figure 5-26 and includes four separate injections from two individual vials:



	Injection Filename	Sample ID	Vial #	Focus Period 1		Focus Period 2	
				Time (min)	Volt (V)	Time (min)	Volt (V)
1	Chem Test 1	Hb	01	1.00	1500V	05.00	3000V
2	Chem Test 2	Hb	01	1.00	1500V	05.00	3000V
3	Chem Test 3	Hb	02	1.00	1500V	05.00	3000V
4	Chem Test 4	Hb	02	1.00	1500V	05.00	3000V

Figure 5-26: *Example of a completed iCE Parameters table using four injections from two vials.*

Step 3 - Set Autosampler Parameters

15. Select the Autosampler Parameters tab (Figure 5-27). Use the scroll bar to view all parameters in the tab.

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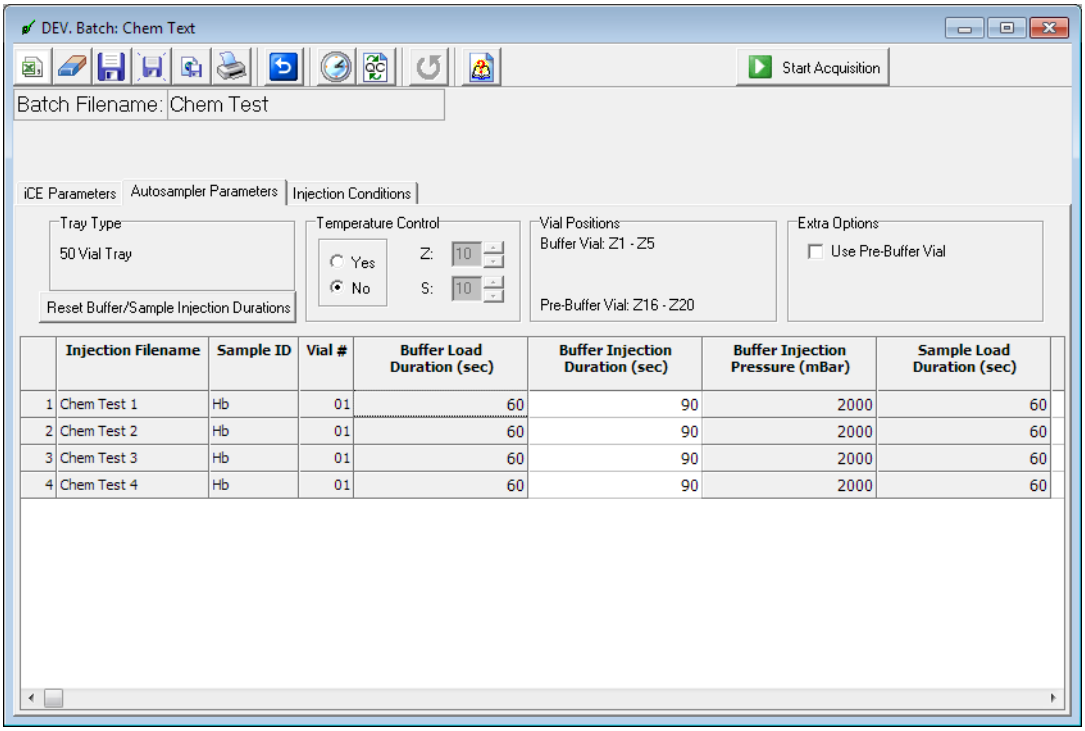


Figure 5-27: *Autosampler Parameters tab.*

16. Set the tray temperature (Figure 5-28). ProteinSimple recommends cooling the sample and buffer trays to 4 °C.
    a. Click **Yes** in the Temperature Control box.
    b. Click the up/down arrows next to Z (buffer tray) and select a temperature between 4 and 40 °C.
    c. Click the up/down arrows next to S (sample tray) and select a temperature between 4 and 40 °C.

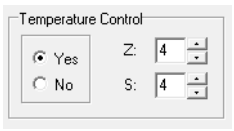


Figure 5-28: *Setting tray temperatures.*

17. ProteinSimple recommends using the default settings for all other autosampler parameters. The following settings can be changed.
    a. **To change the Buffer Injection Duration** - Click in a cell under the column and enter a value in sec-

onds. This is the amount of time the cIEF cartridge is rinsed with methyl cellulose buffer between injections.

b. **To change the Sample Injection Duration** - Click in a cell under the column and enter a value in seconds. Increasing this time will increase the amount of sample used for each injection.

c. **To set Buffer and Sample Injection Durations back to default values** - Click **Reset Buffer/Sample Injection**.

Fill the cells in the table as needed using the right-click menu options described in "Filling the Table" on page 102.

18. **Optional.** To use a pre-buffer step, click **Use Pre-Buffer Vial** in the Extra Options box.

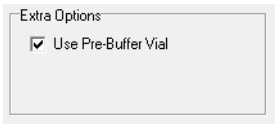


Figure 5-29: *Extra Options box.*

Two new parameters will appear in the table: Pre-Buffer Injection Duration and Pre-Buffer Injection Pressure (Figure 5-30).

	Injection Filename	Sample ID	Vial #	Pre-Buffer Injection Duration (sec)	Pre-Buffer Injection Pressure (mBar)	Buffer Load Duration (sec)	Buffer Injection Duration (sec)
1	Chem Test 1	Hb	01	0	0	60	90
2	Chem Test 2	Hb	01	0	0	60	90
3	Chem Test 3	Hb	01	0	0	60	90
4	Chem Test 4	Hb	01	0	0	60	90

Figure 5-30: *Pre-Buffer parameters.*

a. **To change the Pre-Buffer Injection Duration** - Click in a cell under the column and enter a value in seconds.

b. **To change the Pre-Buffer Injection Pressure** - Click in a cell under the column and enter a value in mBar.

Fill the remaining cells in the table as needed using the right-click menu options described in "Filling the Table" on page 102.

19. When all the information has been entered, you can move on to the next tab. An example of a completed Autosampler Parameters table using the ProteinSimple default parameters is shown in Figure 5-31.

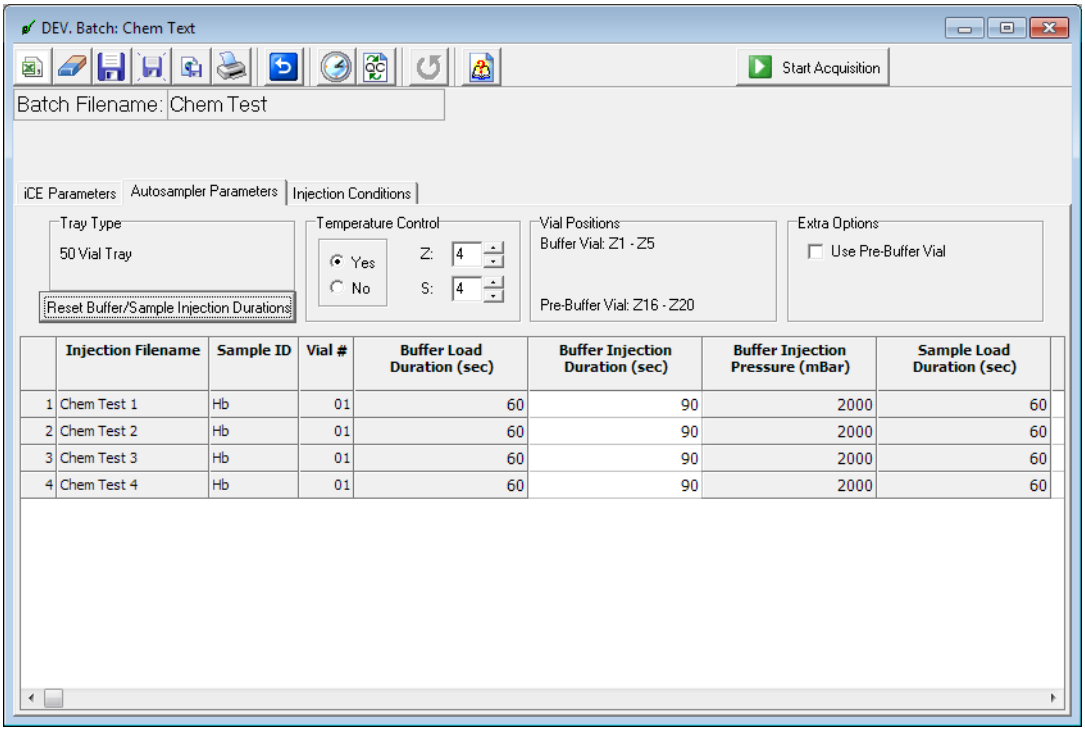


Figure 5-31: *Example of a completed Autosampler parameters table using ProteinSimple default parameters.*

Step 4 - Injection Conditions

20. Select the Injection Conditions tab (Figure 5-32). Use the scroll bar to view all parameters in the tab.

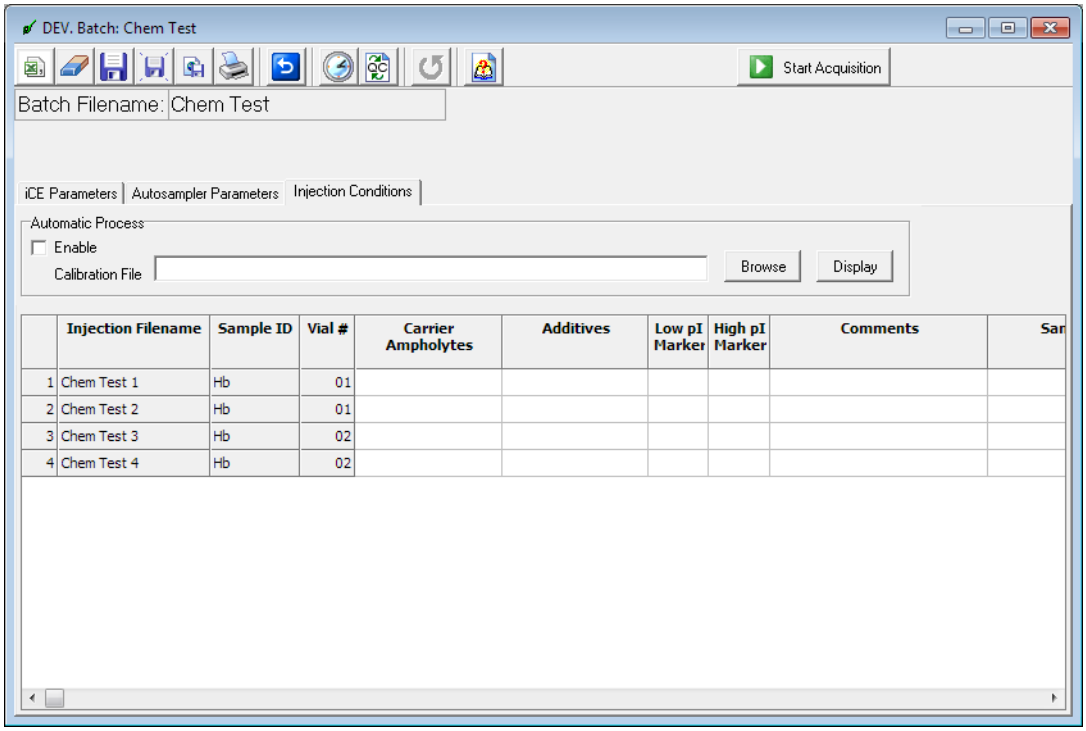
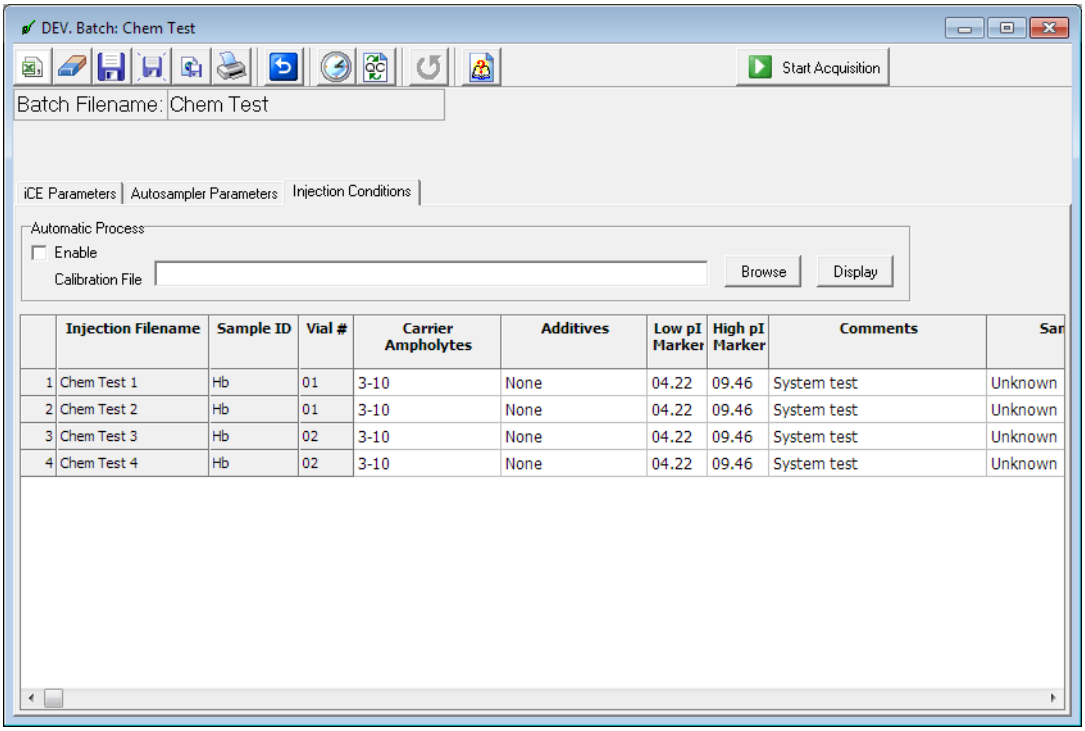


Figure 5-32: *Injection Conditions tab.*

21. Enter the carrier ampholyte range for the first injection. Click the row 1 cell under Carrier Ampholytes and type a range (for example 3-10).
22. Enter names of any additives (optional). Click the row 1 cell under Additives and type a name.
23. Enter the pI for the low pI marker. Click the row 1 cell under Low pI Marker and enter a pI (for example 4.22).
24. Enter the pI for the high pI marker. Click the row 1 cell under High pI Marker and enter a pI (for example 9.46).
25. Enter comments (optional). Click the row 1 cell under Comments to add any additional information.
26. Enter sample type (optional). Click the row 1 cell under Sample Type to and select **Standard** or **Unknown**. Sample type is used for data calibration when the batch file is processed.
27. Enter sample concentration (optional). Click the row 1 cell under Concentration to add concentration in mg/mL.
28. Fill the remaining cells in the table as needed using the right-click menu options described in "Filling the Table" on page 102.

NOTE: iCE CFR versions 4.0 and higher provide automated pI calibration and data export. A saved calibration file that includes processing information can be saved with a batch prior to running. As the data is acquired it will be automatically calibrated and then converted to the selected format for analysis. For more information on automated pI calibration and data export see "Processing Data - Automated pI Calibration and Data Export" on page 181.

An example of a completed Injection Conditions table using the ProteinSimple default parameters for the Chemical Test Kit is shown in Figure 5-33.



	Injection Filename	Sample ID	Vial #	Carrier Ampholytes	Additives	Low pI Marker	High pI Marker	Comments	Sar
1	Chem Test 1	Hb	01	3-10	None	04.22	09.46	System test	Unknown
2	Chem Test 2	Hb	01	3-10	None	04.22	09.46	System test	Unknown
3	Chem Test 3	Hb	02	3-10	None	04.22	09.46	System test	Unknown
4	Chem Test 4	Hb	02	3-10	None	04.22	09.46	System test	Unknown

Figure 5-33: *Example of a completed Injection Conditions table using Chemical Test kit parameters.*

Step 5 - Save the Batch

29. Click Save (disk icon) to save the batch file.

Starting a Batch

1. Place the previously prepared samples in the autosampler:
   - **11 mm vials** - Place the vials in the sample area (S tray, front), so that row 1 is towards the back.
   - **96-well plate** - Place the plate in the sample area (S tray, front) so that well A1 is in the right rear corner.
2. Close the autosampler cover and the iCE3 system door.
3. Click **Start Acquisition**. As the batch runs, progress and sample data is displayed in real-time:

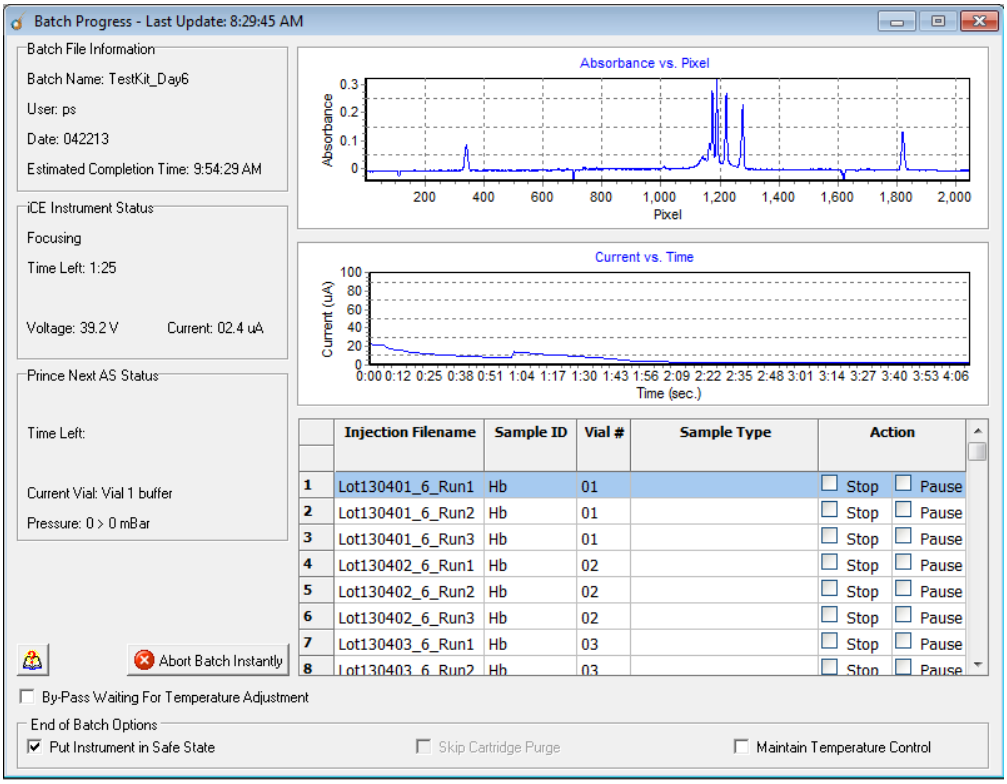


Figure 5-34: Batch in progress.

NOTE: Do not open the iCE3 system door or the autosampler door when a batch is running, this will abort the batch.

- **If using CFR version 3.0 and 4.0x** - make sure to check the **Put Instruments in Safe State** box. When this box is checked, the system will rinse the transfer line with water to minimize blockage of the transfer line. Either a short or long term shutdown procedure should be performed within 24 hours if using the FC Cartridge or within 4 hours if using the HT Cartridge to minimize damage to the cartridge from the anolyte and catholyte.
- **To override the default cartridge purge setting** - check the **Skip Cartridge Purge** box. This lets you disable the cartridge purge in the shutdown sequence whenever the individual needs of your batch change.
- **To maintain tray temperature when the batch completes** - check the **Maintain Temperature Control** box.

To review and analyze data when the batch is complete, see Chapter 7, *"Data Calibration and Conversion"*.

Updating a Running Batch (Batch On-the-Fly)

You can update individual injection information during a batch in progress as long as those injections haven't completed yet.

1. Click **Pause** in the cell under Action for the injection you want to pause at. The batch will pause after that injection is complete.

NOTE: The iCE3 system door can be opened when a batch is paused without aborting the batch.

---

2. Once the selected injection completes, the Batch On-the-Fly window will pop up:

NOTE: Batch On-the-Fly isn't available for QC batches.

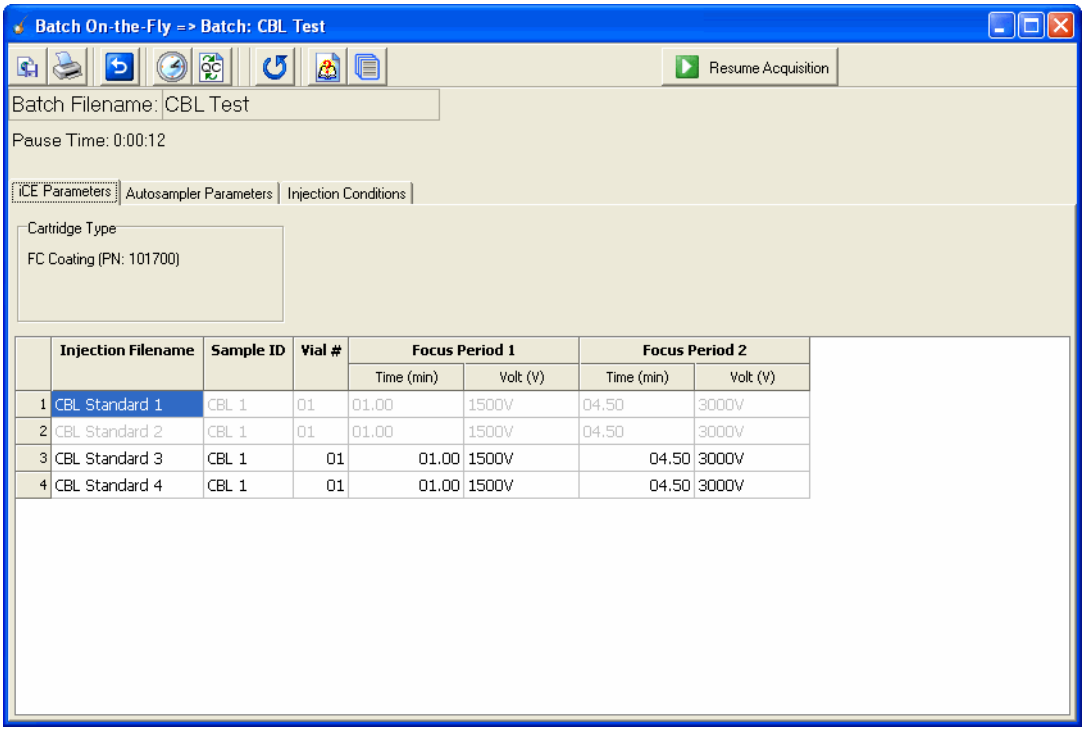


Figure 5-35: Batch On-the-Fly window.

3. You can modify any uncompleted injections or add/delete injections the same way you would when creating a batch. Grey rows indicate completed injections, this part of the batch table can't be modified.
   - **To add injections after the last completed injection** - select the last completed injection row, right-click and select **Insert Row(s) Below**.

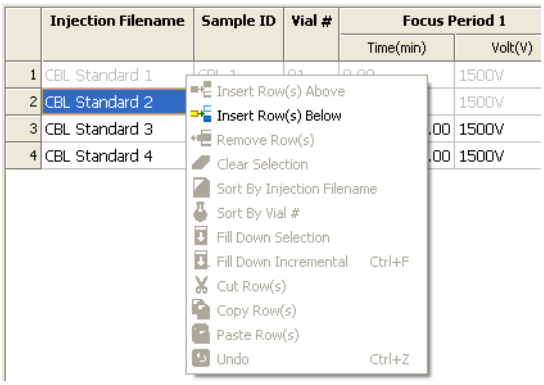


Figure 5-36: Adding rows after a completed injection.

- **To modify the batch at an uncompleted injection** - select an injection row, right-click and select an option from the menu.

NOTE: Sort options aren't available in the Batch On-the-Fly window.

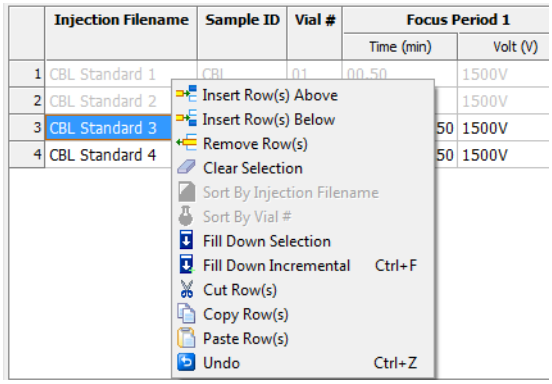


Figure 5-37: *Modifying the batch at an uncompleted injection.*

4. When your batch updates are complete, click **Resume Acquisition** to resume the batch.

Stopping, Pausing or Aborting a Batch

The batch can be paused or stopped after individual injections, and the entire batch can be aborted if needed:

- **To stop the batch after a specific injection** - Click **Stop** in the cell under Action for the injection you want to stop at. The batch will stop after the injection selected is complete. Data collected for injections completed up to this point are saved in the batch file.
- **To pause the batch** - Click **Pause** in the cell under Action for the injection you want to pause at. The batch will pause after the injection selected is complete. To resume the batch, uncheck **Pause**.

NOTE: The iCE3 system door can be opened when a batch is paused without aborting the batch.

- **To abort the batch** - click **Abort Batch Instantly**. Data collected for injections completed up to this point are saved in the batch file.

End of Day Shutdown

If you choose not to perform cartridge purge at the end of the batch, ProteinSimple recommends performing the short-term shutdown procedure at the end of the day. This will make sure the system is left in a safe state and prevents any damage to the cartridge due to incorrect storage. If you will be running the system within the next 7 days, perform a short term shutdown using the procedure that follows. If you will not use the system within the next 7 days or you are unsure when you will use it next, perform the Long Term Shutdown on page 231. To maximize cartridge lifetime, we recommend the cartridge be removed from the system and washed using the "Cartridge Wash Procedure" on page 226.

Short Term Shutdown

1. In iCE CFR Software, select **Operate** from the main menu, then click **Shutdown** and **Less than 7 days**. The short term protocol checklist will display (Figure 5-38):

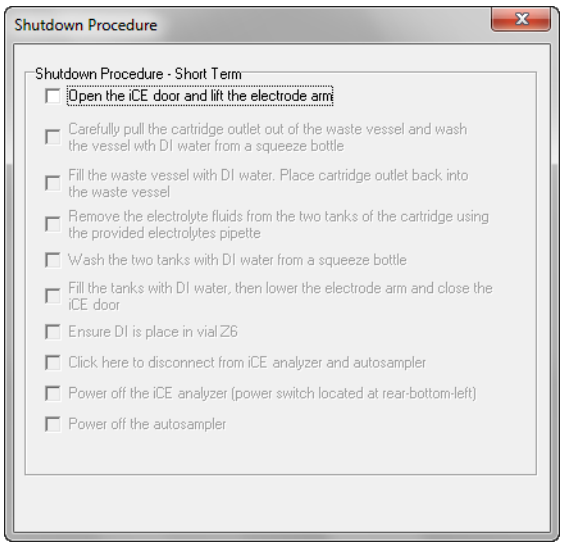


Figure 5-38: *Short term shutdown protocol in iCE CFR Software.*

NOTES:
Before starting the procedure, make sure make sure a vial containing DI water is in position Z6.

The software will step you through the procedure, but software screens may differ depending on the version of iCE CFR software you are using. Click the checkbox as you complete each step to proceed to the next step.

---

2. Perform the steps in the screen as described in the software:
    a. Open the iCE3 system door and lift the electrode arm.
    b. Carefully remove the cIEF cartridge capillary outlet out of the waste vial.
    c. Remove the waste vial and empty its contents. Rinse the vial with HPLC-grade deionized water.
    d. Fill the waste vial with water then reinstall it in the instrument. Insert the cIEF cartridge capillary outlet back into the waste vial.
    e. Aspirate the anolyte and catholyte solutions from the cIEF cartridge electrolyte tanks using the provided electrolyte pipette.
    f. Wash each electrolyte tank with HPLC-grade deionized water and aspirate. Repeat at least 2 more times for a total of 3 washes.
    g. Fill each tank with HPLC-grade deionized water. Lower the electrode arm and close the system door.
    h. Wait for the autosampler to finish washing the transfer line.
    i. Disconnect the iCE3 instrument and autosampler.
    j. Power off the iCE3 instrument and autosampler.

Cartridge Purge

The At the End of Batch Put Instrument in Safe State checkbox is selected by default. This option shuts down the lamp and purges the system. The purge consists of a methyl cellulose purge followed by a water purge and ends with an air purge. The air purge prevents the electrolytes from diffusing across the membrane which can have detrimental effects on the capillary coating.

Chapter 6:

Running the iCE System with the Alcott 720NV Autosampler

Chapter Overview

- System Power Up
- Alcott 720NV Autosampler Vial and Plate Guidelines
- Setting the Sample Tray Type
- Installing a cIEF Cartridge
- Preparing the System to Run a Batch
- Setting up a Batch
- Starting a Batch
- Updating a Running Batch (Batch On-the-Fly)
- Stopping, Pausing or Aborting a Batch
- End of Day Shutdown
- Using the On-Board Sample Mixing Feature

System Power Up

1. Power up the system computer.
2. Login to Windows and wait for the program to initialize.
3. Power up the iCE3 instrument and autosampler.
4. Wait for the system to initialize.
5. Double-click on the iCE CFR Software icon on the computer desktop to open the application.

Alcott 720NV Autosampler Vial and Plate Guidelines

ProteinSimple recommends using only the vials and snap caps shown in Figure 6-1 for samples, and 10 mL amber glass vials for buffer and sample preparation reagents. For a complete list of vial part numbers, please see Table 3-8 on page 30. The Alcott 720NV autosampler can be used with 12 mm vials or a 96-well plate. Any 96-well plate can be used, but when using the autosampler with a microplate a different sample tray is required (P/N 045-058).

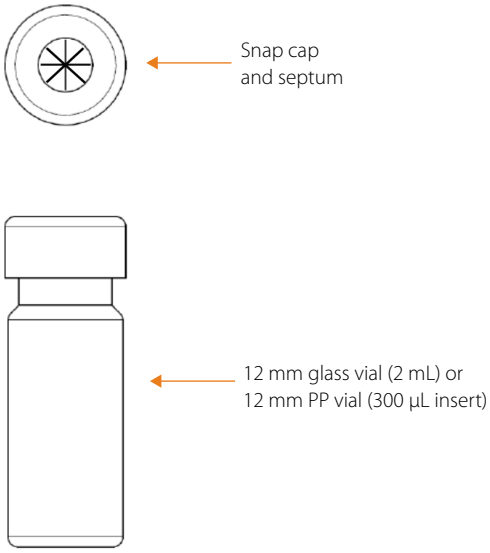


Figure 6-1: *Autosampler vials with snap cap and septa.*

Vial Handling Guidelines

- 12 mm PP vials with 300 µL inserts should be used for samples and TTM solution.

- 10 mL vials should be used for wash buffer (position D on the sample tray) and sample preparation reagents (positions A-C on the sample tray).
- Vials should always be capped before being placed in the autosampler.
- Do not reuse snap caps.
- Do not fill vials more than 80% full.
- A minimum volume of 50 µL is required for vials with 300 µL inserts. Do not under-fill vials or let the liquid level get too low, this can cause air bubbles to be injected with the sample.

96-Well Plate Handling Guidelines

- 96-well plates should only be used for samples.
- 96- well sample plates must be sealed before being placed in the autosampler.
- Do not reuse sealing film.

Setting the Sample Tray Type

Before using the system, you must first select the sample tray type that will be used.

IMPORTANT: Severe autosampler damage can result from using a tray other than the one set in the system configuration file. Make sure you select the correct tray during batch file creation, and insert the correct tray in the autosampler prior to starting a batch.

NOTE: Only administrators can change system configuration settings.

To set the tray type:

1. In iCE CFR Software, click **Utility** in the main menu and select **System Configuration**. The following screen will display:

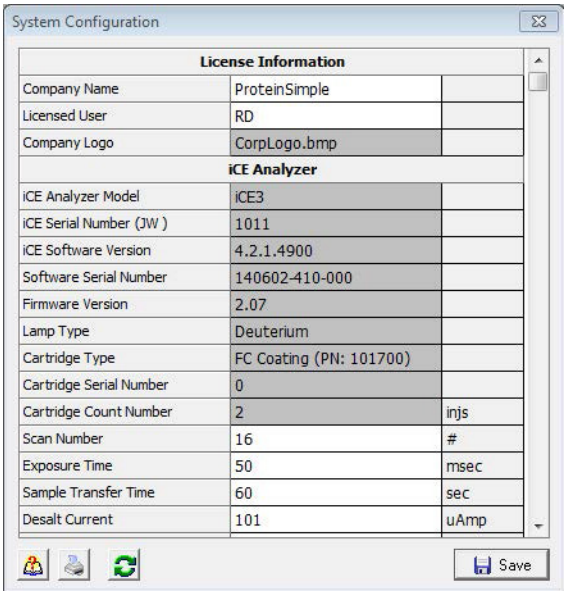


Figure 6-2: *System configuration screen.*

2. Scroll to the end of the configuration file using the scroll bar.
3. Click in the **Tray Type** box and select the tray you want to use:

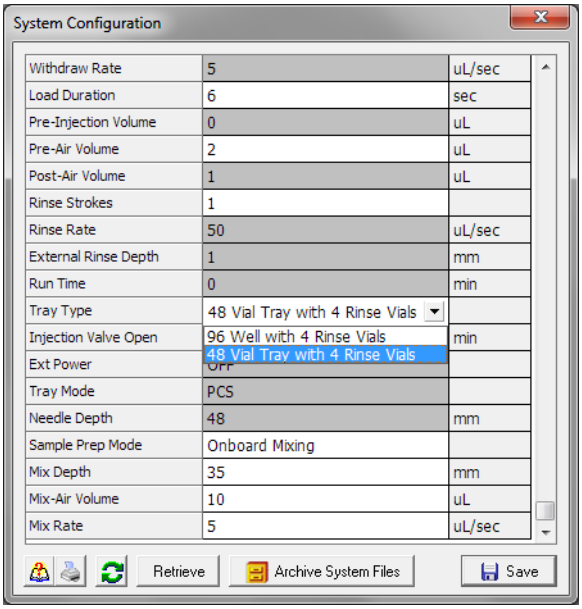


Figure 6-3: Selecting a tray type.

4. Click **Save**.

Installing a cIEF Cartridge

Step 1 - Prepare Reagents

NOTE: Do not fill vials past 80% full. The minimum volume for 2 mL vials is 0.5 mL, and the minimum volume for vials with 300 µL inserts is 50 µL.

1. Prepare the following reagents:
   - 1 vial (10 mL, amber) of 0.5% methyl cellulose buffer, 10 mL
   - TTM solution, 200 µL
   - Refill the autosampler's water supply bottle with HPLC-grade deionized water if needed

NOTES:
Use the appropriate TTM solution for cartridge you will be using. For an FC cIEF Cartridge, use the original FC cIEF Cartridge TTM solution (contains methyl cellulose) P/N 102672. For an HT cIEF Cartridge, use the HT cIEF Cartridge TTM solution (does not contain methyl cellulose) P/N P-0000037-00.

Use fresh 0.5% methyl cellulose solution every day. Bacteria can grow in the solution when it is stored at room temperature.

2. Centrifuge the TTM solution at 10,000 RPM for 3 minutes. Aspirate approximately 75% of the clean supernatant from the top without disturbing the separated layer on the bottom. Then transfer the solution to a sample vial or plate well.
3. Load the buffer vial and the TTM solution into the autosampler tray:
   - **Position D** - 0.5% methyl cellulose buffer
   - **Position 1 (48/4 tray)** or **Well A1 on 96-well plate (96/4 tray)**- TTM solution

Step 2 - Fill the Cartridge Waste Vial

4. Remove the cover on the cartridge waste vial.
5. Take the cartridge waste vial out of the iCE3.
6. Fill the vial with HPLC-grade deionized water until it overflows.
7. Reinstall the vial in the iCE3.
8. Replace the cover.

Step 3 - Insert the cIEF Cartridge in the iCE3

9. Either install a new or previously used cartridge as follows:

   Installing a new cartridge:

   - Remove the new cartridge from its packaging. Wash both electrolyte tanks three times with water and then gently shake the excess water from the cartridge.
   - Clean the cartridge lens with residue-free, oil free condensed air:
     - Place the can's nozzle, or tube opening, 10 to 12 inches away from the top surface of the cIEF Cartridge.
     - Depress the aerosol actuator down halfway to generate a gentle flow of air.
     - Sweep the air flow across the entire space between the electrolyte tanks.

- Turn the cIEF Cartridge over. Sweep the air flow across the back surface between the two electrolyte tanks.
- Turn the cIEF Cartridge over again, and gently clean the top surface again.

Installing a previously used cartridge:

- Rinse the top and bottom of the cIEF Cartridge with HPLC-grade deionized water and allow the cartridge to air dry.
- Wash both electrolyte tanks three times with water, then gently shake the excess water from the cartridge.
- Clean the lens on the cartridge holder using residue-free, oil-free condensed air per the instructions above.

IMPORTANT: Do not touch the metal optical slit on the cIEF cartridge or the area beneath it.

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10. Open the front door of the iCE3 instrument to access the cartridge holder (Figure 6-4).

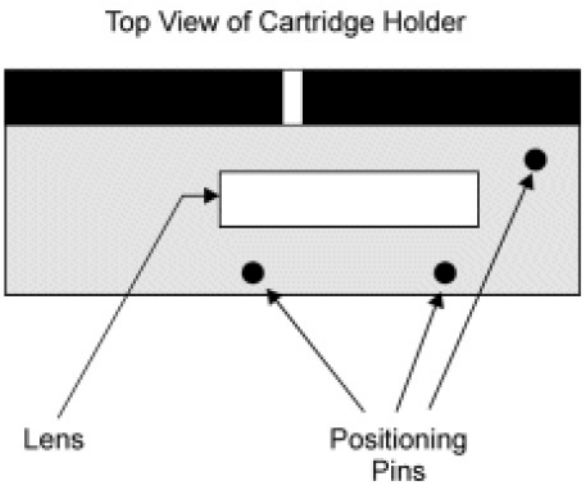


Figure 6-4: *Cartridge holder top view.*

11. Hold the cartridge above the front positioning pins and about 1-2 mm away from the pin on the right (Figure 6-5).

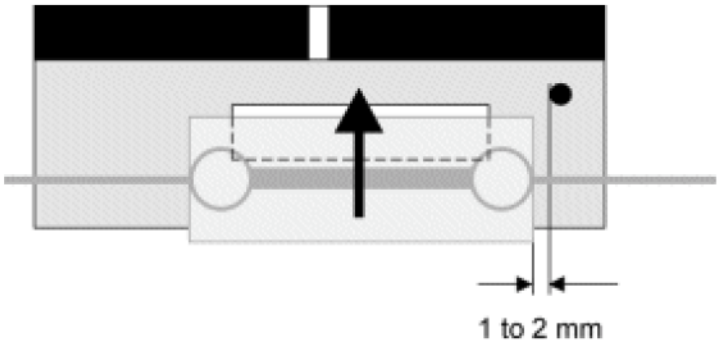


Figure 6-5: *Positioning the cartridge for installation.*

12. Tilt the cartridge at a 30-degree angle so it can be pushed towards the plunger at the back of the holder and still clear the front pins (Figure 6-6).

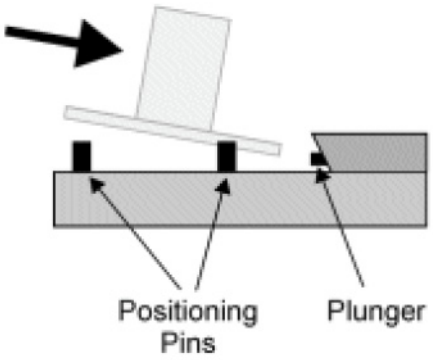


Figure 6-6: *Tilting the cartridge for installation.*

13. Push the cartridge against the plunger and then set it down gently on the lens so its positioned between the plunger and the two front pins (Figure 6-7).

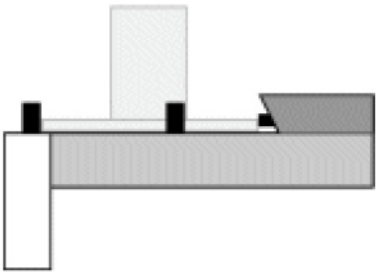


Figure 6-7: *Cartridge inserted between plunger and front pins.*

14. Gently slide the cartridge to the right until it stops at the right positioning pin (Figure 6-8).

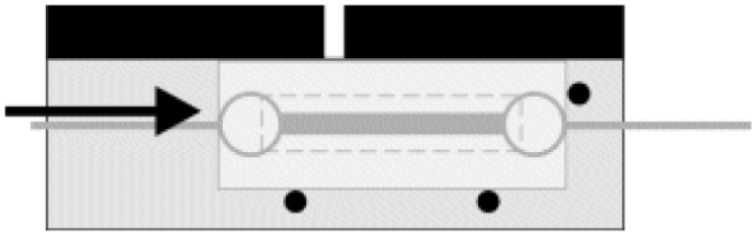


Figure 6-8: *Slide cartridge against the right pin.*

15. Make sure the cartridge is sitting firmly on the glass surface of the lens in the cartridge holder.

Step 4 - Run the Cartridge Installation Procedure

16. In iCE CFR Software, select **Utility** from the main menu, click **Maintenance** and then **Install/Replace Cartridge**. The following page displays:

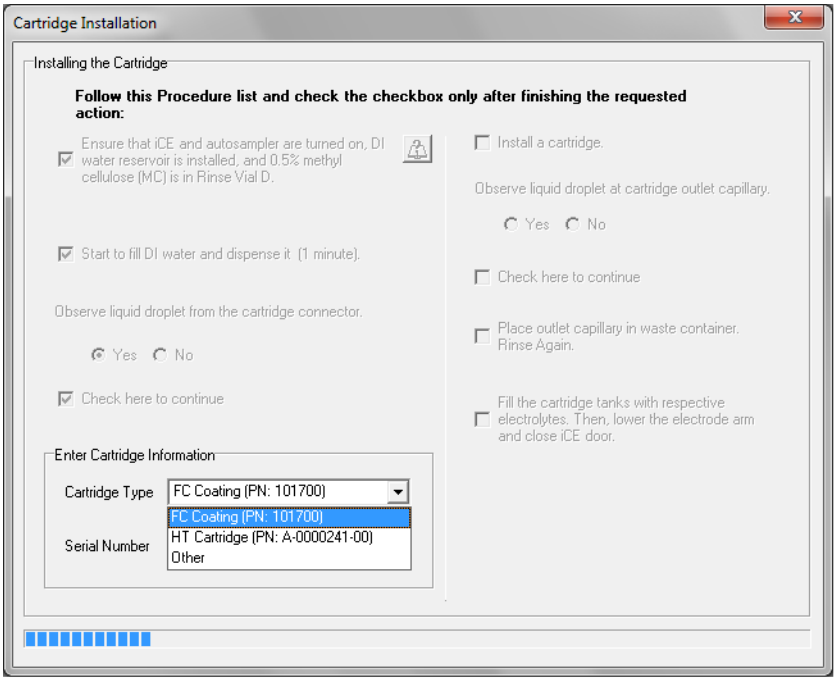


Figure 6-9: *Cartridge installation page 1.*

NOTE: The software will step you through the procedure. Click the checkbox as you complete each step to proceed to the next step.

---

17. Make sure the iCE3 and autosampler are turned on.
18. Place the prepared reagents in the autosampler, making sure that the 10 mL reagent vial is placed in position D located towards the back of the tray.
19. Wait for the system to perform the protocol and monitor the following as it progresses:
    - Droplets should be seen exiting the main waste line (from port 4 of the iCE valve) during the load step. If no droplets are seen:
      - **Check fluid connections.** A fitting that is either overtightened or too loose at port 1 on the iCE valve can result in no droplets at the waste line. Check port 1 for leaks. In both cases, simply remove the fitting and reinsert as instructed in "Making Fluid Connections" on page 68.
      - **Check vials.** Confirm all vials are in their correct location and filled adequately.

    If none of these resolve the issue, exit the procedure and refer "Troubleshooting Fluid Path and Flow Issues" on page 243.

- Droplets should be seen exiting the cartridge inlet sleeve fitting (port 2) during the inject step. If droplets are not seen during this step please see "Troubleshooting Fluid Path and Flow Issues" on page 243.

20. Select the correct cartridge from the drop down menu (FC Cartridge or HT Cartridge). Enter the cartridge serial number and click **OK**.
21. Remove the cartridge inlet sleeve and finger nut fitting from port 2 of the iCE valve.
22. Hold the cartridge inlet sleeve with two fingers of one hand, and hold the cartridge inlet capillary with two fingers of your other hand (Figure 6-10).

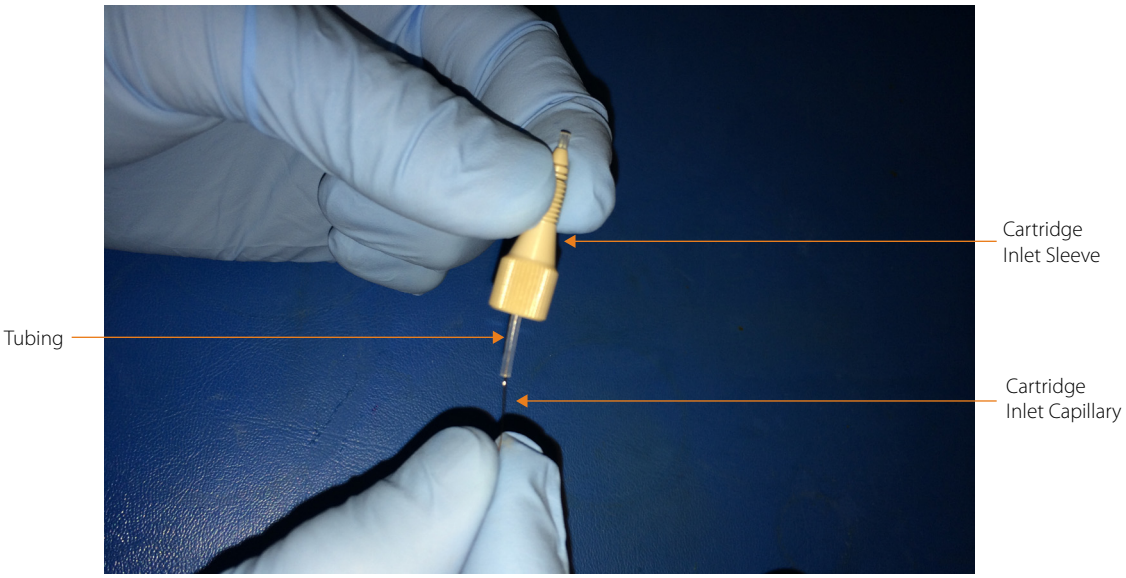


Figure 6-10: *Holding the cartridge inlet sleeve and cartridge inlet capillary.*

23. Insert the cartridge inlet capillary into the opening at the bottom of the tubing (Figure 6-10).
24. Gently push the cartridge inlet capillary through the tubing until the end of the capillary protrudes approximately 2.0 mm past the cartridge inlet sleeve (Figure 6-11).

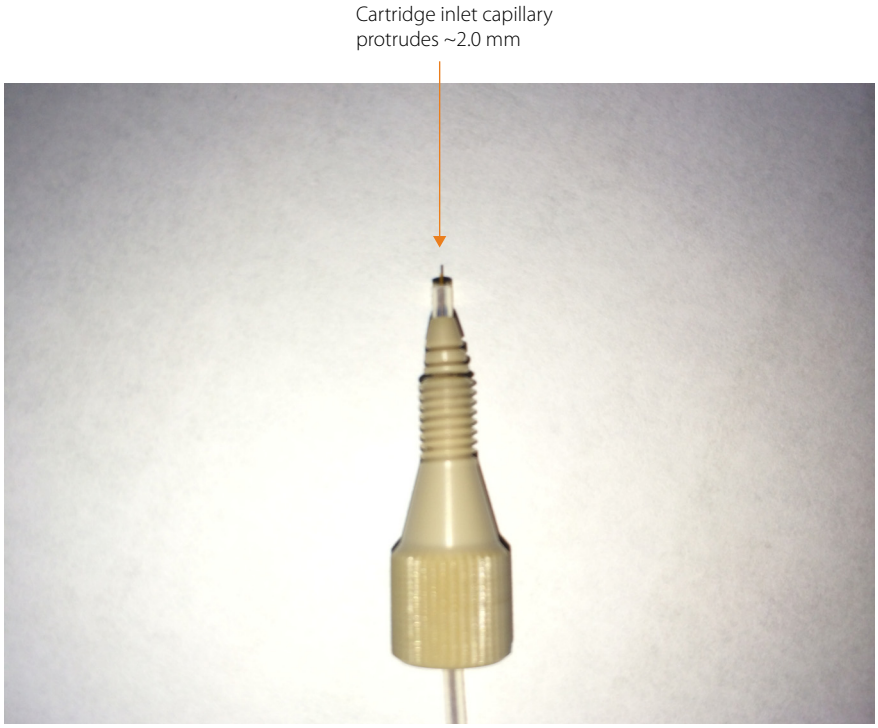


Figure 6-11: *Cartridge inlet capillary approximately 2.0 mm past the cartridge inlet sleeve.*

25. Hold the cartridge inlet sleeve and the cartridge inlet capillary and gently push inward while gently threading the cartridge inlet sleeve fitting into port 2 (Figure 3) until it reaches a hard stop.

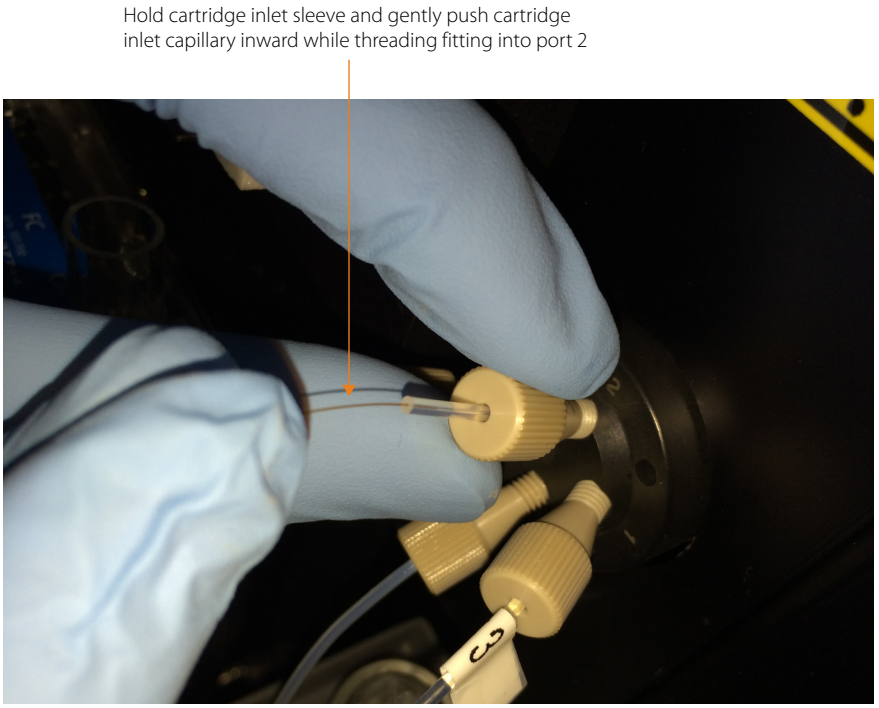


Figure 6-12: *Cartridge inlet installation.*

26. Tighten the fitting finger-tight plus approximately a 1/8 turn.
27. Once the cartridge is installed, check the box indicated in the cartridge installation screen. Wait for the system to perform the protocol and monitor the following as it progresses:
    - Droplets should be seen exiting the main waste line (port 4) during the load step.
    - Droplets should be seen exiting the cartridge outlet capillary (it will take a few minutes for this to occur).

NOTE: If droplets are not observed exiting the cartridge outlet capillary, remove the cartridge from the system and wash the cartridge using the Cartridge Wash Procedure to determine if the cartridge is clogged. If the capillary cartridge is clogged, a new cartridge should be installed.

---

28. Insert the cartridge outlet capillary into the cartridge waste vial, making sure that the end is completely below the liquid level in the vial.
29. Add anolyte to the left cartridge tank, and catholyte to the right tank. Fill each until the level is approximately 2 mm from the top of the tank.

30. Lower the electrode arm.
31. Close the iCE door.

Cartridge Light Intensity Calibration

32. The system will perform the cartridge UV light intensity calibration. The following screen will display:

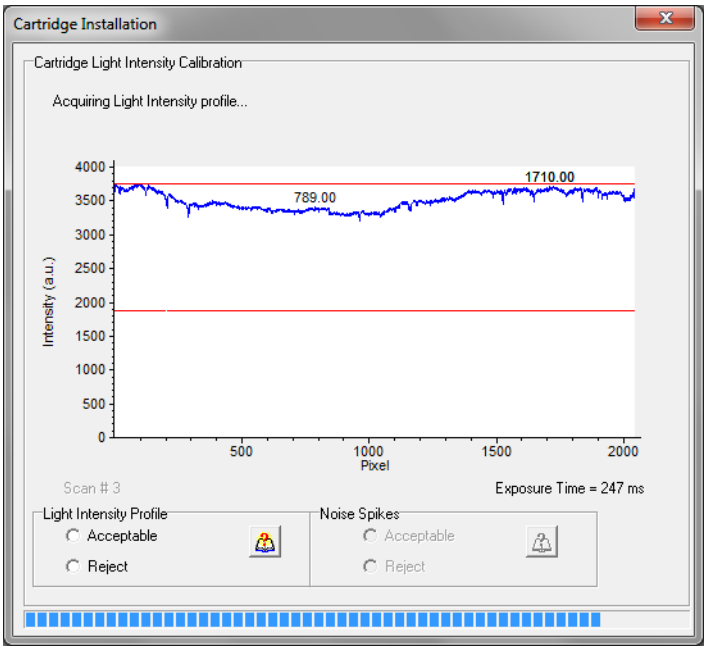


Figure 6-13: *Cartridge UV light intensity calibration screen.*

A good cartridge light intensity calibration profile will have no or very few spikes, and will maintain a signal between the red lines, as shown in Figure 6-13.

33. Click **Acceptable** to accept the calibration, or **Reject** to rerun the calibration.
34. Click **Acceptable** to accept the number of noise spikes, or **Reject** to rerun the calibration.

Sample Transfer Time Measurement

35. The software will now display the sample time transfer measurement screen.

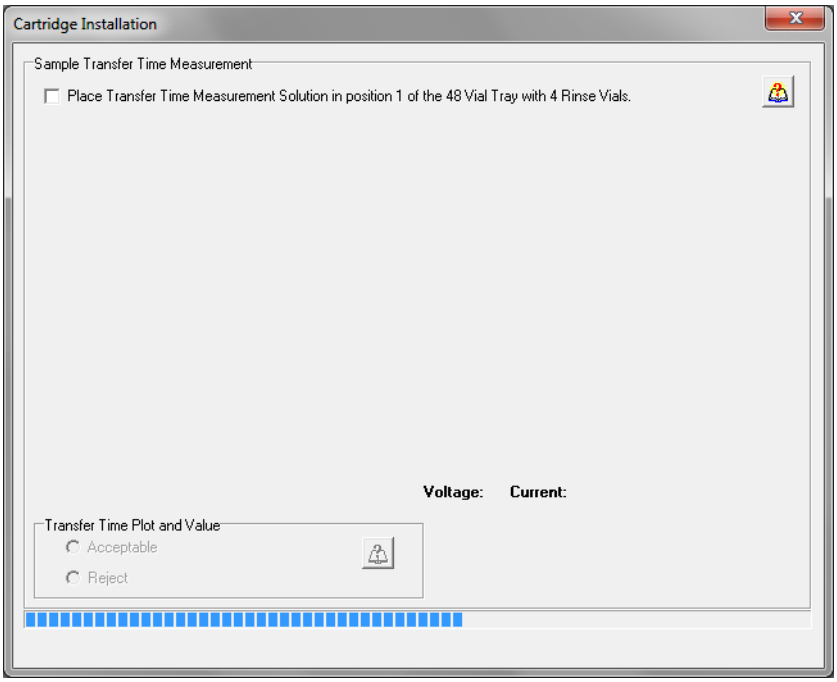


Figure 6-14: *Sample transfer time measurement screen.*

Wait for the measurement to complete, or click **Stop TTM** when an acceptable plot has been obtained:

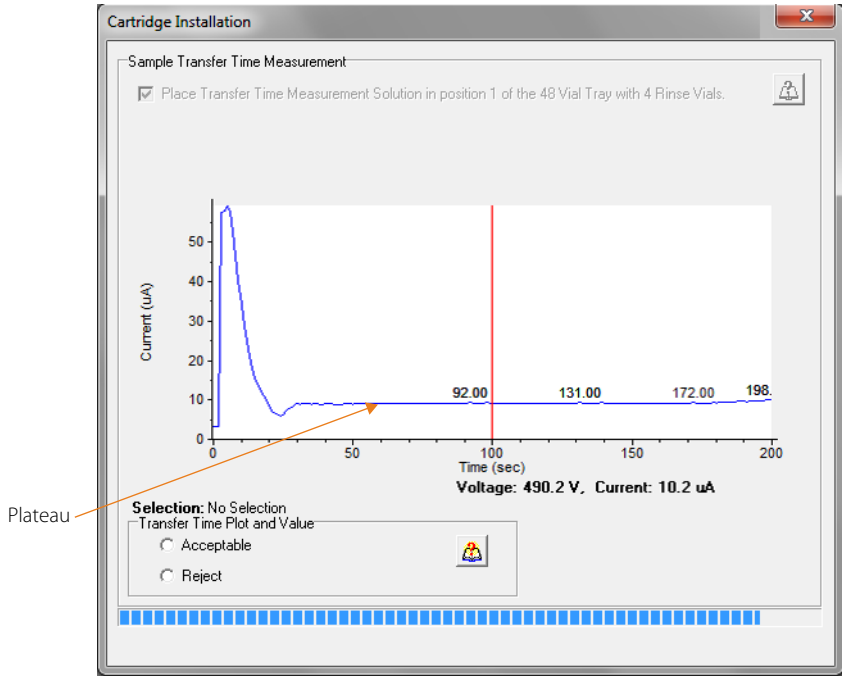


Figure 6-15: Example transfer time plot.

Accepting the Transfer Time Value and Plot

The transfer time value is indicated by the red vertical line. The transfer time value should be on the plateau section but not close to the start of the plateau to ensure complete sample loading. You can adjust the transfer line by right clicking on the plateau and selecting **Set Transfer Time**. If the plot meets the requirements below, click **Accept**. If it does not meet these requirements, click **Reject** to rerun the measurement.

An acceptable transfer time plot for the Alcott 720NV autosampler has the following characteristics (Figure 6-15):

- The plateau begins prior to 60 seconds for TTM solutions containing methyl cellulose and prior to 30 seconds for methyl cellulose-free TTM solutions.
- Height of the plateau is higher than 5.0 uA (when the ProteinSimple TTM sample is used).

Preparing the System to Run a Batch

Step 1 - Prepare Your Samples

1. Prepare samples and centrifuge at 10,000 RPM for 3 minutes. Aspirate approximately 75% of the clean supernatant from the top without disturbing the separated layer on the bottom. Then transfer this solution to a sample vial or plate well.
2. Add samples to individual vials or a 96-well plate:

NOTE: Do not fill vials past 80% full.

- **12 mm PP vials (300 µL insert)** - Use a minimum volume of 50 µL per vial
- **96-well plate** - Use a minimum volume of 50 µL per well

3. Load the vials or 96-well plate into the autosampler's sample tray:
   - **12 mm vials** - Load sample vials into a 48/4 tray starting at position 2 (if a TTM solution **will** be used) or position 1 (if a TTM solution **will not** be used).
   - **96-well plate** - Load samples starting in well A2 (if a TTM solution **will** be used) or well A1 (if a TTM solution **will not** be used). Place the plate in a 96/4 tray.

Step 2 - Prepare Assay Reagents

1. If you've just completed the installation of a new cartridge, the reagents are already in the autosampler and you can move on to "Setting up a Batch" on page 137 If you are starting the system after a short term shutdown prepare the following reagents vials and load them into the autosampler.

NOTE: Do not fill vials past 80% full. The minimum volume for 2 mL vials is 0.5 mL, and the minimum volume for vials with 300 µL inserts is 50 µL.

- 1 vial (10 mL, amber) of 0.5% methyl cellulose buffer, 5 mL
- TTM solution, 200 µL (optional)
- Refill the autosampler wash bottle with HPLC-grade deionized water if needed

---

NOTES:
Prepare a TTM sample only if you want to run a sample time transfer measurement before starting a batch.

Use the appropriate TTM solution for cartridge you will be using. For an FC cIEF Cartridge, use the original FC cIEF Cartridge TTM solution (contains methyl cellulose) P/N 102672. For an HT cIEF Cartridge, use the HT cIEF Cartridge TTM solution (does not contain methyl cellulose) P/N P-0000037-00.

Use fresh 0.5% methyl cellulose solution every day. Bacteria can grow in the solution when it is stored at room temperature.

---

2. If TTM solution will be used, centrifuge at 10,000 RPM for 3 minutes. Aspirate approximately 75% of the clean supernatant from the top without disturbing the separated layer on the bottom. Then transfer the solution to a sample vial or plate well.
3. Load buffer and reagent vials and the TTM solution as follows, the tray will be placed in the autosampler in an upcoming step:
   - **Position D** - 0.5% methyl cellulose buffer
   - **Position 1 (48/4 tray)** or **Well A1 on 96-well plate (96/4 tray)**- TTM solution (optional)

Step 3 - Run the Startup Procedure

4. If you've just completed the installation of a new cartridge, you can skip the startup procedure and move on to "Setting up a Batch" on page 137. If you are starting the system after a short term shutdown, proceed to the next step.

NOTE: If you are using iCE CFR Software version 4.0 or higher, you can run either the startup procedure or the quick startup procedure. The quick startup eliminates the three-minute rinse with methyl cellulose. The quick startup is not available with iCE CFR Software version 3.0, so the startup procedure must be used.

---

5. In iCE CFR Software, select **Operate** from the main menu and click **Startup**. The following screen will display:

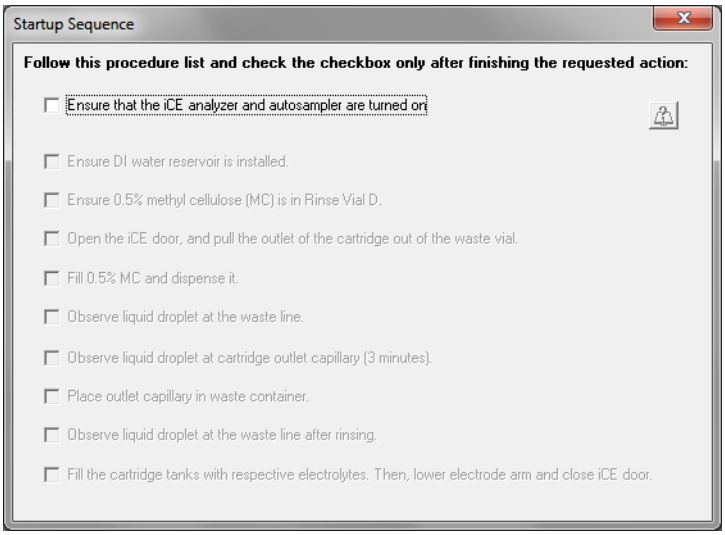


Figure 6-16: *Startup sequence screen.*

NOTE: The software will step you through the procedure. Click the checkbox as you complete each step to proceed to the next step.

---

6. Confirm reagents are in the correct position.
7. Open the iCE3 system door and remove the cartridge outlet capillary from the cartridge waste vial (if it is not already).
8. Wait for the system to perform the cartridge wash protocol. Droplets should be seen exiting the cartridge outlet capillary during the wash.
9. Insert the outlet capillary into the cartridge waste vial, making sure that the end is completely below the liquid level in the vial.
10. Add anolyte to the left cartridge tank, and catholyte to the right tank. Fill each until the level is approximately 2mm from the top of the tank.
11. Lower the electrode arm.
12. Close the iCE3 door.
13. Optional. The software will allow you to run a new time transfer measurement. To skip and move on to the next step, select **Accept current transfer time and complete startup**. To proceed, click **Place time transfer measurement solution in position 1 of the 48 vial tray with 4 rinse vials.**

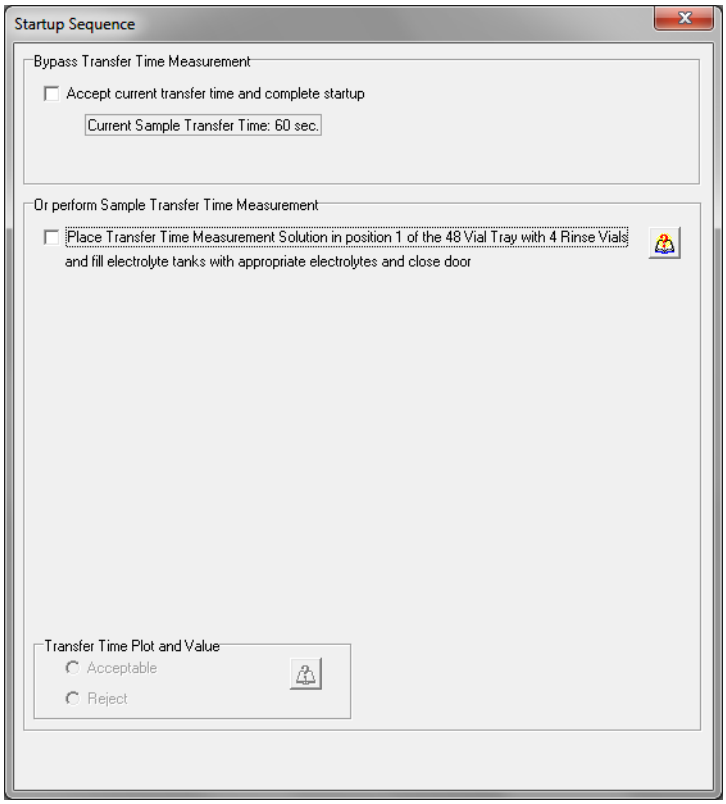


Figure 6-17: *Time transfer measurement option in startup sequence.*

14. Optional. The software will allow you to run a new cartridge light intensity profile. To skip this step and complete the startup sequence, select **Don't acquire one scan to check the cartridge intensity**. To proceed select **Acquire one image scan.**

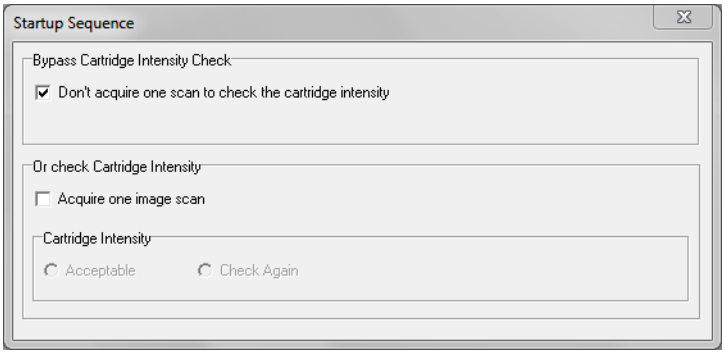


Figure 6-18: *Cartridge light intensity profile option in startup sequence.*

Setting up a Batch

Step 1 - Create a Batch File

1. Select **Batch/Data** from the main menu and click **Development**. The following screen will display:

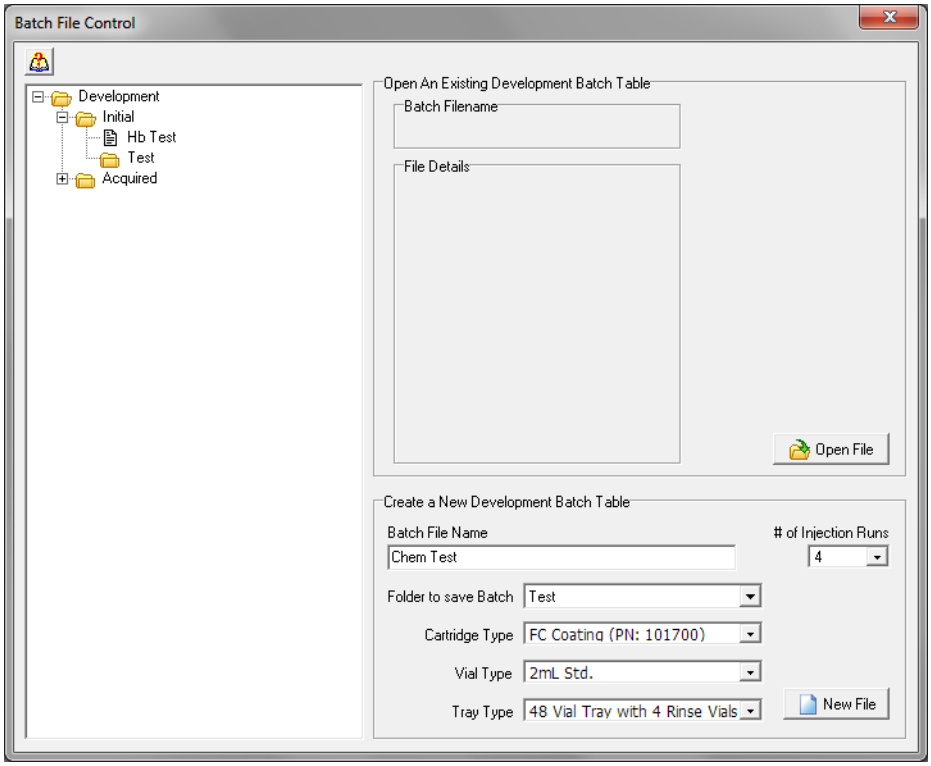


Figure 6-19: *Batch file control screen.*

2. To use a previously created batch file, expand the directory tree on the right, select the batch file and click **Open File**. Otherwise proceed to the next step.
3. Enter a name for the batch in the Batch File Name box.

NOTE: The batch name including its file path cannot exceed 255 characters.

---

4. Select a folder to save the batch file to. If the Folder to Save Batch box is left empty or NONE is selected, the batch file will automatically save to the Initial folder. To save the batch in a different folder or create a new batch folder, click the arrow. Select a folder or click **Create new folder** and enter a new name.

5. Set the number of injections. Click the arrow in the # of Injection Runs box and select the number of injections for the batch.

NOTES:
Multiple injections can be made from the same sample vial.

If needed, more injections can be added in the iCE Parameters table as described in the next section.

---

6. Select the sample tray type. Click the arrow in the Tray Type box and select **48 vial tray with 4 rinse vials** or **96-wells with 4 rinse vials**.

IMPORTANT: Severe autosampler damage can result from using a tray other than the one set in the system configuration file. Make sure you select the correct tray during batch file creation, and then insert the correct tray in the autosampler prior to starting a batch.

---

7. Select the sample vial type. Click the arrow in the Vial Type box and select **2 mL Std** or **2 mL with 300 µL insert**.
8. Click **New File**.

Step 2 - Set iCE Parameters

The iCE Parameters tab will display (Figure 6-20). The table contains one row per injection, based on the number of injections selected previously. Each injection is saved as an individual file in the batch.

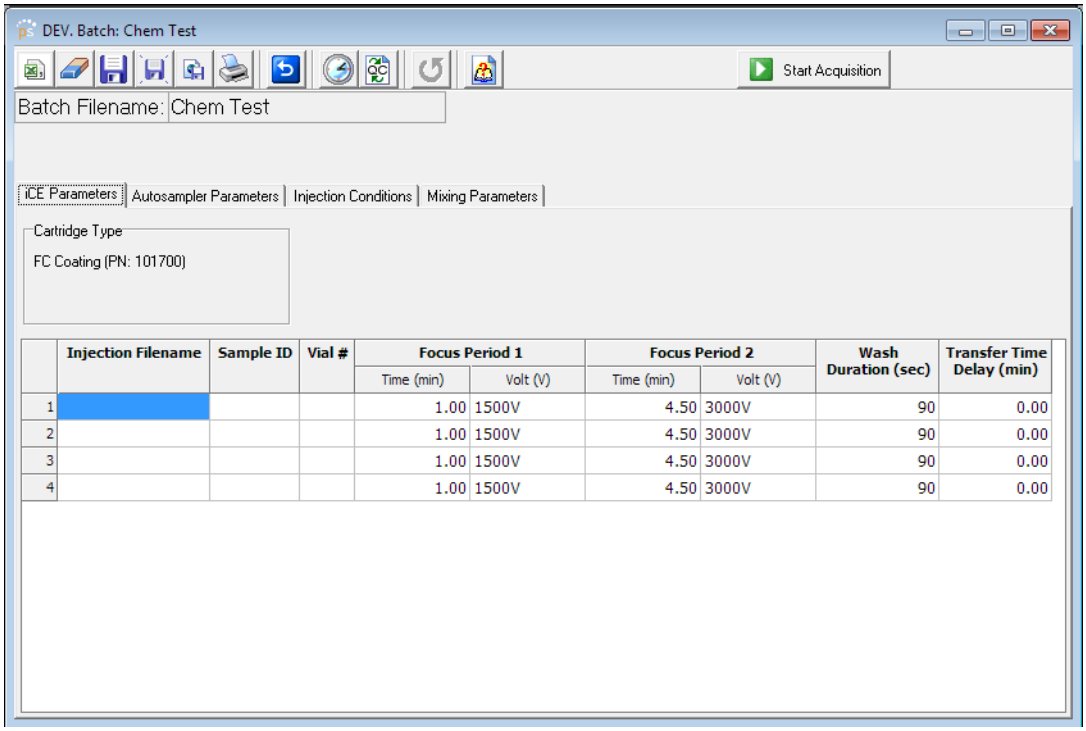


Figure 6-20: *iCE parameters.*

9. Enter a file name for the first injection. Click the row 1 cell under Injection Filename and type a name.
10. Enter a sample ID for the first injection. Click the row 1 cell under Sample ID and type a name.
11. Select the vial/well number for the first injection. Double-click the first cell under Vial # and select a vial number or plate well.
12. Set focus period 1 values for the first injection. ProteinSimple recommends using the default settings. To change these values, click in the row 1 cell under Time and enter a new value, then do the same for the Volt setting.
13. Set focus period 2 values for the first injection. ProteinSimple recommends using the default settings. To change these values, click in the row 1 cell under Time and enter a new value, then do the same for the Volt setting.
14. Set a wash duration for the first injection. ProteinSimple recommends using the default setting. To change this value, click in the row 1 cell under Wash Duration and enter a new value in seconds. This is the length of time the cartridge will be washed.

15. Set a transfer time delay for the first injection. ProteinSimple recommends using the default setting. To change this value, click in the row 1 cell under Transfer Time Delay and enter a new value in minutes. If a delay time is entered, the autosampler will load the solution and then hold until the entered time has elapsed before injecting.

Filling the Table

16. You can now enter information for the remaining injections manually, or use the table's right-click menu to auto-fill rows.
    a. **To copy a row and use it to fill other rows** - Click the first cell of the row you want to fill from. Hold the mouse button down and select the rest of the cells in the row. Right click and select a fill option (Figure 6-21 top).
    b. **To copy a cell and use it to fill other cells in the column** - Click the cell you want to fill from. Hold the mouse button down and select the other cells in the column you want to fill. Right click and select a fill option (Figure 6-21 bottom).

Filling the Table: Copy/Paste

a. **Copy/paste for file name, sample ID and vial number** - The first three columns can be filled in from a spreadsheet using copy and paste.
b. **Review imported values** - Review imported values to ensure they are correct.

Filling the Table: CSV Import

a. **Click the CSV button in Development Mode** - This fills the cells with the same information in the selected .csv template. (Figure 6-22).
b. **Use the navigation window to select your updated template** - Modify the ProteinSimple template to your method and sample names.
c. **Review imported values** - Review imported values to ensure they are correct.

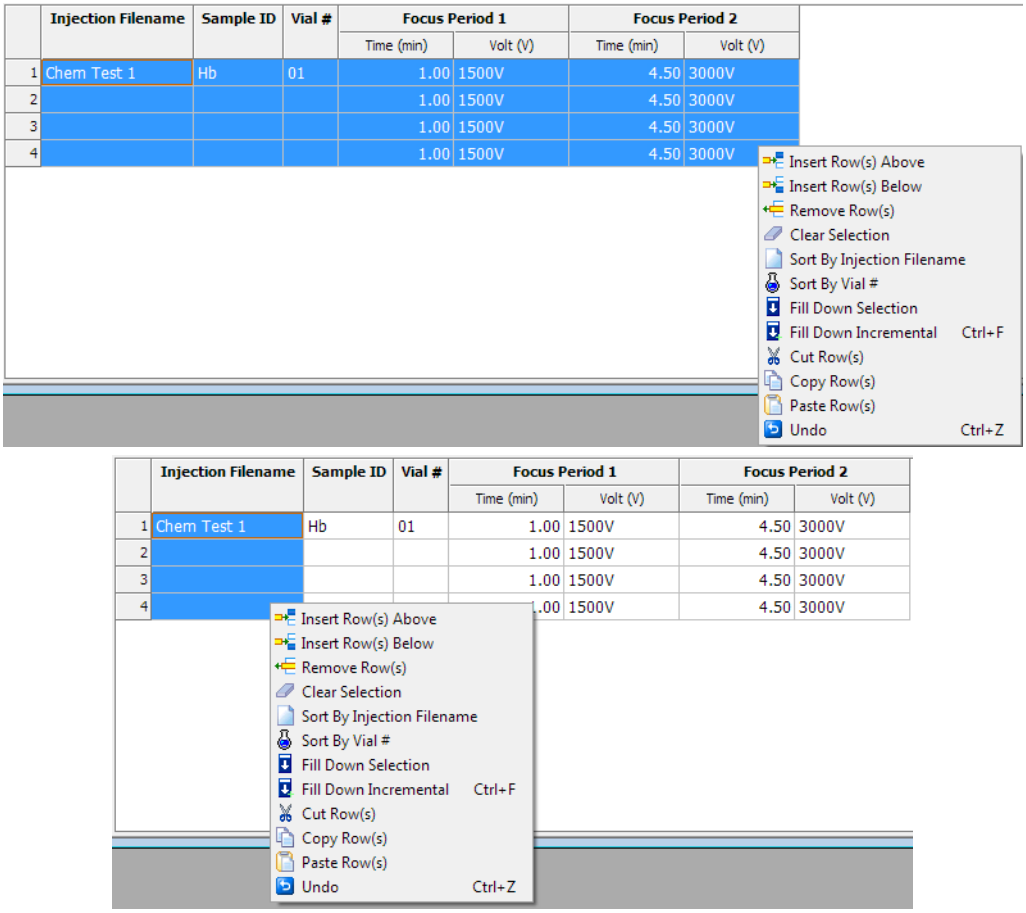


Figure 6-21: *Selecting rows and cells to auto-fill the table.*

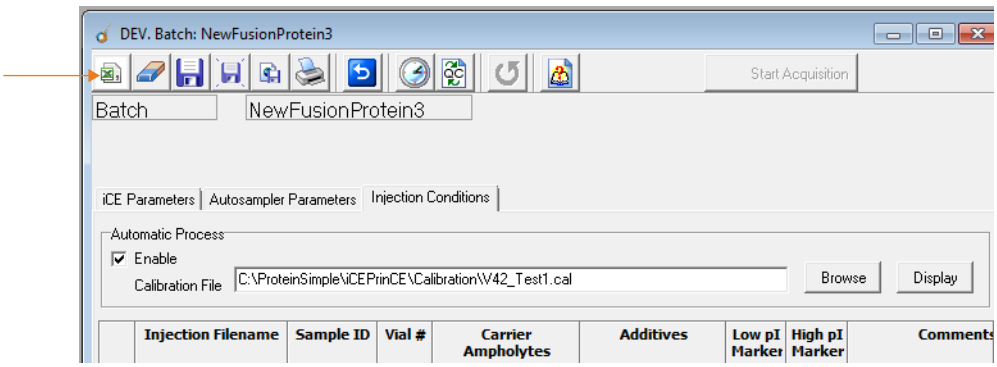
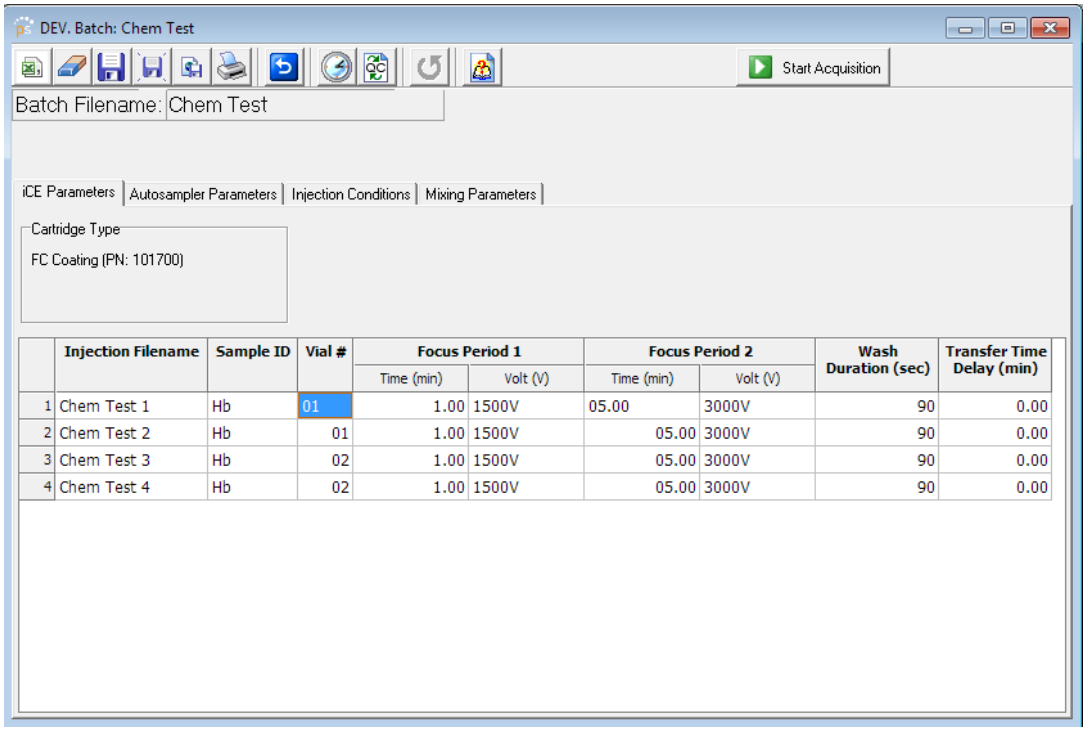


Figure 6-22: *Filling table with CSV button.*

The following fill and other right click table menu options are available:

- **Fill Down Selection** - Fills the selected cells with the same information.
- **Fill Down Incremental** - Fills the selected cells with the same information, but adds numbered increments in the Injection Filename, Sample ID and Vial # rows. If a number is entered as the last value in the cell to fill from, the remaining cells will increment from that value.
- **Insert Row(s) above** - Inserts a user-entered number of rows above the current selection. The new rows will populate using the selected row's parameters.
- **Insert Row(s) below** - Inserts a user-entered number of rows below the current selection. The new rows will populate using the selected row's parameters.
- **Remove Row(s)** - Removes the selected rows. Only one cell in the row needs to be selected.
- **Clear Selection** - Clears the information in the selected cells.
- **Sort by Injection Filename** - Sorts the table so rows with the same injection file names are grouped together.
- **Sort by Vial #** - Sorts the table so rows using the same vial or well number are grouped together.
- **Cut Row(s)** - Removes the currently selected row. Only one cell in the row needs to be selected.
- **Copy Row(s)** - Copies the currently selected row. Only one cell in the row needs to be selected.
- **Paste Row(s)** - Pastes the copied row into the selected row. Only one cell in the row needs to be selected.
- **Undo** - Reverts the last row-related action. You can continue to click undo to revert multiple actions.

17. When all the information has been entered, you can move on to the next tab. An example of a completed iCE Parameters table is shown in Figure 6-23 and includes four separate injections from two individual vials:



	Injection Filename	Sample ID	Vial #	Focus Period 1		Focus Period 2		Wash Duration (sec)	Transfer Time Delay (min)
				Time (min)	Volt (V)	Time (min)	Volt (V)		
1	Chem Test 1	Hb	01	1.00	1500V	05.00	3000V	90	0.00
2	Chem Test 2	Hb	01	1.00	1500V	05.00	3000V	90	0.00
3	Chem Test 3	Hb	02	1.00	1500V	05.00	3000V	90	0.00
4	Chem Test 4	Hb	02	1.00	1500V	05.00	3000V	90	0.00

Figure 6-23: *Example of a completed iCE Parameters table using four injections from two vials.*

Step 3 - Set Autosampler Parameters

18. Select the Autosampler Parameters tab (Figure 6-24). Use the scroll bar to view all parameters in the tab.

.

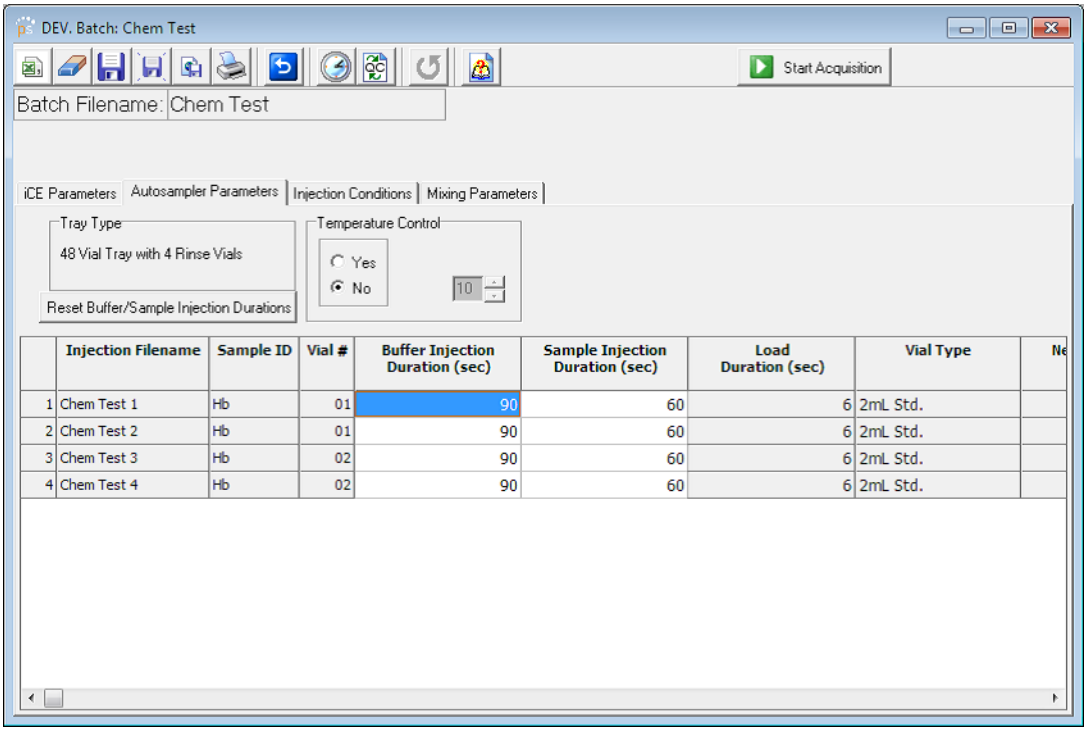


Figure 6-24: *Autosampler Parameters tab.*

19. Set the tray temperature (Figure 6-25). ProteinSimple recommends cooling the tray to 4 °C.
    a. Click **Yes** in the Temperature Control box.
    b. Click the up/down arrows and select a temperature between 4 and 40 °C.

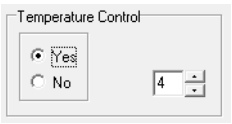


Figure 6-25: *Setting tray temperature.*

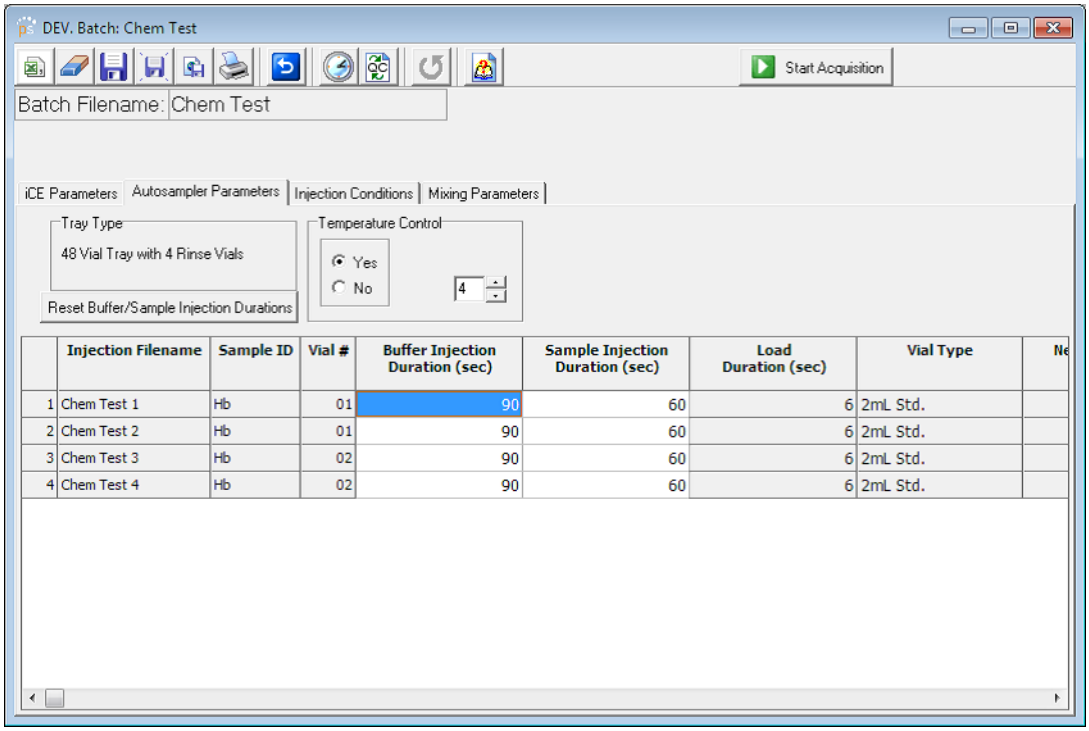
20. ProteinSimple recommends using the default settings for all other autosampler parameters. The following settings can be changed.
    a. **To change the Buffer Injection Duration** - Click in a cell under the column and enter a value in seconds. This is the amount of time the cIEF cartridge is rinsed with methyl cellulose buffer between injections.

b. **To change the Sample Injection Duration** - Click in a cell under the column and enter a value in seconds. Increasing this time will increase the amount of sample used for each injection.

c. **To set Buffer and Sample Injection Durations back to default values** - Click **Reset Buffer/Sample Injection**.

Fill the cells in the table as needed using the right-click menu options described in "Filling the Table" on page 140.

21. When all the information has been entered, you can move on to the next tab. An example of a completed Autosampler Parameters table using the ProteinSimple default parameters is shown in Figure 6-26.



	Injection Filename	Sample ID	Vial #	Buffer Injection Duration (sec)	Sample Injection Duration (sec)	Load Duration (sec)	Vial Type	Ne
1	Chem Test 1	Hb	01	90	60	6	2mL Std.	
2	Chem Test 2	Hb	01	90	60	6	2mL Std.	
3	Chem Test 3	Hb	02	90	60	6	2mL Std.	
4	Chem Test 4	Hb	02	90	60	6	2mL Std.	

Figure 6-26: *Example of a completed Autosampler parameters table using ProteinSimple default parameters.*

Step 4 - Injection Conditions

22. Select the Injection Conditions tab (Figure 6-27). Use the scroll bar to view all parameters in the tab.

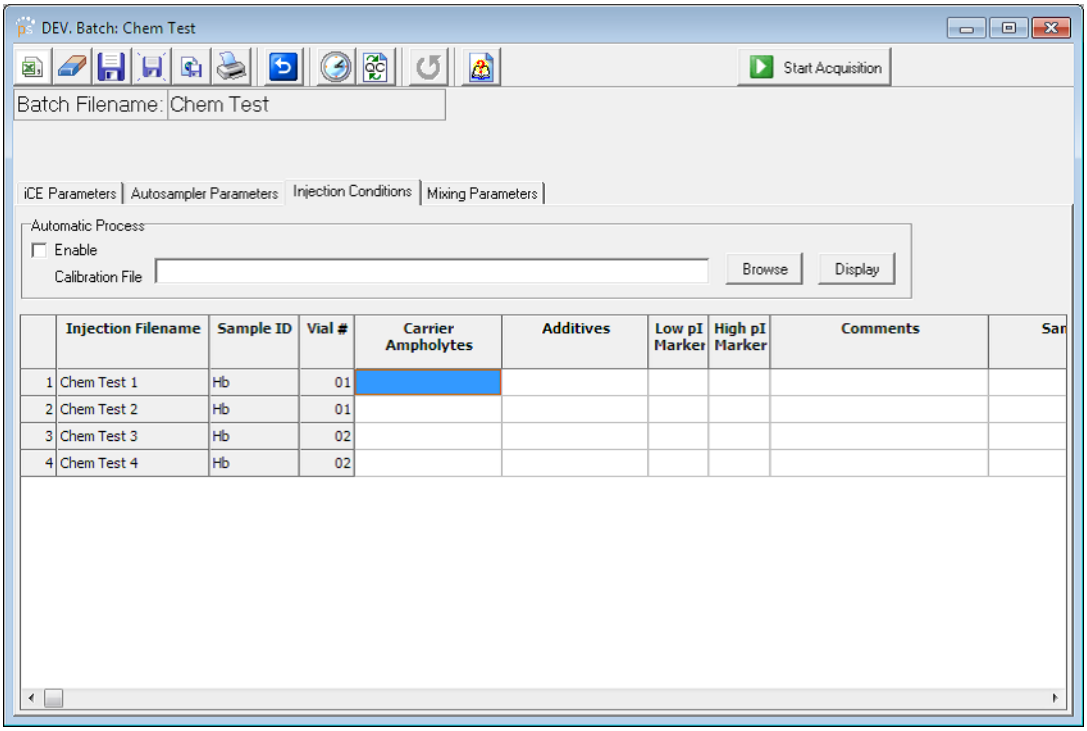
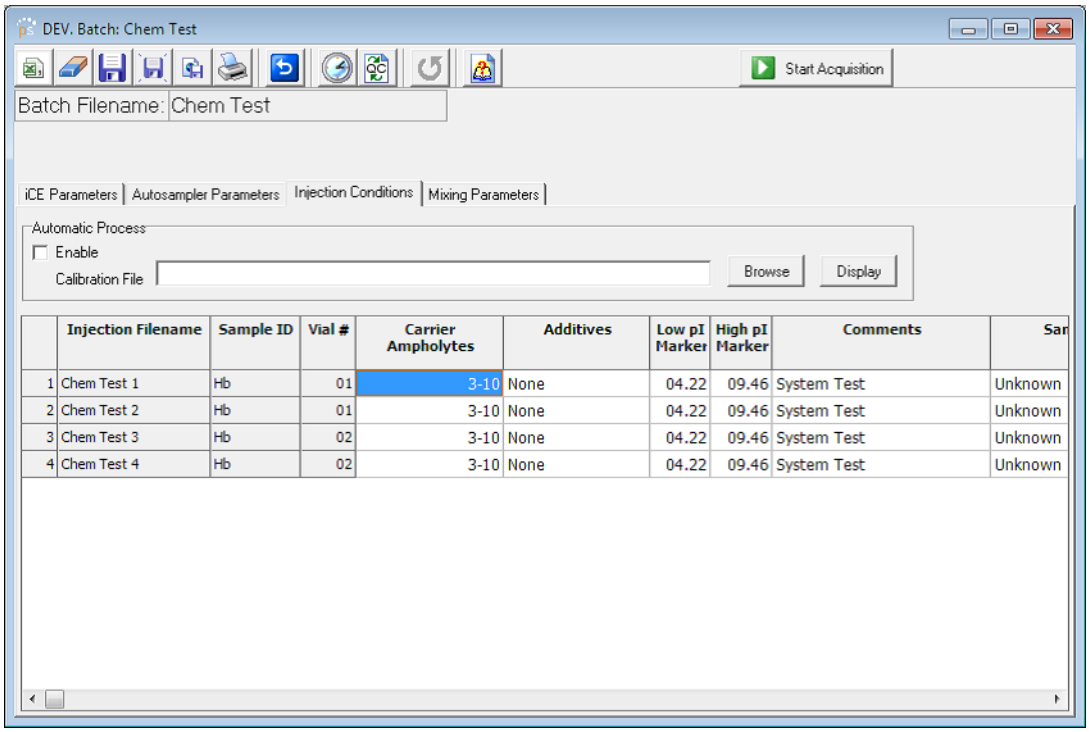


Figure 6-27: *Injection Conditions tab.*

23. Enter the carrier ampholyte range for the first injection. Click the row 1 cell under Carrier Ampholytes and type a range (for example 3-10).
24. Enter names of any additives (optional). Click the row 1 cell under Additives and type a name.
25. Enter the pI for the low pI marker. Click the row 1 cell under Low pI Marker and enter a pI (for example 4.22).
26. Enter the pI for the high pI marker. Click the row 1 cell under High pI Marker and enter a pI (for example 9.46).
27. Enter comments (optional). Click the row 1 cell under Comments to add any additional information.
28. Enter sample type (optional). Click the row 1 cell under Sample Type to and select **Standard** or **Unknown**. Sample type is used for data calibration when the batch file is processed.
29. Enter sample concentration (optional). Click the row 1 cell under Concentration to add concentration in mg/mL.
30. Fill the remaining cells in the table as needed using the right-click menu options described in "Filling the Table" on page 140.

NOTE: iCE CFR versions 4.0 and higher provide automated pI calibration and data export. A saved calibration file that includes processing information can be saved with a batch prior to running. As the data is acquired it will be automatically calibrated and then converted to the selected format for analysis. For more information on automated pI calibration and data export see "Processing Data - Automated pI Calibration and Data Export" on page 181.

An example of a completed Injection Conditions table using the ProteinSimple default parameters for the Chemical Test Kit is shown in Figure 6-28.



	Injection Filename	Sample ID	Vial #	Carrier Ampholytes	Additives	Low pI Marker	High pI Marker	Comments	Sar
1	Chem Test 1	Hb	01	3-10	None	04.22	09.46	System Test	Unknown
2	Chem Test 2	Hb	01	3-10	None	04.22	09.46	System Test	Unknown
3	Chem Test 3	Hb	02	3-10	None	04.22	09.46	System Test	Unknown
4	Chem Test 4	Hb	02	3-10	None	04.22	09.46	System Test	Unknown

Figure 6-28: *Example of a completed Injection Conditions table using Chemical Test kit parameters.*

Step 5 - Mixing Parameters (Optional)

31. The Mixing Parameters tab is used for the On-Board Sample Mixing feature. Before using this option, please first review "Using the On-Board Sample Mixing Feature" on page 153 for instructions on proper set up and accurate determination of parameter values. If you will not be using this option, skip to the next step.

Step 6 - Save the Batch

32. Click Save (disk icon) to save the batch file.

Starting a Batch

1. Place the prepared tray in the autosampler, making sure that 10 mL reagent vials are located towards the back.
2. Close the autosampler cover and the iCE3 system door.
3. Click **Start Acquisition**. As the batch runs, progress and sample data is displayed in real-time:

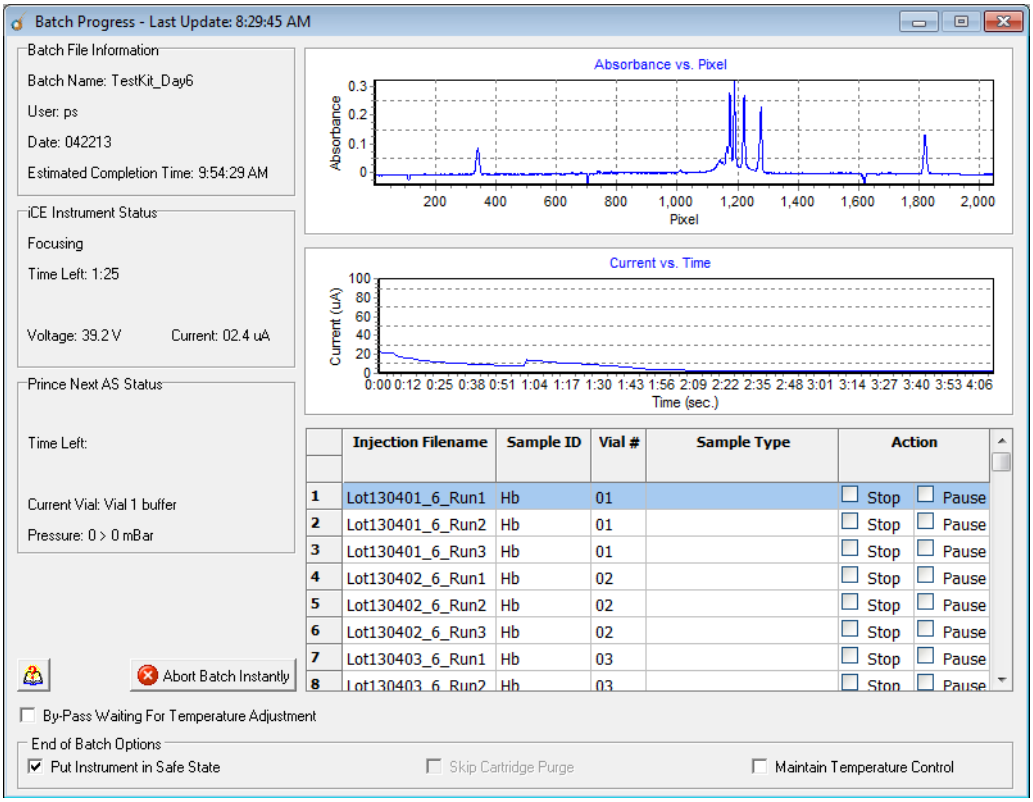


Figure 6-29: *Batch in progress.*

NOTE: Do not open the iCE3 system door when a batch is running, this will abort the batch.

- **If using CFR version 3.0 and 4.0x** - make sure to check the **Put Instruments in Safe State** box. When this box is checked, the system will rinse the transfer line with water to minimize blockage of the transfer line. Either a short or long term shutdown procedure should be performed within 24 hours if using the FC Cartridge or within 4 hours if using the HT Cartridge to minimize damage to the cartridge from the anolyte and catholyte.
- **To override the default cartridge purge setting** - check the **Skip Cartridge Purge** box. This lets you disable the cartridge purge in the shutdown sequence whenever the individual needs of your batch change.
- **To maintain tray temperature when the batch completes** - check the **Maintain Temperature Control** box.

To review and analyze data when the batch is complete, see Chapter 7, *"Data Calibration and Conversion"*.

Updating a Running Batch (Batch On-the-Fly)

You can update individual injection information during a batch in progress as long as those injections haven't completed yet.

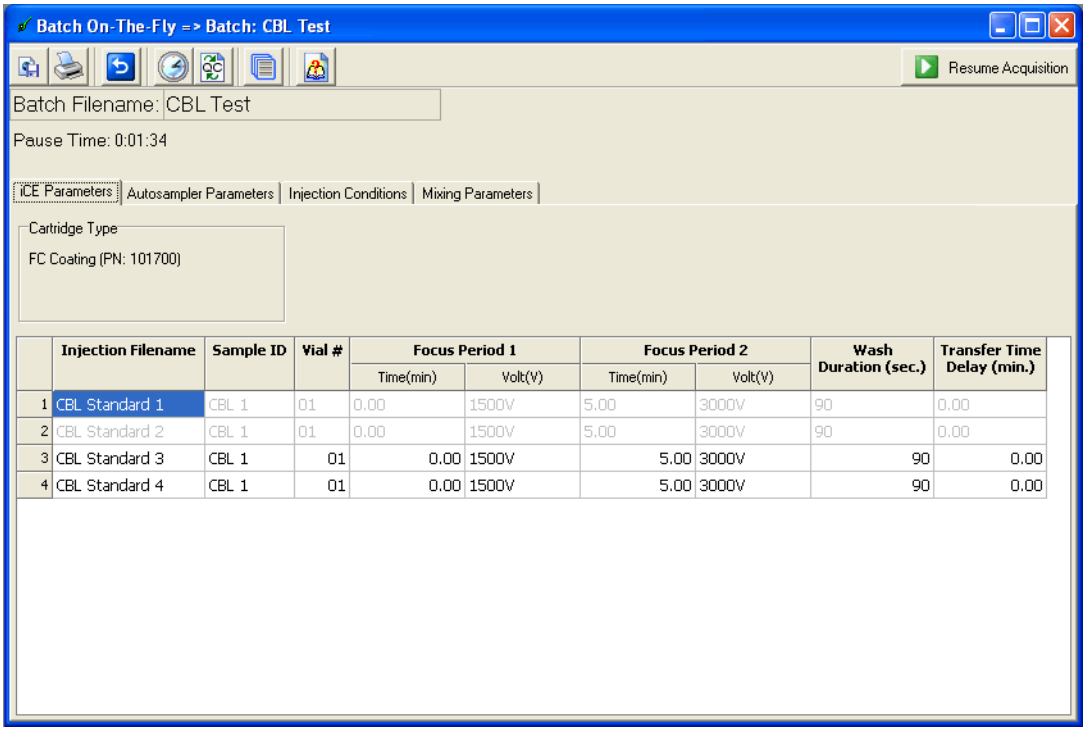
1. Click **Pause** in the cell under Action for the injection you want to pause at. The batch will pause after that injection is complete.

NOTE: The iCE3 system door can be opened when a batch is paused without aborting the batch.

---

2. Once the selected injection completes, the Batch On-the-Fly window will pop up:

NOTE: Batch On-the-Fly isn't available for QC batches.



	Injection Filename	Sample ID	Vial #	Focus Period 1		Focus Period 2		Wash Duration (sec.)	Transfer Time Delay (min.)
				Time(min)	Volt(V)	Time(min)	Volt(V)		
1	CBL Standard 1	CBL 1	01	0.00	1500V	5.00	3000V	90	0.00
2	CBL Standard 2	CBL 1	01	0.00	1500V	5.00	3000V	90	0.00
3	CBL Standard 3	CBL 1	01	0.00	1500V	5.00	3000V	90	0.00
4	CBL Standard 4	CBL 1	01	0.00	1500V	5.00	3000V	90	0.00

Figure 6-30: Batch On-the-Fly window.

3. You can modify any uncompleted injections or add/delete injections the same way you would when creating a batch. Grey rows indicate completed injections, this part of the batch table can't be modified.

   - **To add injections after the last completed injection** - select the last completed injection row, right-click and select **Insert Row(s) Below**.

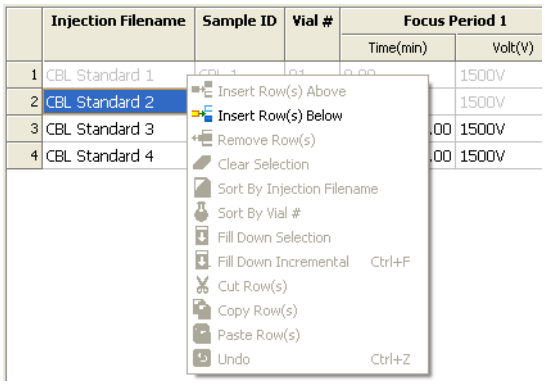


Figure 6-31: Adding rows after a completed injection.

- **To modify the batch at an uncompleted injection** - select an injection row, right-click and select an option from the menu.

NOTE: Sort options aren't available in the Batch On-the-Fly window.

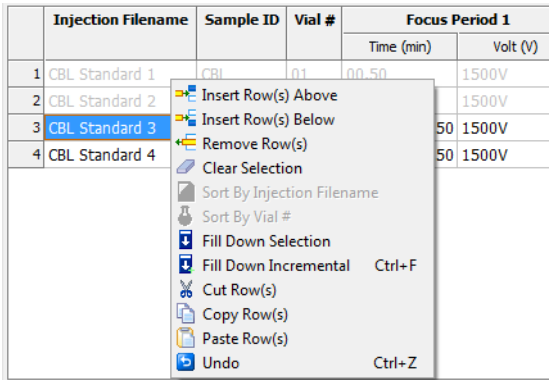


Figure 6-32: *Modifying the batch at an uncompleted injection.*

When your batch updates are complete, click **Resume Acquisition** to resume the batch.

Stopping, Pausing or Aborting a Batch

The batch can be paused or stopped after individual injections, and the entire batch can be aborted if needed:

- **To stop the batch after a specific injection** - Click **Stop** in the cell under Action for the injection you want to stop at. The batch will stop after the injection selected is complete. Data collected for injections completed up to this point are saved in the batch file.
- **To pause the batch** - Click **Pause** in the cell under Action for the injection you want to pause at. The batch will pause after the injection selected is complete. To resume the batch, uncheck **Pause**.

NOTE: The iCE3 system door can be opened when a batch is paused without aborting the batch.

- **To abort the batch** - click **Abort Batch Instantly**. Data collected for injections completed up to this point are saved in the batch file.

End of Day Shutdown

If you choose not to perform cartridge purge at the end of the batch, ProteinSimple recommends performing the short-term shutdown procedure at the end of the day. This will make sure the system is left in a safe state and prevents any damage to the cartridge due to incorrect storage. If you will be running the system within the next 7 days, perform a short term shutdown using the procedure that follows. If you will not use the system within the next 7 days or you are unsure when you will use it next, perform the Long Term Shutdown on page 231. To maximize cartridge lifetime, we recommend the cartridge be removed from the system and washed using the "Cartridge Wash Procedure" on page 226.

Short Term Shutdown

1. In iCE CFR Software, select **Operate** from the main menu, then click **Shutdown** and **Less than 7 days**. The short term protocol checklist will display (Figure 6-33):

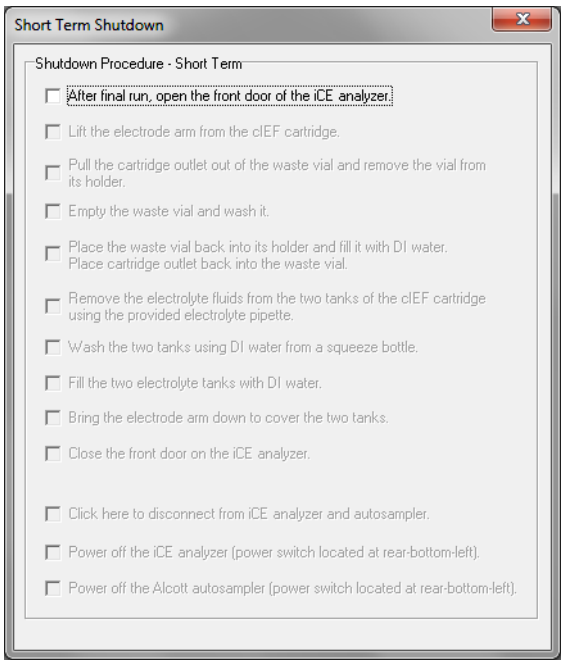


Figure 6-33: *Short term shutdown protocol in iCE CFR Software.*

NOTES:

Before starting the procedure, make sure the water reservoir is connected and the fluid level is adequate.

The software will step you through the procedure, but software screens may differ depending on the version of iCE CFR software you are using. Click the checkbox as you complete each step to proceed to the next step.

2. Perform the steps in the screen as described in the software:
   a. Open the iCE3 system door and lift the electrode arm.
   b. Carefully remove the cIEF cartridge capillary outlet out of the waste vial.
   c. Remove the waste vial and empty its contents. Rinse the vial with HPLC-grade deionized water.
   d. Fill the waste vial with water then reinstall it in the instrument. Insert the cIEF cartridge capillary outlet back into the waste vial.
   e. Aspirate the anolyte and catholyte solutions from the cIEF cartridge electrolyte tanks using the provided electrolyte pipette.
   f. Wash each electrolyte tank with HPLC-grade deionized water and aspirate. Repeat at least 2 more times for a total of 3 washes.
   g. Fill each tank with HPLC-grade deionized water. Lower the electrode arm and close the system door.
   h. Wait for the autosampler to finish washing the transfer line.
   i. Disconnect the iCE3 instrument and autosampler.
   j. Power off the iCE3 instrument and autosampler.

Using the On-Board Sample Mixing Feature

Although the iCE3 system offers high sample throughput and rapid analysis, proteins can still sometimes degrade when exposed to carrier ampholytes and additives for extended periods of time. The Alcott 720 NV autosampler can prepare samples immediately prior to injection using the on-board sample mixing feature. This limits sample exposure to cIEF buffers and additives which helps prevent degradation.

Reagent Positions

Alcott 720 NV autosampler trays are available in two configurations, 48 individual vials or 96-well plate. The 48-vial tray is shown in Figure 6-34. Both trays include four large vial positions at the back of the tray that hold 10 mL vials. Sample preparation reagents used for on-board sample mixing must be in 10 mL vials and placed in positions A-C.

NOTE: The 0.5% methyl cellulose cIEF buffer should be placed in position D.

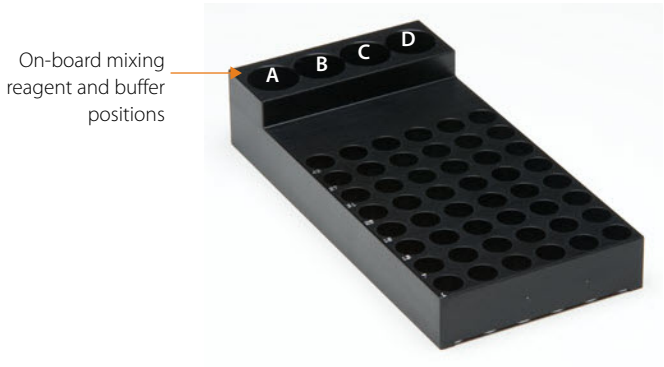


Figure 6-34: *On-board sample mixing reagent positions (48/4 tray shown).*

Principles of Automated Sample Preparation

During automated sample preparation, the protein samples in their original formulations are first loaded in sample vials. The sample vials are then placed in the chilled autosampler sample tray as shown in Figure 6-35.

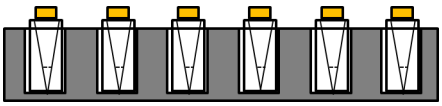


Figure 6-35: *Sample vials in sample tray.*

Just prior to sample injection, the autosampler needle aspirates the IEF buffers stored in the 10 mL vials and dispenses them into the bottom of a sample vial as shown in Figure 6-36.

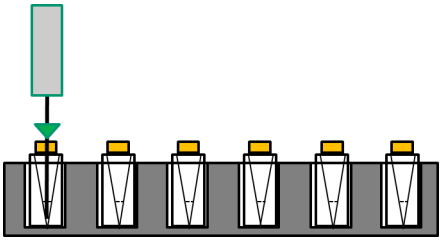


Figure 6-36: *Needle dispenses IEF buffer into the sample vial.*

The dispensed buffers are mixed with the protein sample solution in the sample vial by the autosampler needle. In each mixing stroke, the needle aspirates 75% of the solution in the vial. The aspirated solution is dispensed back into the vial as the needle moves up, as shown in Figure 6-37.

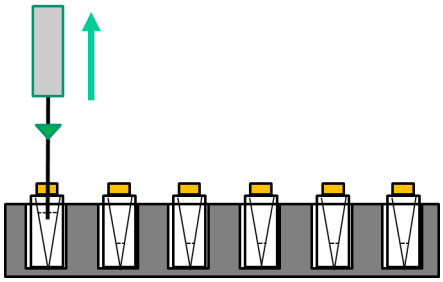


Figure 6-37: *Sample is mixed when the needle aspirates the solution and dispenses it back in the vial.*

Optimizing Mixing Parameters

There are several important method parameters such as Mixing Rate, Mixing Strokes and Mixing Depth that require optimization. This section describes an optimization strategy for on-board sample preparation and provides relevant examples.

Buffer Dispensing Rate

During automated sample preparation, the autosampler needle aspirates IEF buffers stored in the 10 mL vials and dispenses them into the bottom of a sample vial.

Dispensing speed impacts mixing efficiency, and a higher dispensing speed provides better mixing efficiency. The highest speed of the fluid delivery of the autosampler is 100 µL/second and should be used as the Dispensing Rate.

Mixing Rate

After the buffer is dispensed into the sample vial, the buffer and sample are mixed by the needle. In each mixing stroke, the needle aspirates 75% of the liquid in the vial and then dispenses it back into the vial.

Mixing Rate also impacts mixing efficiency, and a higher mixing rate provides better mixing efficiency. The optimal Mixing Rate is 100 µL/second.

Mixing Depth

For each mixing stroke, the needle dispenses the aspirated solution from the sample vial back into the vial at the Mixing Rate while the needle moves up in 10 steps, as shown in Figure 6-38.

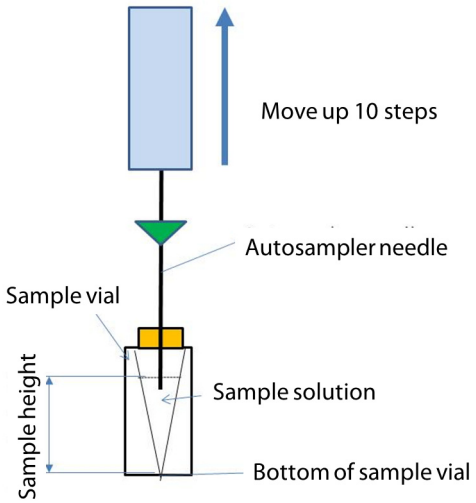


Figure 6-38: *Needle mixing depth.*

When the needle dispenses solution back into the sample vial, it starts at the bottom of the vial (Needle Depth) and moves up as it dispenses. When it's finished dispensing, the needle stops at a position higher than the bottom of the vial as shown in Figure 6-38.

The distance between the vial bottom and the needle stopping point at the end of a mixing stroke is: Needle Depth (vial bottom) - Mixing Depth. So, the optimal distance should be slightly less than the sample height as shown in Figure 6-38. This will ensure the dispensed solution is always mixed into the solution in the vial. To determine the Mixing Depth, first measure the sample height of the final sample volume in the vial as shown in Figure 6-38. Subtract 1 – 2 mm from this height, and use this corrected height value to calculate the Mixing Depth: Mixing Depth = Needle Depth (vial bottom) - corrected height value. This final result should be entered as the Mixing Depth. This parameter is dependent on the final sample solution volume in the vial and the type of vial used in the experiments.

Number of Mixing Strokes

Thorough sample mixing depends on the parameters already discussed and the Number of Mixing Strokes. Once the above parameters and Number of Mixing Strokes are set, tests need to be done to see if the mixing is complete and the sample is reproducible. A good starting value for Number of Mixing Strokes is 4.

To test the mixing parameters, perform three injections from the same sample vial after mixing. The peak heights of the three injections should be reproducible as shown in Figure 6-39.

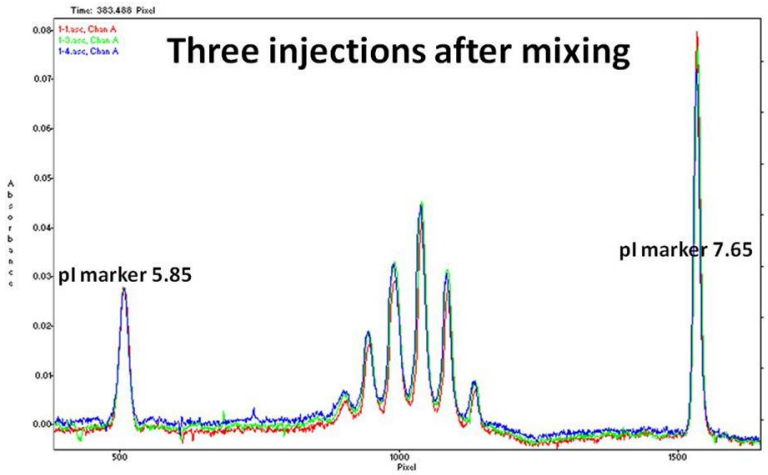


Figure 6-39: Three injections of a mixed sample will confirm if the sample and solution are completely mixed.

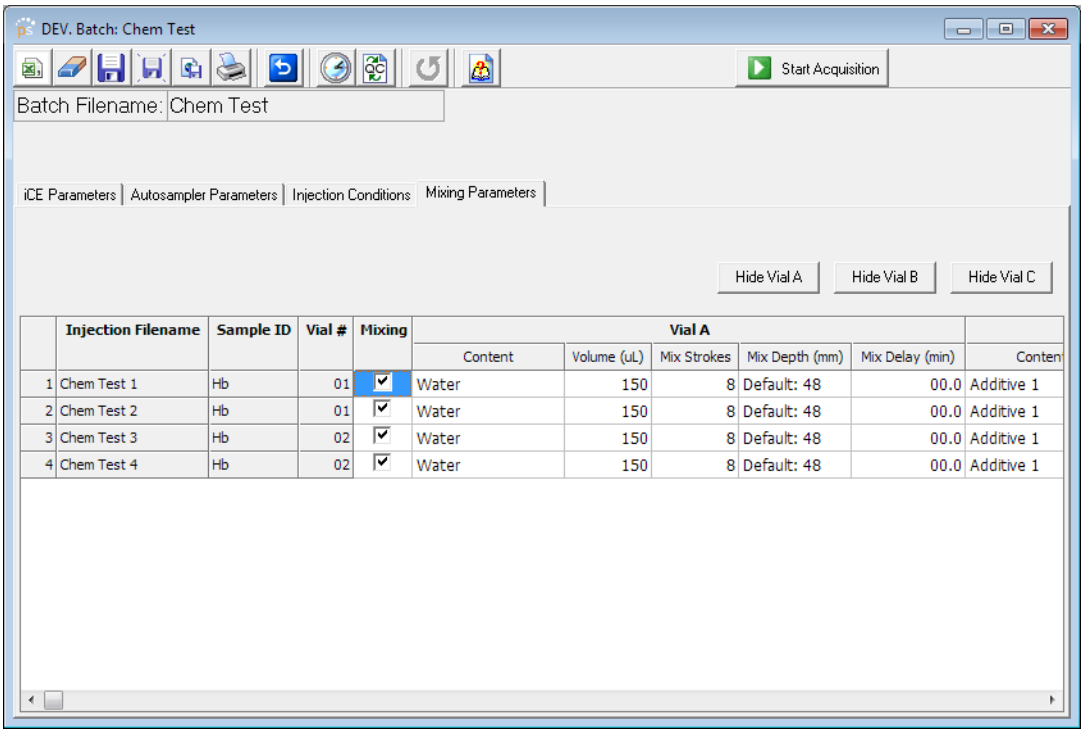
If the results are not reproducible, the Number of Mixing Strokes should be increased.

Overview of On-Board Sample Mixing Parameters

The following batch settings and system configuration values are used for on-board mixing.

Batch Settings

- **Vial Volume** - This is the volume of the on-board reagent the autosampler adds to the sample vial. This setting is entered in the **Volume (µL)** column in the batch mixing parameters tab for the on-board reagent location (A, B or C) as shown in Figure 6-40.
- **Mix Strokes** - The number of mix strokes used is entered in the **Mix Strokes** column in the batch mixing parameters tab (Figure 6-40). For details on how to optimize Mix Strokes for your application, see "Number of Mixing Strokes" on page 156.



	Injection Filename	Sample ID	Vial #	Mixing	Vial A					
					Content	Volume (uL)	Mix Strokes	Mix Depth (mm)	Mix Delay (min)	Conten
1	Chem Test 1	Hb	01	☑	Water	150	8	Default: 48	00.0	Additive 1
2	Chem Test 2	Hb	01	☑	Water	150	8	Default: 48	00.0	Additive 1
3	Chem Test 3	Hb	02	☑	Water	150	8	Default: 48	00.0	Additive 1
4	Chem Test 4	Hb	02	☑	Water	150	8	Default: 48	00.0	Additive 1

Figure 6-40: Mixing parameters tab in batch file.

System Configuration Parameters

The parameters described in this section are set in the System Configuration and can be modified by the user. For information on how to change these values, see "Changing On-Board Mixing System Configuration Values" on page 161.

- **Mix Depth** - The default value for Mix Depth is 35 mm, and the factory set position for Needle Depth is 48 mm. This assumes a sample solution height of approximately 13 mm which works well for 200 µL sample volumes. For details on how to optimize Mix Depth for your application, see "Mixing Depth" on page 156.

NOTE: ProteinSimple recommends using the default Mix Depth of 35 mm for sample volumes of 200 µL and up. For sample volume less than 200 µL, the Mix Depth should be set higher.

- **Mix-Air Volume** - The air gap between the sample and the water in the needle. This value is similar to the Pre-Air Volume setting, but applies specifically to on-board mixing procedure. The default value is 10 µL.
- **Mix Rate** - Aspirate and dispense rate used for mixing. In a mixing stroke, the aspirate and dispense rates are separate parameters. The default aspirate rate is 5 µL/second, and the default dispense rate is 100 µL/second. For details on how to optimize Mix Rate for your application, see "Mixing Rate" on page 155.

NOTE: ProteinSimple recommends using the default values for Mix-Air Volume and Mix Rate.

Using the On-Board Sample Mixing Feature

1. If you will be using the autosampler for automated sample preparation, select the Mixing Parameters tab (Figure 6-41) during the Batch setup.

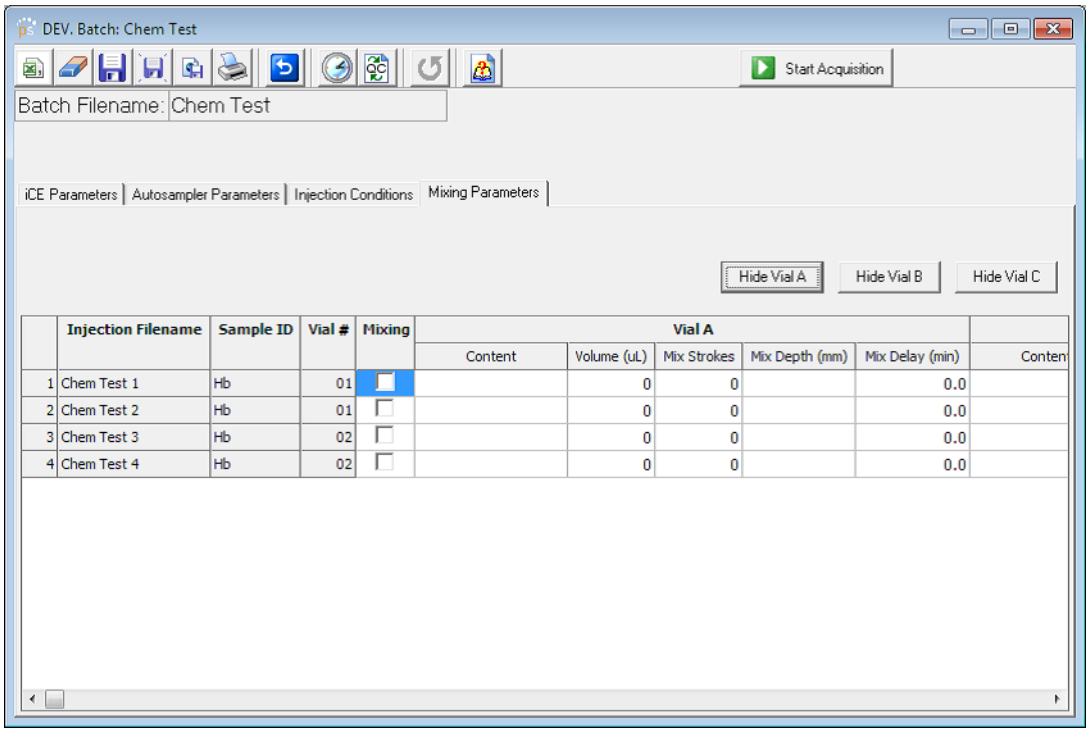


Figure 6-41: *Mixing Parameters tab.*

2. Select the checkbox in the Mixing column for all the injections you want to set up sample preparation for.

NOTE: The Mixing column cannot be auto-filled.

---

3. Enter names for the sample preparation reagents you are using. Click the row 1 cell under Content for Vial A, B or C and type a name.

NOTE: You can hide a reagent vial you aren't using in the table by clicking its Hide Vial button.

---

4. Enter the volume of the reagent to add to the sample vial. Click the row 1 cell under Volume for Vial A, B or C and enter a volume in µL.
5. Enter the mix strokes to be used for on-board mixing. Click the row 1 cell under Mix Strokes for Vial A, B or C and enter a value. ProteinSimple recommends using 8 mix strokes to ensure thorough mixing.
6. Enter the mix depth. Click the row 1 cell under Mix Depth for Vial A, B or C and select a value. The default value entered in the System Configuration file will be listed first. ProteinSimple recommends using a mix depth of 35 mm.

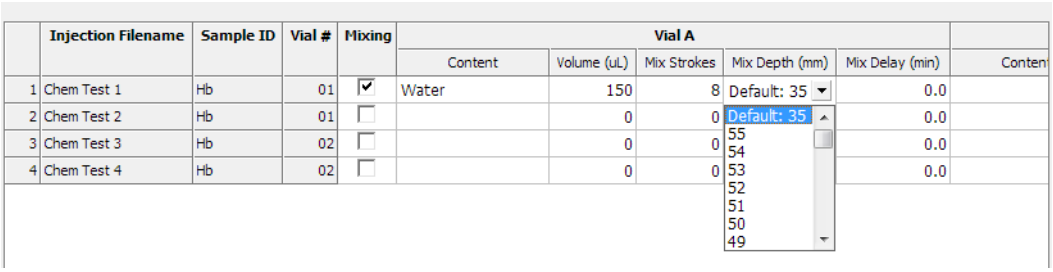


Figure 6-42: *Selecting mix depth.*

NOTE: The mix depth values will automatically update if more than one reagent is used to accommodate volume level changes in the sample vial. This ensures proper mixing as each additional reagent is added.

---

7. Enter a mix delay. Click the row 1 cell under Mix Delay for Vial A, B or C and enter a value in minutes. When a time is entered here, the autosampler will hold to allow solutions to react before starting the mixing steps.

8. Fill the remaining cells in the table as needed using the right-click menu options described in "Filling the Table" on page 140.

   An example of a completed Mixing Parameters table is shown in Figure 6-43.

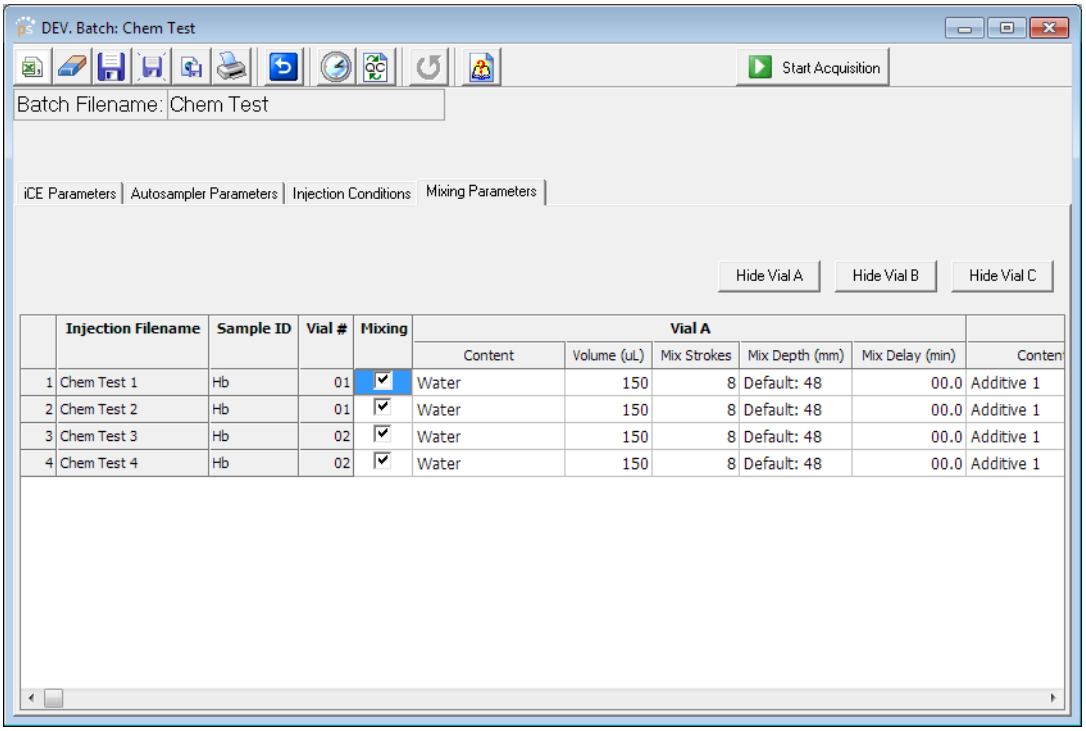


Figure 6-43: *Example of a completed Mixing Parameters table.*

Changing On-Board Mixing System Configuration Values

Mix depth, mix-air volume and mix rate values are set in the System Configuration. To change these values:

NOTE: Only administrators can change system configuration settings.

---

1. In iCE CFR Software, click **Utility** in the main menu and select **System Configuration**. The following screen will display:

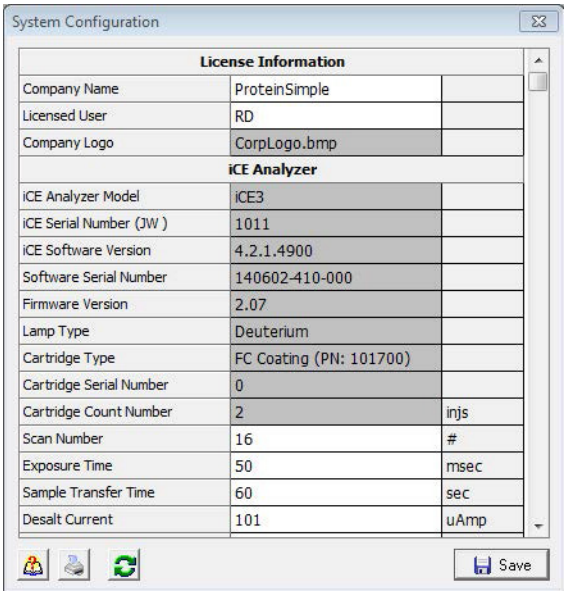


Figure 6-44: *System configuration screen.*

2. Scroll to the end of the configuration file using the scroll bar.
3. Click in the **Mix Depth**, **Mix-Air Volume** or **Mix Rate** boxes and enter the value you want to use:

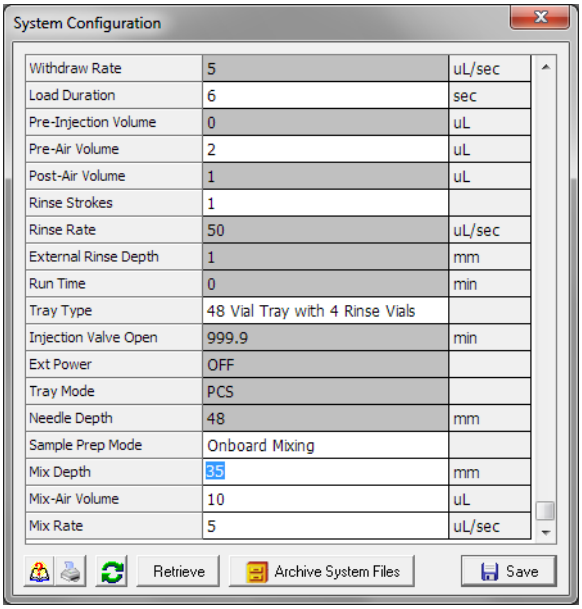


Figure 6-45: Changing on-board mixing parameters.

4. Click **Save**.

Chapter 7:

Data Calibration and Conversion

Chapter Overview

- Calibrating Batch Data
- Opening an Acquired Batch File
- Batch Book Overview
- Processing Data - Manual pI Calibration
- Converting Data Files for External Processing
- Processing Data - Automated pI Calibration and Data Export

Calibrating Batch Data

Once a batch is complete, the data must be calibrated to determine sample pI values. This is done by identifying both the low and high pI marker peaks in the electropherogram for each sample injection. After identification, iCE CFR Software uses the pI entered for the markers in the batch file to calculate sample pI. Automated pI calibration is available in iCE CFR version 4.x.

Opening an Acquired Batch File

1. To calibrate a batch file, select **Batch/Data** in the main menu and click **Review and Process**. The Batch Review screen will display:

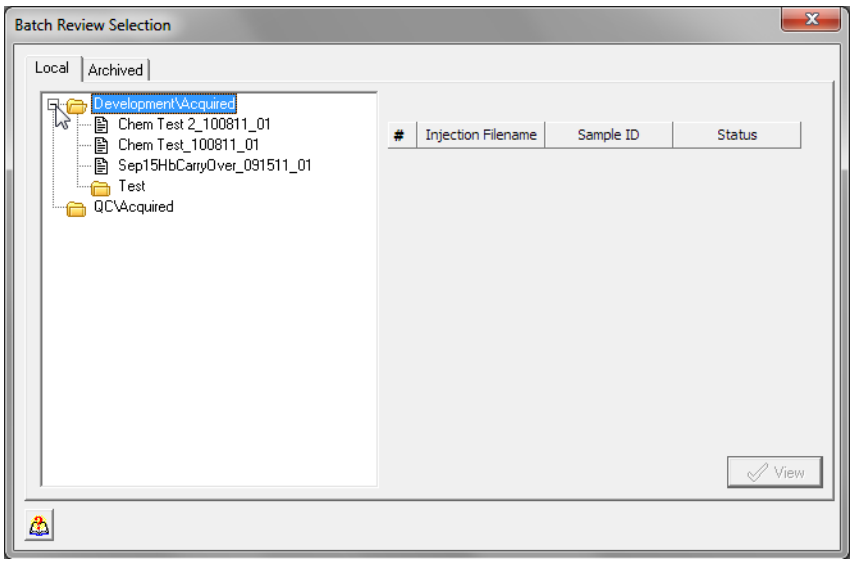


Figure 7-1: Batch selection.

Expand the tree on the right to view all batch files containing acquired data. Clicking a batch file will display details and processing status for each sample injection in the batch:

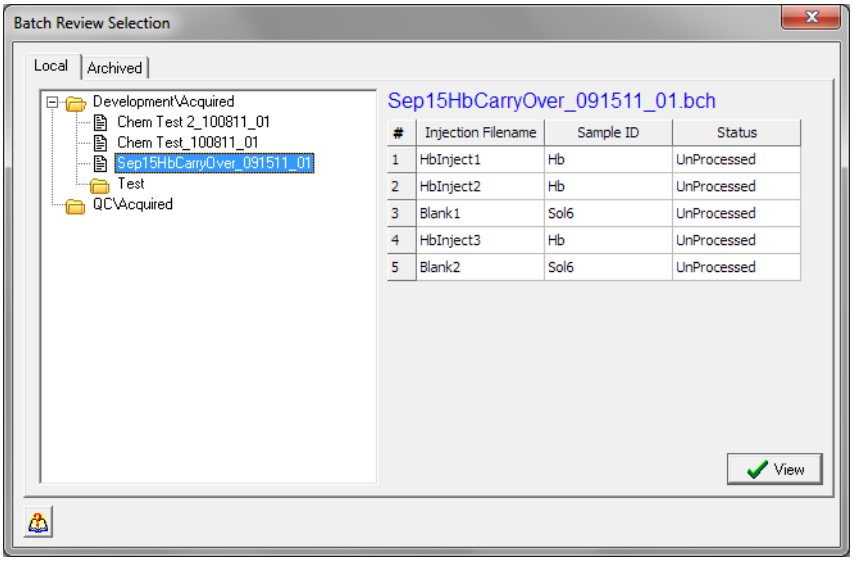


Figure 7-2: Batch status.

2. To view the data for or process (calibrate) a file, select a batch and click **View**.

Batch Book Overview

Viewing Sample Data and File Details

The Injection Information tab lets you review sample electropherograms and calibrate data. File details for the data displayed are shown in the File Information box and processing status is shown in the Injection box.

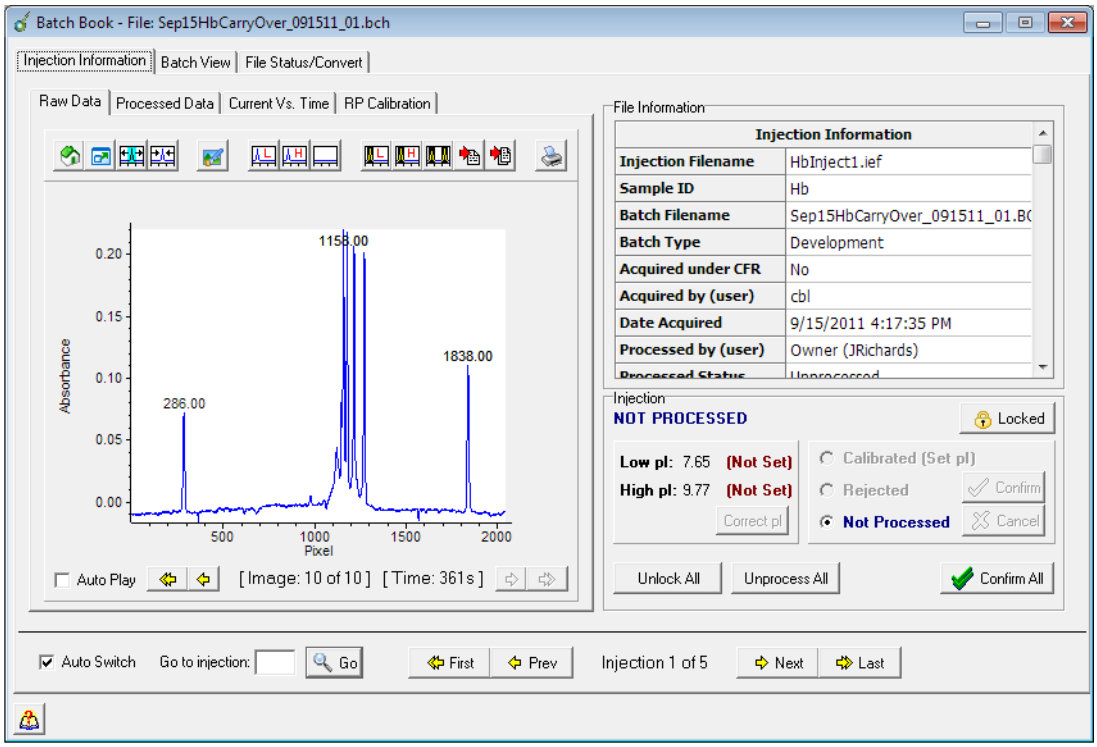


Figure 7-3: *Injection Information tab.*

Sample data can be viewed in one of four data tabs. You can view data as follows:

- **To view data for individual injections** - Click **Next**, **Prev**, **First**, or **Last** to page through each injection file in the batch.
- **To view data for a specific injection:**
  - Select the **File Status/Convert** tab and select an injection row. Select the **Injection Information** tab again to display data for the selected injection.
  - Enter the injection number in the Go to injection box and click **Go**.
- **To view images captured during sample focusing** - Click the single and double arrows below the electropherogram to page through each image. Click the **Auto Play** checkbox to have the software automatically play all the images sequentially.

Buttons in the data tabs let you do the following:


Top view. Displays the electropherogram at the default scale.


Full screen view. Maximizes the electropherogram to a full screen view.


Zoom selection. Lets you zoom in on the area selected in the electropherogram.


Zoom out. Returns the zoom level to the previous level.


Save as bitmap. Lets you save the data displayed as a bitmap file.


Set low pI. Sets the peak selected in the electropherogram as the low pI marker (Raw Data tab only).


Set high pI. Sets the peak selected in the electropherogram as the high pI marker (Raw Data tab only).


Clear pI settings. Lets you clear the pI marker selections (Raw Data tab only).


Set low pI window. Sets the window selected in the electropherogram as the low pI window when doing automated pI marker assignment (Raw Data tab only).


Set high pI window. Sets the window selected in the electropherogram as the high pI window when doing automated pI marker assignment (Raw Data tab only).

Process settings. Opens the Process Settings window where you can set calibration and file conversion settings (Raw Data tab only).

Process single run. Applies the selected pI windows to the data currently displayed (Raw Data tab only).

Process all. Applies the selected pI windows to all data in the batch (Raw Data tab only).

Print. Lets you print the information for the sample injection currently displayed.

Viewing Raw Data

Click the **Raw Data** tab to view unprocessed sample electropherograms. You can also calibrate pIs in this tab.

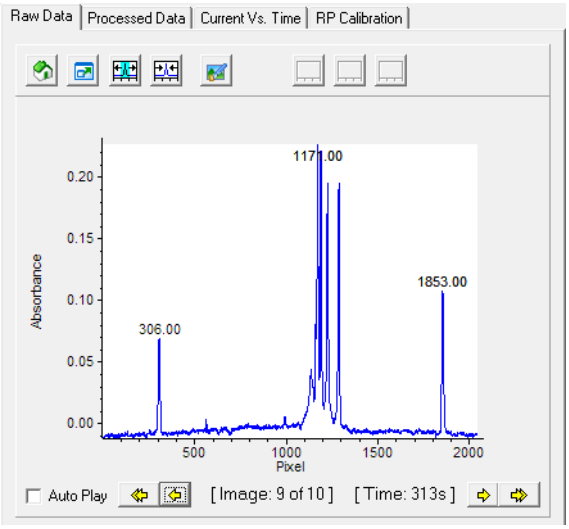


Figure 7-4: *Raw Data tab.*

Viewing Processed Data

Click the **Processed Data** tab to view the calculated peak pIs for calibrated electropherograms.

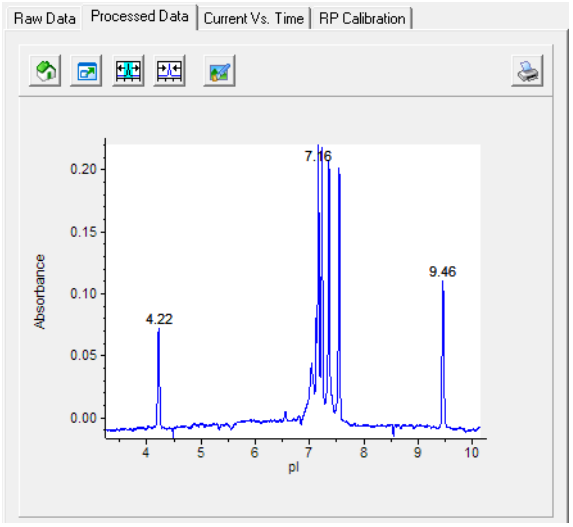


Figure 7-5: *Processed Data tab.*

Viewing Focus Current Plots

Click the **Current vs Time** tab to view a plot of the current during the sample focusing period.

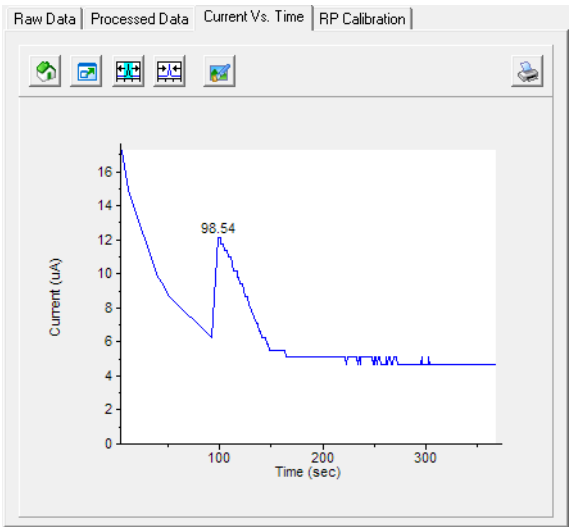


Figure 7-6: *Current Vs. Time tab.*

Viewing RP Calibration Data

Click the **RP Calibration** tab to view the calibrated data in relative pixel units (RPU) rather than pI.

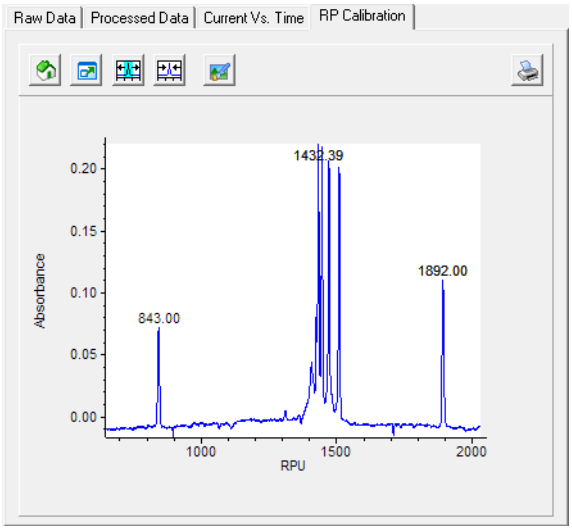
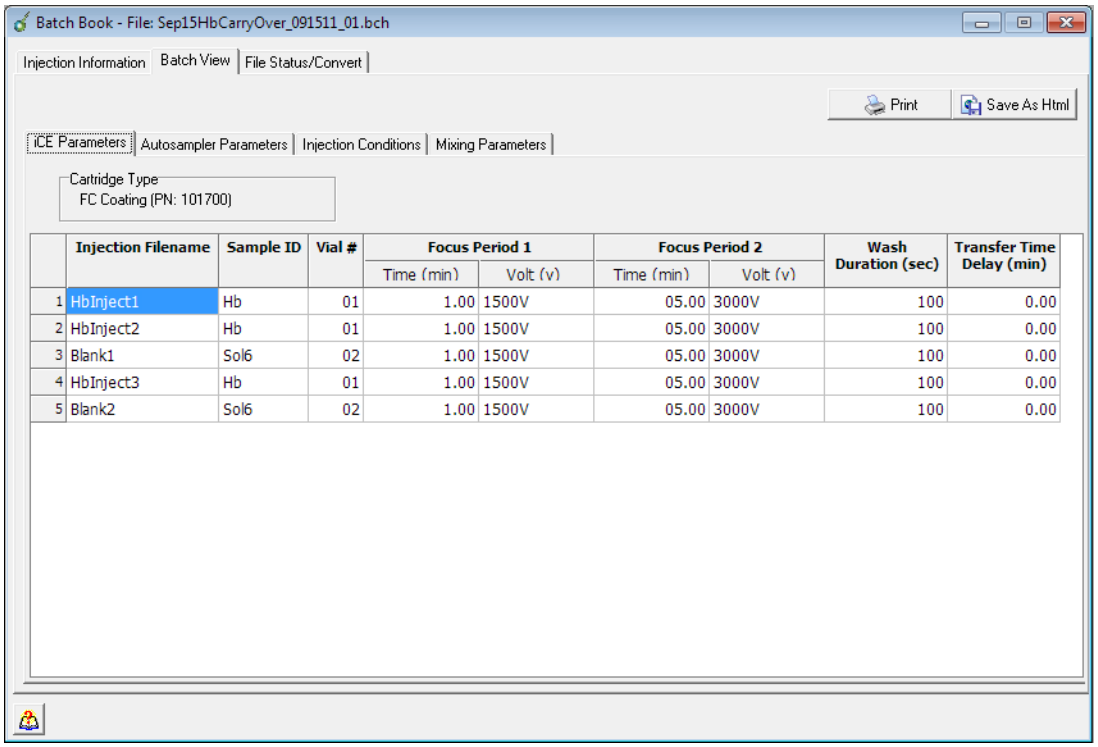


Figure 7-7: *RP Calibration tab.*

Viewing Batch File Details

To view the original batch file details, click the **Batch View** tab:



	Injection Filename	Sample ID	Vial #	Focus Period 1		Focus Period 2		Wash Duration (sec)	Transfer Time Delay (min)
				Time (min)	Volt (v)	Time (min)	Volt (v)		
1	HbInject1	Hb	01	1.00	1500V	05.00	3000V	100	0.00
2	HbInject2	Hb	01	1.00	1500V	05.00	3000V	100	0.00
3	Blank1	Sol6	02	1.00	1500V	05.00	3000V	100	0.00
4	HbInject3	Hb	01	1.00	1500V	05.00	3000V	100	0.00
5	Blank2	Sol6	02	1.00	1500V	05.00	3000V	100	0.00

Figure 7-8: *Batch View tab.*

Viewing Batch File Process Status

To view the full table of injections in the batch and the processing status for each, select the **File Status/ Convert** tab. This tab is also used to convert batch files to other formats for further processing in other analysis programs. This will be described in more detail in “Converting Data Files for External Processing” on page 178.

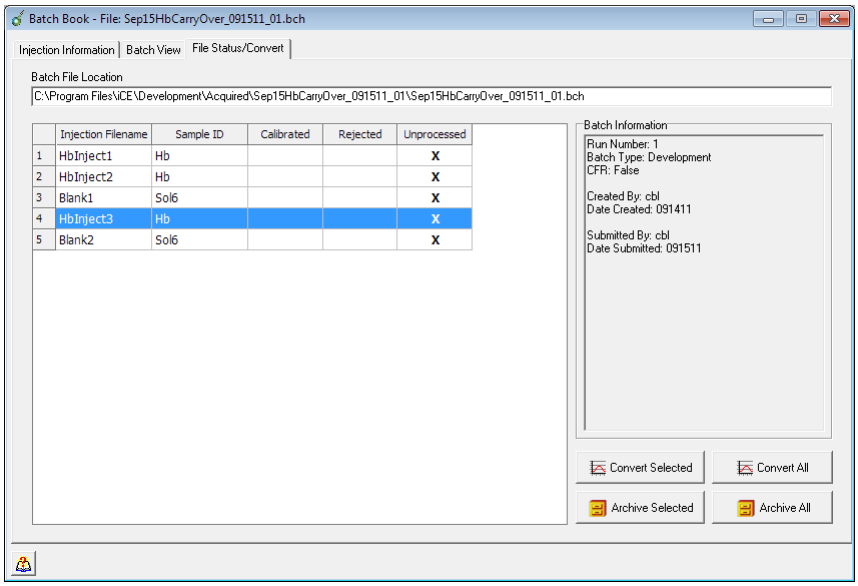


Figure 7-9: *File Status/Convert tab.*

Processing Data - Manual pI Calibration

1. Set the low pI marker. Click in the electropherogram and drag the mouse over the low pI marker peak (typically the first peak) as shown in Figure 7-10 (left). If there are multiple peaks at the beginning of the data, the low pI marker will be the largest peak.
2. Click the **Set low pI** icon or right click and select **Set First pI Marker** (Figure 7-10, right).

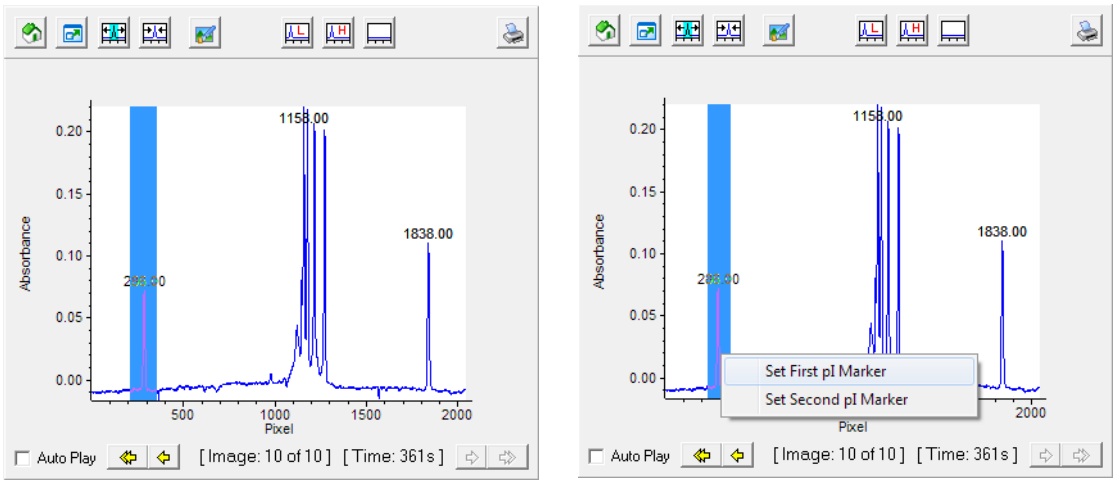


Figure 7-10: Selecting the lower pI marker peak (left) and setting as the first pI marker (right).

The status of the low pI marker in the Injection box will change from Not Set to Set:

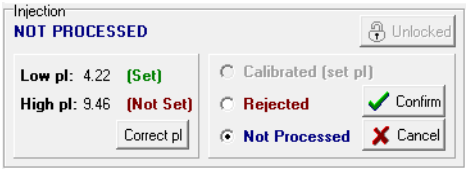


Figure 7-11: Lower pI marker process status.

3. Set the high pI marker. Click in the electropherogram and drag the mouse over the high pI marker peak (typically the last peak) as shown in Figure 7-12. If there are multiple peaks at the end of the data, the high pI marker will be the largest peak.
4. Click the **Set high pI** icon or right click and select **Set Second pI Marker**.

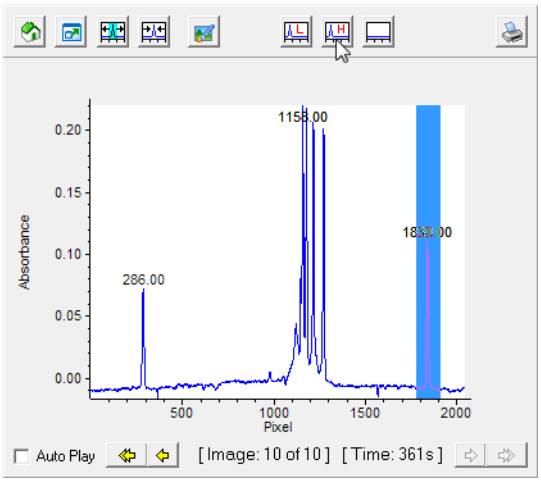


Figure 7-12: *Setting the high pI marker.*

The status of the high pI marker in the Injection box will change from Not Set to Set and the file will be marked as Calibrated. The electropherogram data will now display in the Processed Data tab, and pI marker peaks will be identified, and sample peaks will display calculated pI values (Figure 7-13).

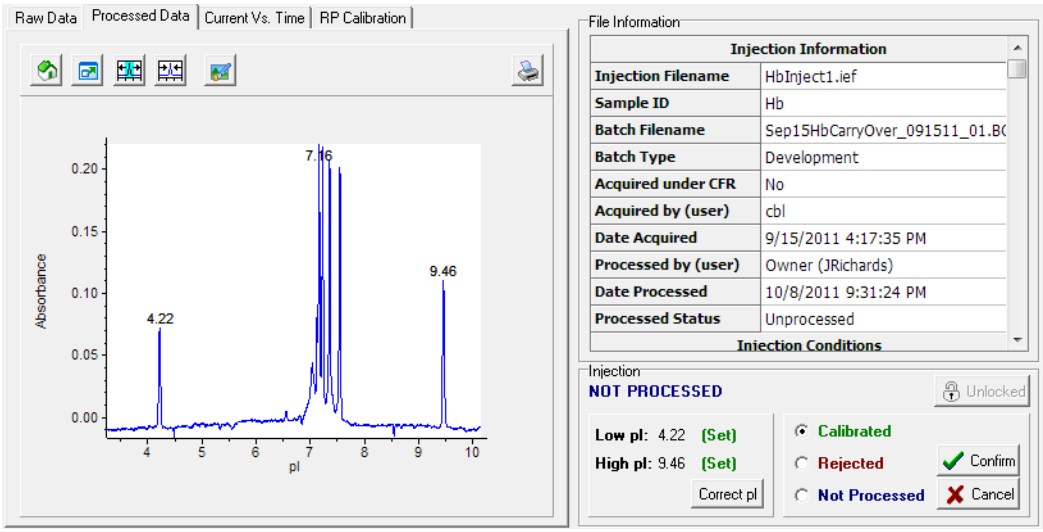


Figure 7-13: *Calibrated data.*

5. To accept the calibration, make sure Calibrated is selected in the Injection box then click **Confirm**.

The file status will change from NOT PROCESSED to CALIBRATED:

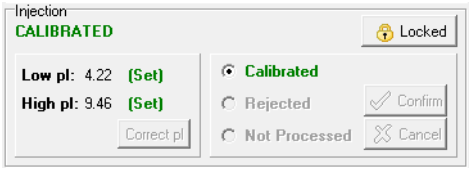


Figure 7-14: *File status calibrated.*

NOTE: To confirm a calibration you must enter your e-signature. To avoid having to do this for each injection file, you can click Next and process each of the remaining files first. When calibration of all injection files is complete, click Confirm All to accept all calibrated files at once.

- **To reject the data completely** - Click **Rejected** and then click **Confirm**.
- **To remove the calibration and reprocess the data** - Click **Not Processed** and then click **Confirm**.

6. Click **Next** to process the next injection file.
7. Repeat the previous steps for the remaining injection files in the batch.
8. Once the data is calibrated it will be locked.
   - If you need to recalibrate, the data file will need to be unlocked. Data files can be either be unlocked one at a time by clicking the **Locked** button or you can unlock an entire batch by clicking **Unlock All** (Figure 7-15).
   - If you need to reprocess the batch, click **Unprocess All** (Figure 7-15). This will unlock all data files in the batch and reset the file status for each to Not Processed.

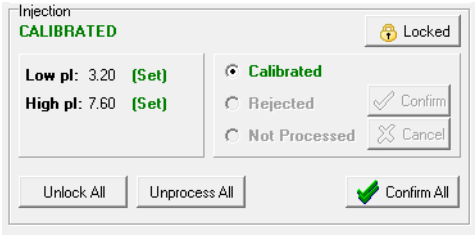


Figure 7-15: *Unlock All and Unprocess All buttons.*

Converting Data Files for External Processing

Batch injection files can be converted to several formats for external processing in other analysis programs. The following file format conversions are available in iCE CFR Software:

- Chrom Perfect Seven
- EZChrom 6.8 (Agilent Technologies)
- Empower 2 and 3 (Waters)
- Chromeleon 6.8, 7.1 and 7.2 (Dionex Corporation)
- EZChrom Elite 3.0 (Agilent Technologies, formerly Scientific Software)
- Standard ANDI format (for other analysis programs)

To convert files:

1. Click the **File Status/Convert** tab.

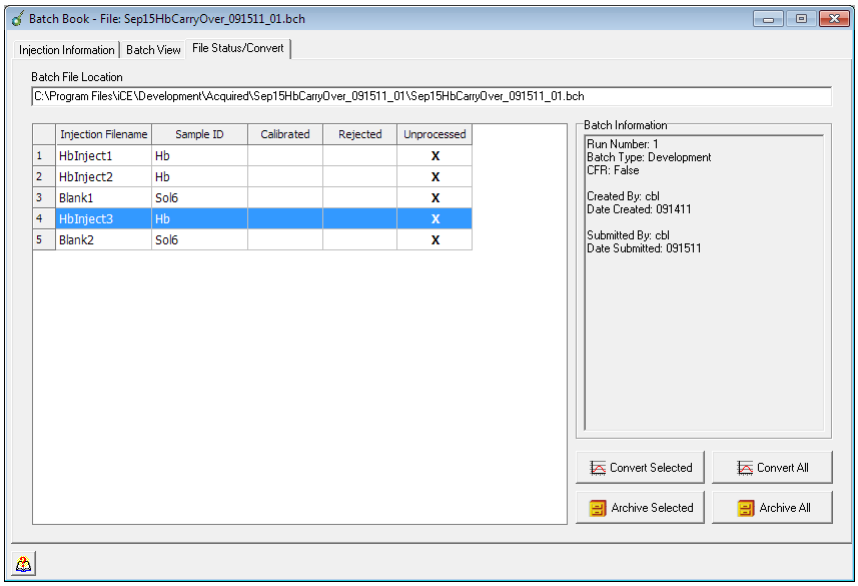


Figure 7-16: *File Status/Convert tab.*

2. Select the injection files to convert.
   a. **To select all or a sequential group of files** - Click the first row in the group. Hold the mouse button down and select the rest of the injection files. Click **Convert Selected**.
   b. **To select a non- sequential group of files** - Hold the Shift key and select the injection files individually. Click **Convert Selected**.
   c. **To select all the files in the batch** - Click **Convert All**.

The File Conversion screen will display:

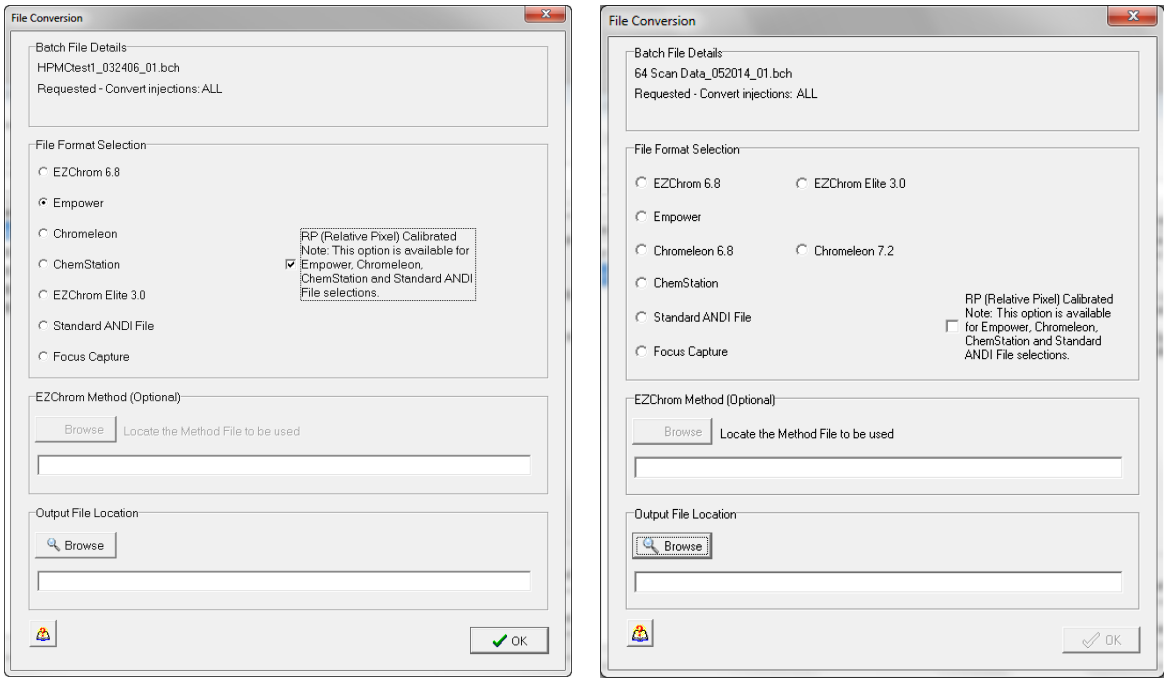


Figure 7-17: *File conversion screen, iCE CFR software version 4.0 (left) and 4.1 (right).*

3. Select an option in the File Format Selection box:
   a. **Chrom Perfect** - Select **Standard ANDI File**. For more information on analyzing iCE CFR batch files in Chrom Perfect, see Appendix A.
   b. **EZ Chrom 6.8** - Select **EZChrom 6.8**. To attach a method to the converted files, click **Browse** in the EZChrom Method (Optional) box and select a method. When this is done, the method file is automatically opened in EZChrom with the files. Converted files will be saved in .asc format and a .seq file will also be created. For more information on analyzing iCE CFR batch files in EZChrom, see ProteinSimple's Guide for EZChrom 6.8 (P/N 102167).
   c. **Empower** - Select **Empower**. Converted files will be saved in .cdf format. For more information on analyzing iCE CFR batch files in Empower see ProteinSimple's Guide to File Conversion for Empower Software (P/N 102323).

NOTES:

To enable automated import of data for Empower, you can utilize the Waters Data Converter 2 software application. For further information, please contact Waters directly.

The sample_name field required for Empower files is a combination of two fields from the iCE CFR file and will use the following format: Injection Filename-Sample ID.

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d. **Chromeleon**:

With iCE CFR software v3.0 and 4.0x - Select **Chromeleon**. Converted files will be saved in.cdf format.

With iCE CFR software 4.1 and higher - Select **Chromeleon 6.8** or **Chromeleon 7.2**, both versions are supported in iCE CFR software version 4.1 and higher. Chromeleon version 7.0 and 7.1 do not support ANDI file import which means iCE files cannot be directly imported into them. You must either import into Chromeleon 6.8 and then move them to version 7.0 or 7.1 or upgrade to Chromeleon 7.2.

NOTE: The sample_name field required for Chromeleon files is a combination of two fields from the iCE CFR file and will use the following format: Injection Filename-Sample ID.

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e. **EZChrom Elite 3.0 (Agilent Technologies, formerly Scientific Software)** - Select **EZChrom Elite 3.0**. To attach a method to the converted files, click **Browse** in the EZChrom Method (Optional) box and select a method. When this is done, the method file is automatically opened in EZChrom with the converted injection files. Converted files will be saved in .asc format and a .seq file will also be created.

f. **Standard ANDI Conversion** - Select **Standard ANDI File**. This format converts the files to a standard AIA (.cdf) format that can be used in other analysis programs.

g. **ChemStation Conversion** - iCE CFR Version 4.0 and higher offers the ability to convert iCE data files for use with Agilent ChemStation software.

4. Click **Browse** in the Output File Conversion box. Select a directory to save the converted files to. Files will be saved in this directory in a folder with the original batch name.

5. Optional. You can choose to convert only the RP Calibrated files. To do this select the **RP (Relative Pixel) Calibrated** checkbox (Figure 7-18).

NOTE: This option is only available for Chrom Perfect, Empower, Chromeleon and Standard ANDI formats.

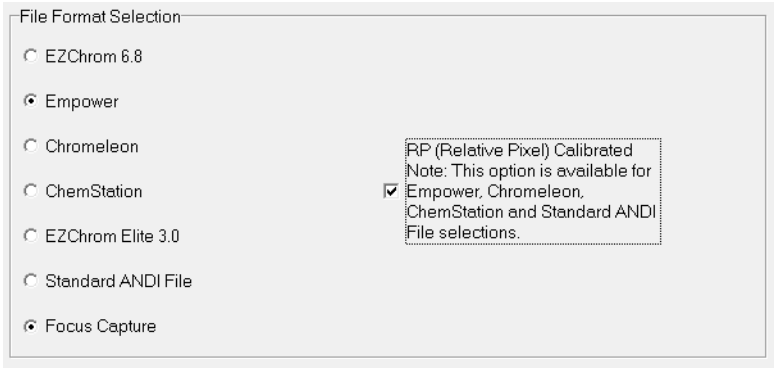

Figure 7-18: *Selecting Relative Pixel Calibration, iCE CFR software version 4.0.*

6. Click **OK** to convert the files.

Processing Data - Automated pI Calibration and Data Export

iCE CFR version 4.0 and higher offers automated pI calibration and data export. Detection windows are set for both the high and low pI marker windows. The software will then identify the highest peak in the window as the pI marker and assign the value specified in the batch table.

Buttons in the data tabs used for automated pI calibration and export:


Set low pI window. Sets the window selected in the electropherogram as the low pI window (Raw Data tab only).


Set high pI window. Sets the window selected in the electropherogram as the high pI window (Raw Data tab only).


Process settings. Opens the Process Settings window where you can set calibration and file conversion settings (Raw Data tab only).


Process single run. Applies the selected pI windows to the data currently displayed (Raw Data tab only).


Process all. Applies the selected pI windows to all data in the batch (Raw Data tab only).

To use the automated pI calibration option:

1. Set the low pI marker window by clicking in the electropherogram and dragging the mouse over the low pI marker peak (typically the first peak) as shown in Figure 7-19. If there are multiple peaks at the beginning of the data, the low pI marker will be the largest peak.

2. Click the **Set Low pI Window** button or right click and select **Set Low pI Marker Window** (Figure 7-19).

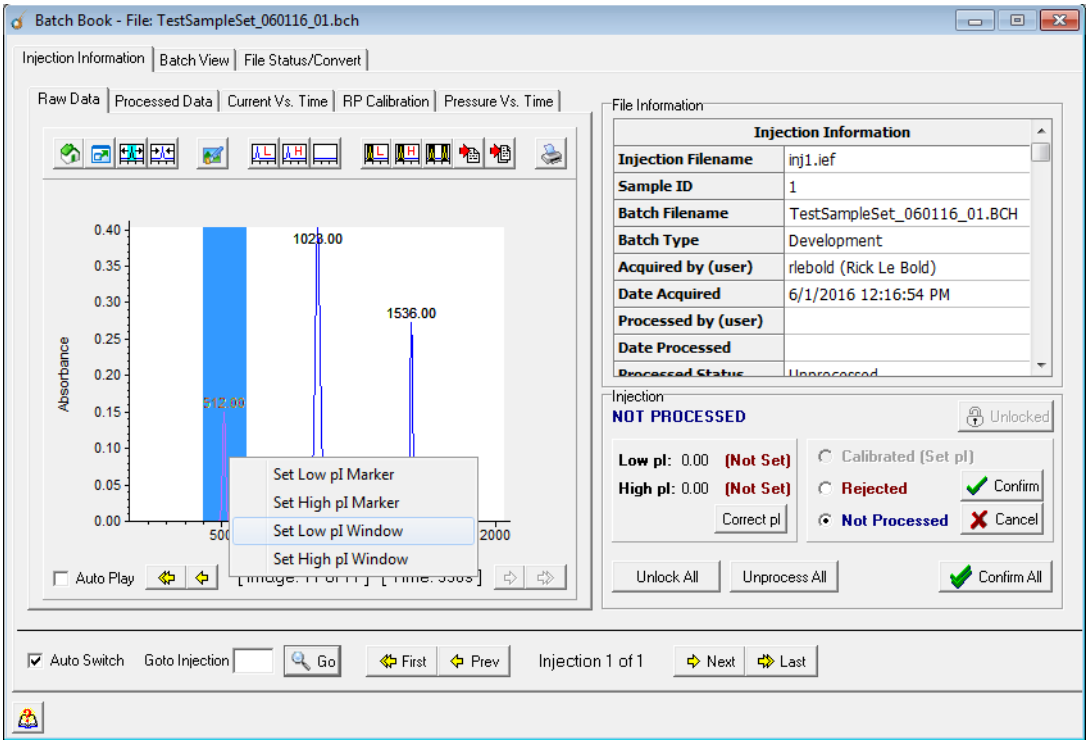

Figure 7-19: Setting low pI window in the Injection Information tab.

3. Set the high pI marker window by clicking in the electropherogram and dragging the mouse over the high pI marker peak (typically the last peak) as shown in Figure 7-20. If there are multiple peaks at the end of the data, the high pI marker will be the largest peak.

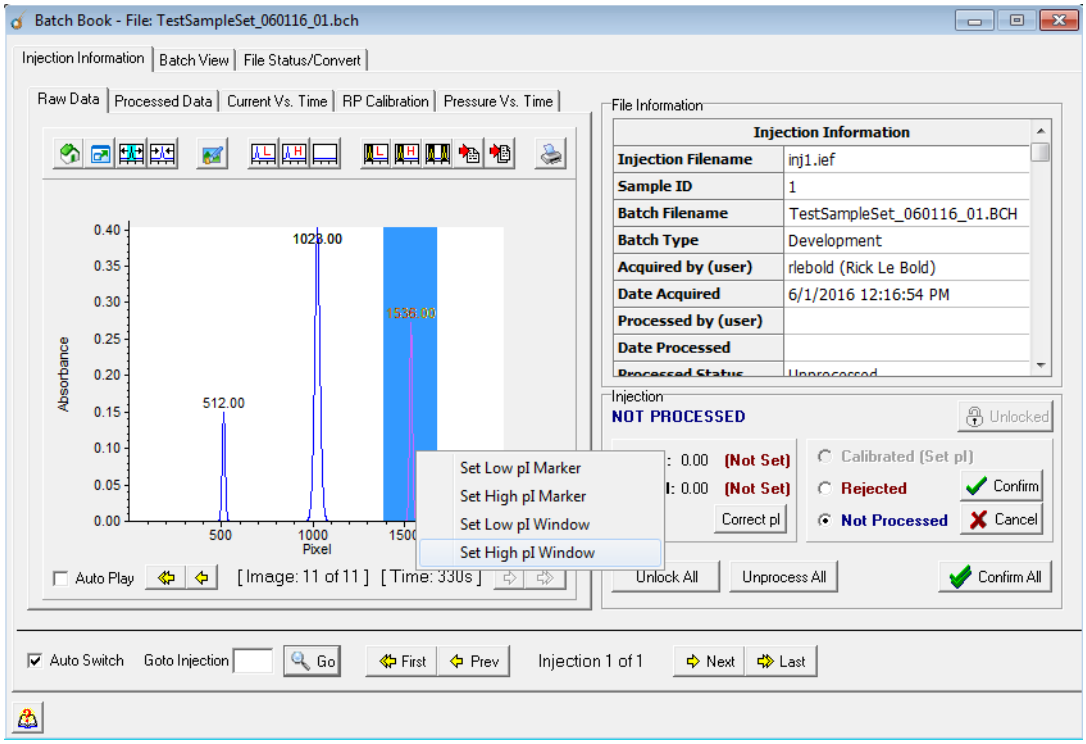

Figure 7-20: Setting high pI window in the Injection Information tab.

4. Click the **Set High pI Window** button or right click and select **Set High pI Window**.
5. To enable automated pI calibration and/or data export, click the **Process Settings** button.
6. In the Process Settings window (Figure 7-21), select **Enable** under Calibration to enable automated pI calibration. Manually adjust the pI windows if desired.

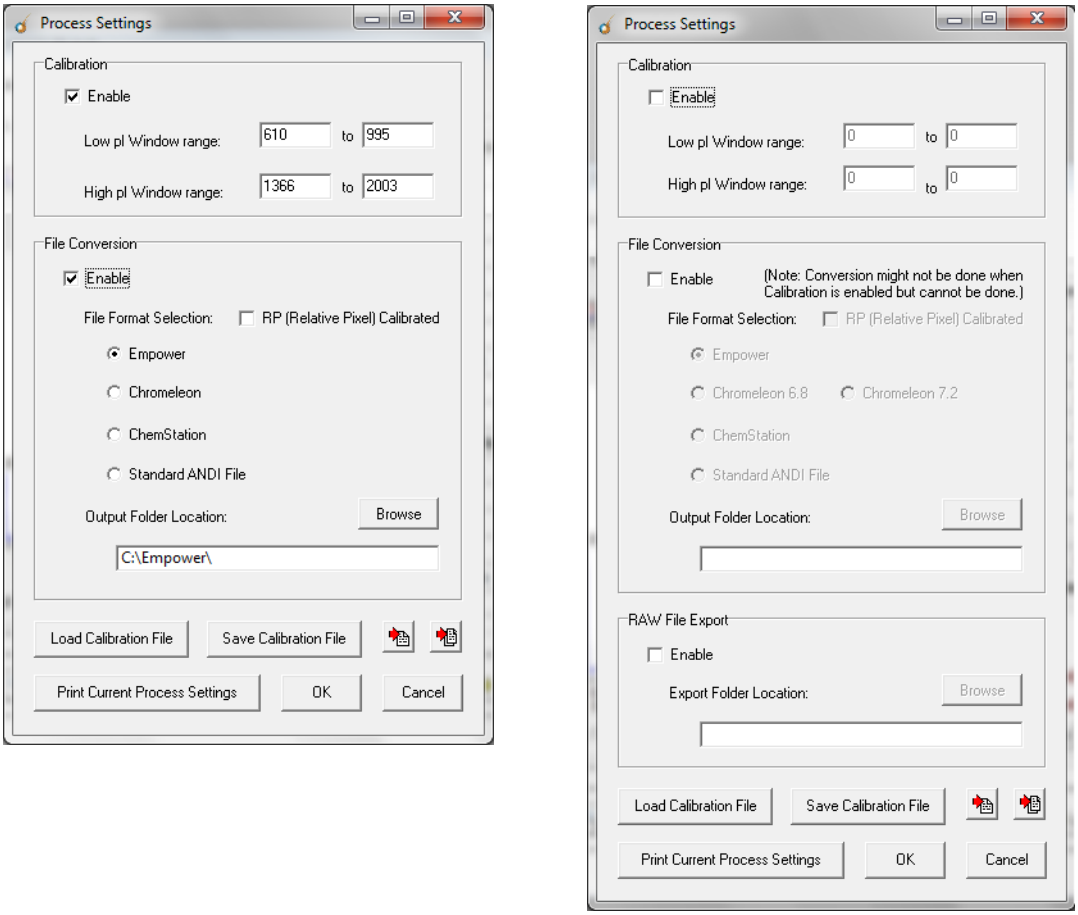

Figure 7-21: Process Settings window, iCE CFR software version 4.0 (left) and 4.1 (right).

7. If automated file conversion is needed, select **Enable** under File Conversion and specify the file and export format, then select the output folder for the converted files. Files can be saved to the iCE local PC or to a network drive.
8. Save the calibration file if you'd like to use the file for analysis of future batches.
9. **Optional for iCE CFR software version 4.1 and higher.** The Process Settings window has been updated to allow for automated backup of the raw data during sample acquisition. The data can be exported to a local drive and folder or a network shared folder.
10. Click **OK** to close the Process Settings window.
11. Click the **Process All** button. The selected pI marker windows will be applied to all data in the batch and data will be exported if this option was enabled.

12. Once the data is calibrated it will be locked.
    - If you need to recalibrate, the data file will need to be unlocked. Data files can either be unlocked one at a time by clicking the **Locked** button or you can unlock an entire batch by clicking **Unlock All**(Figure 7-20).
    - If you need to reprocess the batch, click **Unprocess All**. This will unlock all data files in the batch and reset the file status for each to Not Processed.

Focus Capture Text File Conversion

Users can export the focus capture images taken during the IEF separation as text files. Images are taken at approximately 30 second intervals, and each image is captured as a raw data file (.ief).

To do this:

1. Click the **File Status/Convert** tab.

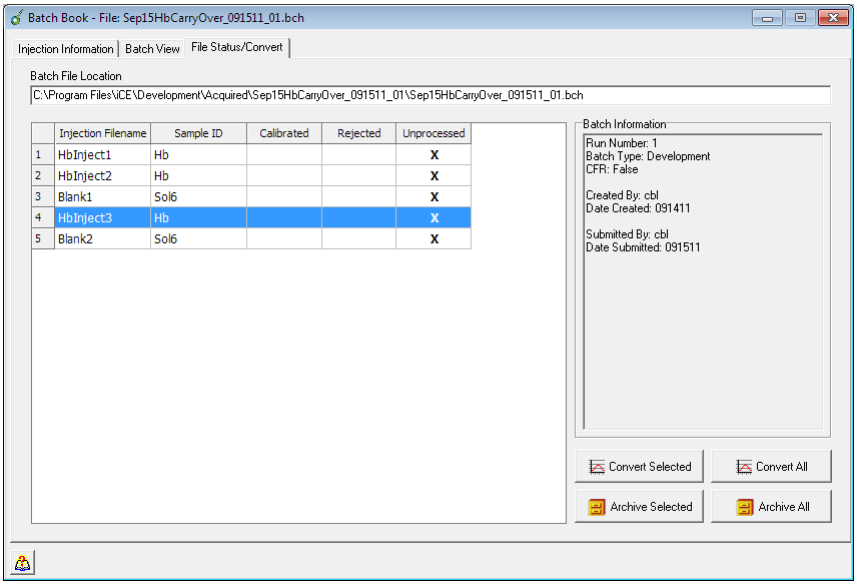

Figure 7-22: *File Status/Convert tab.*

2. Select the injection files to convert.
    - **To select all or a sequential group of files** - Click the first row in the group. Hold the mouse button down and select the rest of the injection files.
    - **To select a non- sequential group of files** - Hold the Ctrl key and select the injection files individually.

h. **To select all the files in the batch** - Click **Convert All**.

The File Conversion screen will display:

Figure 7-23: *File conversion screen, iCE CFR software version 4.2.*

3. Select **Focus Capture** in the File Format Selection box.

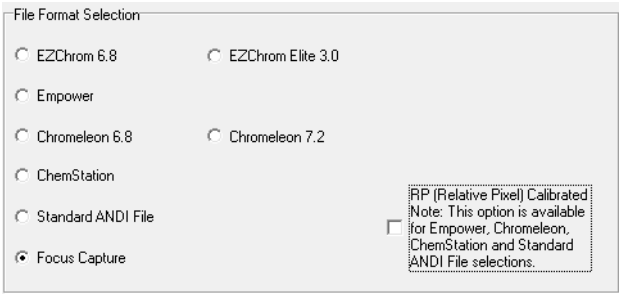

Figure 7-24: *Selecting Relative Pixel Calibration.*

4. Click **Browse** in the Output File Conversion box. Select a directory to save the converted files to. Files will be saved in this directory in a folder with the original batch name. Files are saved in .txt format.
5. Click **OK** to convert the files.

Chapter 8:

Method Development

Chapter Overview

- Results Optimization
- Initial Conditions
- Method Development
- Chemical Interferences in cIEF
- Peak Identification and pI Calibration
- Procedure for Peak Identification

Results Optimization

A successfully defined and optimized cIEF method will provide the following:

- A highly reproducible peak pattern
- Satisfactory peak resolution for the targeted applications

Initial Conditions

The most important condition in a cIEF method is the carrier ampholytes. The specific vendor (or brand name), pH range (narrow or wide), and concentration of the carrier ampholytes in final sample solutions are critical. Sample concentration, sample additives, focusing time and voltage must also be considered.

Carrier Ampholytes

Choosing the Appropriate Carrier Ampholyte

There are only three carrier ampholytes (CAs) with different molecular structures commercially available: Pharmalytes (GE Healthcare), Servalyts (Serva) and Biolytes (Bio-Rad). All carrier ampholytes currently offered today are from one of these three vendors. They are repackaged and sold under various other brand names.

Background. Use of Pharmalytes is advantageous due to their lower optical absorption at 280 nm (the cIEF detection wavelength), thus the background noise produced is lower. Pharmalytes should be the first choice for unknown samples for this reason.

Resolution. Optimal resolution can only be determined by running multiple experiments. For some glycosylated proteins and all fusion proteins ProteinSimple has tested, it has been determined that Servalyts provide much better resolution compared to Pharmalytes and Biolytes. However, Servalyts have a greater absorption at 280 nm compared to the other vendors. As an example, we have found that Servalyts absorption at 280 nm was approximately 5X higher than Pharmalytes.

pH range. Both Servalyts and Pharmalytes offer a wide selection of pH ranges and gradients. However, the pH ranges for Biolytes are limited, and therefore the reported applications for their use is also limited.

Summary of carrier ampholyte characteristics:

- Pharmalytes (GE)
  - Low background at 280 nm
  - First choice for unknown samples
- Servalyts (Serva)
  - Better resolution for some glycosylated proteins and fusion proteins
  - High background noise at 280 nm (5X higher than Pharmalytes)
- Biolytes (Bio-Rad)

- Limited applications reported

Initial Carrier Ampholyte Conditions

For an unknown sample, ProteinSimple recommends starting with a wide pH range such as pH 3-10 Pharmalytes with a carrier ampholyte concentration of 2-8% as an initial starting point. This will provide the best buffer capacity and sample sensitivity at 280 nm. For the first run, a 4% concentration should be used.

Once concentration has been optimized, different carrier ampholytes with narrower pH ranges should be used to enhance resolution as needed.

Summary of starting carrier ampholyte conditions:

- When sample pI is unknown:
  - Use Pharmalytes pH 3-10
  - Start with a 2-8% concentration
- Resolution
  - Try different CAs if higher resolution is required
  - CA selection should be made based on experimental results
- pH range
  - Start with a wide pH range for unknown samples
  - Use CAs with a narrower pH range if higher resolution is required

Sample Concentration

Use a sample concentration of approximately 0.2 mg/mL initially if only one major peak is expected. If multiple major peaks are expected, increase the sample concentration by approximately 0.05 mg/mL per major peak.

Note: Major peaks are considered to be any peak that equals at least 20% of the total peak area.

Summary of initial sample concentration requirements:

- One major peak: ~0.2 mg/mL in the final solution
- Multiple major peaks: ~0.2 mg/mL plus (number of major peaks X 0.05 mg/mL)

Additives

For initial method development conditions, only use methyl cellulose as an additive. Methyl cellulose provides a dynamic coating in the column and must be added to all samples and wash solutions. Other additives should only be used when needed.

Summary of initial additive use:

- Use Methyl cellulose for all samples
  - Modifies the hydrophobic surface of FC coated column
  - Enhances resolution by reducing the sample's diffusion coefficient
- Other additives used for protein stabilization during IEF are optional

Initial Assay and Focus Settings

- Carrier ampholytes
  - pH 3-10 Pharmalytes, 4% concentration
- Sample concentration
  - 0.2 mg/mL or higher
- Additives
  - 0.35% methyl cellulose (no other additives)
- Focusing voltage and time
  - Pre-focus 1 minute at 1.5 kV
  - Focus 3 kV (600 V/cm) for 6-8 minutes

Always use a pre-focusing period of 1 minute at 1.5 kV. Since focusing time is generally related to the carrier ampholytes used, a 1-minute prefocus with a 6-minute focus is a good starting point for the majority of samples. You can easily tell if the focusing time is correct as the iCE3 monitors the focusing process.

Extended Unattended Operation (100 Runs)

NOTE: The option to extend operation to 100 unattended runs is only available for iCE3 systems that have and updated electrode arm with thicker, closed foam pads. If you are not sure if your system has the updated electrode arm, please see Figure 4-4 on page 36, or contact ProteinSimple Technical Support.

During isoelectric focusing separation, electroosmotic, isotachophoretic (ITP) and hydrodynamic forces act on the free solution pH gradient. Traditionally, the net effect of these forces is a drift towards higher pixel positions once the gradient is formed along with a stepwise increase in the pixel position between consecutive runs. Although the cathodic drift towards higher pixel numbers cannot be fully eliminated, ProteinSim-

ple has been able to dramatically reduce its effects and improve gradient stability. Resilient capillary coatings have been developed to minimize electroosmotic flow, and the recent incorporation of the iCE valve on the iCE3 instrument has eliminated the sources of hydrodynamic flow.

Through a redesign of the electrode assembly to better seal the electrolyte vials from the atmosphere, isotachophoretic effects are now addressed. Eliminating the source for carbonic acid formation in the catholyte preserves the solution's conductivity, generating a more consistent ITP rate between runs and provides safe operation of up to 100 runs using the same catholyte.

Combined, these system features and improvements have dramatically reduced cathodic drift, and the minor drift that still remains has been extensively characterized using methods with normal sample conductivity < 20 µA maximum current and focusing times up to 11 minutes. Using the results of this systematic study, ProteinSimple has created the following guidance for a basic marker's pixel position in run 1 and the risk of losing that marker before run 100.

- 5% Failure Rate = 1893 pixel position
- 1% Failure Rate = 1863 pixel position
- 4 Sigma; 1/10000 = 1812 pixel position
- 6 Sigma; 1/3.4 million = 1740 pixel position

For those with methods that have basic pI markers outside of this range, ProteinSimple suggests the addition of a cathodic spacer such as 5-10 mM arginine or 1-2% Pharmalytes 8-10.5 to the sample solution to adjust the position of the basic marker.

Method Development

Figure 8-1 shows the flow chart for method optimization. After first trying the initial recommended conditions:

1. **Adjust sample concentration** accordingly if sample peak absorbance is above 0.60 AU or below 0.05 AU.
2. **Obtain a reproducible peak pattern**, this is the most important step of method development. Try different additives and/or carrier ampholytes until a reproducible peak pattern can be maintained.
3. **Optimize resolution** as needed. Try narrow pH gradient carrier ampholytes and those from different vendors to enhance resolution. Once satisfactory resolution is obtained, pI calibration can be performed by spiking pI markers into the sample.

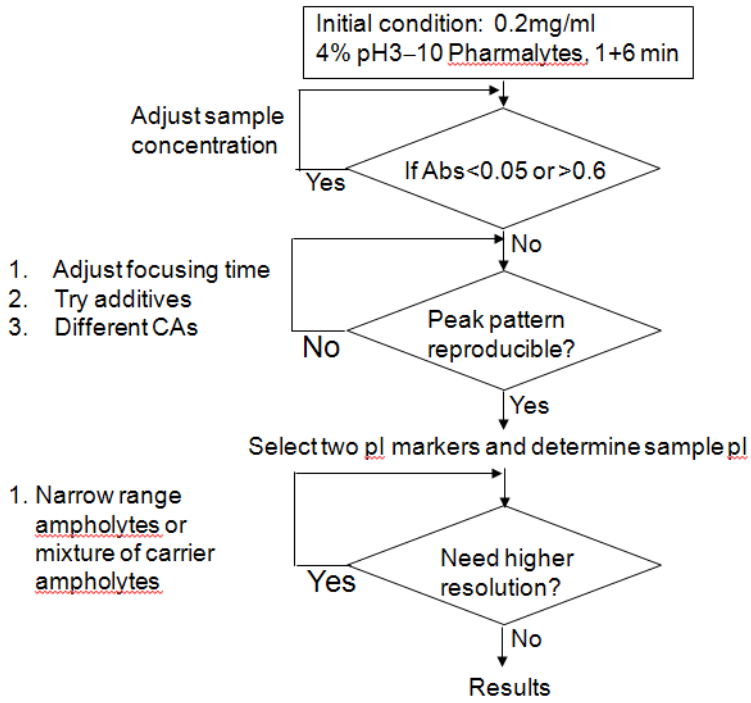

Figure 8-1: Method Development Flow Chart

Non-Reproducible Peak Patterns

The most important step in method development is the reproducibility of the peak pattern. There are two major causes of a non-reproducible peak pattern: sample aggregation and sample precipitation. It should be pointed out that the sample aggregation is induced by isoelectric focusing, and does not necessarily originate in the sample. More than 50% of non-antibody proteins may precipitate during isoelectric focusing. Some cases of non-reproducible peak patterns are still not fully understood and may be caused by protein interactions.

Sample Aggregation

Typical symptoms of sample aggregation are shown in Figure 8-2. When focusing begins, peak shapes are normal. Later in the focusing process, sharp spikes begin to appear. These spikes will never stabilize during the focusing process. However, part of the peak pattern other than the spikes may stabilize and become somewhat reproducible.

A 4 M urea additive solves a sample aggregation problem close to100% of the time. If adding this concentration of urea works, gradually reduce the urea concentration until the lowest concentration that can be used is found. In some cases, 0.5 M urea may be enough.

If urea can't be used in the assay, sucrose or glucose may also be used as additives. Start with a 5% solution of either initially, then increase to 10-25% as needed. It should be mentioned that sucrose and glucose do not work as well as urea, and will typically only resolve sample aggregation issues about 80% of the time.

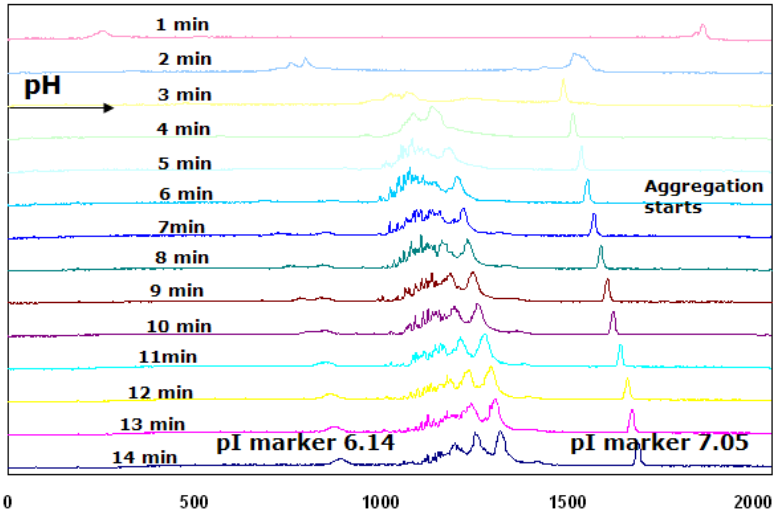

Figure 8-2: Sample Aggregation example. A mAb at 0.28 mg/mL in 4% Pharmalytes pH 5-8. 2 M urea used as an additive resolved the problem.

Sample Precipitation

The symptoms of sample precipitation differ from sample aggregation. If the sample precipitates during focusing, spikes will appear almost immediately as shown in Figure 8-3. The peak pattern will not be reproducible at all.

There are only a few possible solutions to eliminate sample precipitation. Adding 8 M urea to create a sample denaturing condition will resolve the problem 80% of the time. In rare cases, using a different vendor for the carrier ampholytes can also rectify the problem.

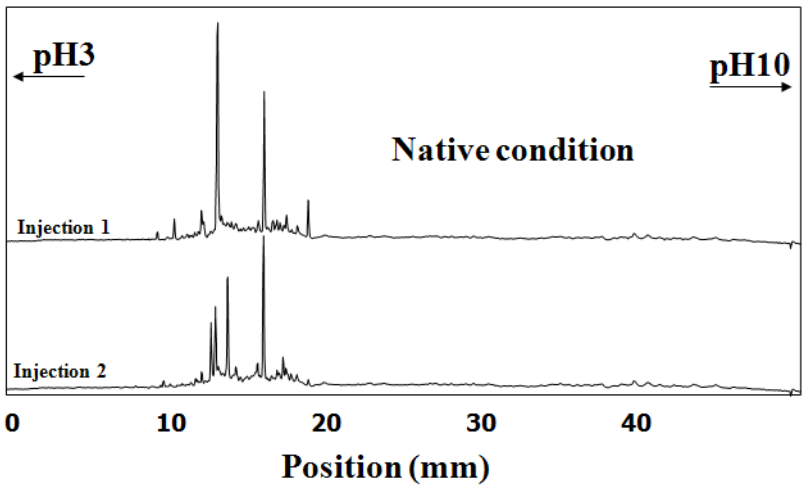

Figure 8-3: *Sample precipitation example. Soya protein extraction, 0.25 mg/mL in 4% pH 3-10 Pharmalytes, 5-minute focusing. 8 M urea used as an additive resolved the problem.*

Recommended Approach to Using Additives

Based on 15 years of experience with more than 800 protein samples, ProteinSimple has found that 95% of all non-reproducible peak pattern issues will be resolved by the following:

1. First, try 4 M urea to address aggregation.
    a. If 4 M urea works, try lower concentrations
    b. If urea is not an option, try sucrose (5-25%)
2. Denature proteins with 8 M urea - no need to try other concentrations

Summary of Additives used to Stabilize Proteins

- Prevention of aggregation and interaction of proteins:
    - Required with 15% of mAbs
    - <4 M urea
    - Sucrose or glucose (5%-25%)
- Denaturing sample conditions
    - Required for 50% of recombinant proteins
    - 8 M urea

- Reducing samples in a denatured condition (rare)
  - Applicable for non-soluble proteins such as membrane proteins
  - Proteins denatured using 8 M urea
  - Add DTT (<100 mM)

Note: If a gel IEF method exists for your sample, first try the additives used in this method.

Denaturing Samples

When sample denaturing is required, the following preparation should be used to ensure the final urea concentration in the sample is 8 M.

- The total sample volume should be 200 µL. This requires adding 96 mg of urea powder to 120 µL of liquid.
- **Example preparation:** Add 96 mg urea powder to a solution containing:
  - 22 µL of HPLC-grade deionized water
  - 70 µL of 1% methyl cellulose
  - 8 µL of carrier ampholytes (total)
  - 20 µL of sample

Enhancing Resolution

Once the peak pattern is reproducible, enhancement of peak resolution may be needed. In general, higher resolution is usually required.

Use of Narrow pH Range Carrier Ampholytes

An immediate way to enhance peak resolution is to use narrow pH range carrier ampholytes. When using these carrier ampholytes, always mix them with wide pH range carrier ampholytes or other narrow pH range carrier ampholytes. The mixture ratio of narrow to wide pH range carrier ampholytes can be anywhere from 1:1 to 5:1, with 3:1 being the most common.

When testing narrow pH range carrier ampholytes, the same anolytes and catholytes can be used. When these types of carrier ampholytes are used, the focusing time used should be substantially longer. Initially, try doubling the focusing time compared to what would be used with wide pH range ampholytes. Then adjust the focusing time as needed based on actual results.

Summary of narrow pH range carrier ampholytes usage:

- Use narrow pH CAs mixed with wide pH CAs
  - A 3:1 mixture ratio is commonly used
  - Provides pH gradient stability and focusing speed
- Use the same anolyte and catholyte
  - Simple electrolyte system
- Use a longer focusing time compared to wide pH range CAs
  - Double the focusing time for the first run

Try Carrier Ampholytes from a Different Vendor

Another way to enhance resolution is to try carrier ampholytes from a different vendor. For example, Servalyts have shown better resolution for most fusion proteins (see Figure 8-4) as well as heavily glycosylated proteins. For some monoclonal antibodies, Servalyts improve resolution for the acidic side of these peaks (see Figure 8-5).

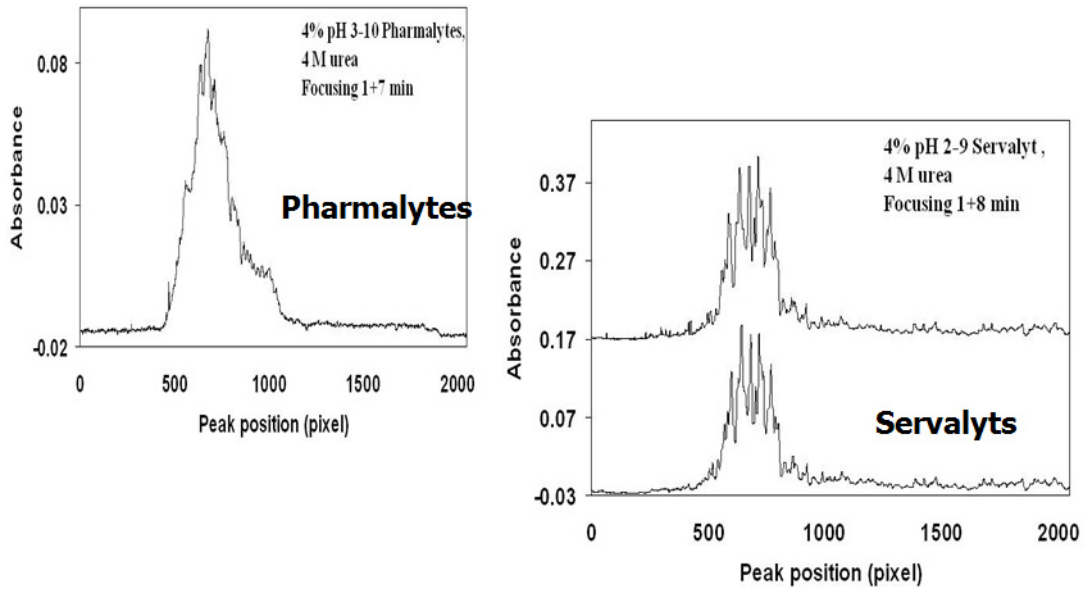

Figure 8-4: *Example of the resolution difference between Pharmalytes and Servalyts for a fusion protein.*

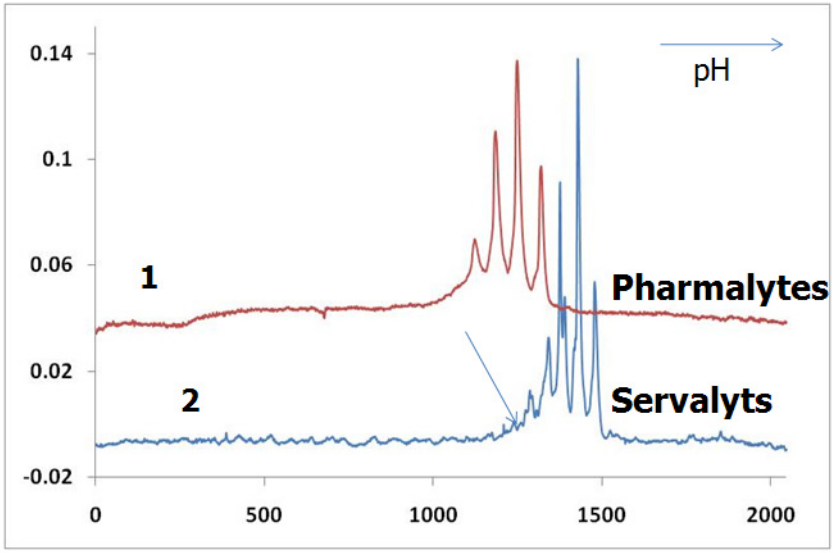

Figure 8-5: *Example of improved resolution at the acidic side of the sample peaks from an mAb using Servalyts.*

Chemical Interferences in cIEF

Some chemical interferences should be considered during cIEF method development.

High Sample Salt Concentration

The biggest interference in cIEF analysis is high salt concentration in samples. High salt can cause the focusing current to overheat the sample. As shown in Figure 8-6, the pH gradient created by the carrier ampholytes is compressed at higher salt concentrations, thus reducing resolution. Overheating also creates air bubbles, leading to spikes and baseline noise. This combination of overheating and pH gradient compression reduces the overall pH gradient stability.

When sample protein concentration is high enough, dilute samples by 10X in running buffer. This generally dilutes the final salt concentration to less than 15 mM. However, ProteinSimple recommends maintaining salt concentrations in final sample solutions at under 10 mM. For samples with lower protein concentrations, desalting may be necessary.

Effects of high salt concentration in samples:

- Samples overheat
- Resolution deteriorates due to compression of pH gradient
- Increased baseline noise (such as spikes)

- Reduces pH gradient stability

To eliminate salt effects:

- Ensure salt concentration in final sample solution is <15 mM (<10 mM recommended)
- Dilute samples at higher protein concentrations 10X with running buffer
- Desalt samples if protein concentration is low and dilution is not an option

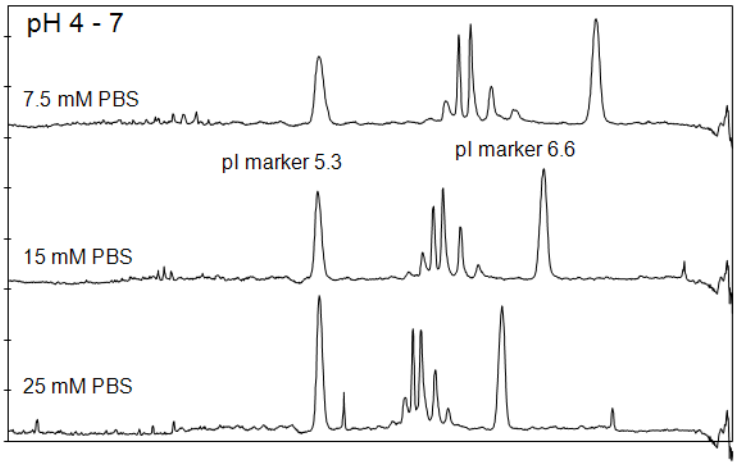

Figure 8-6: *pH gradient compression ad various salt concentrations.*

Other Sample Matrix Chemicals

Histidine is commonly used in antibody buffers, and focuses at approximately pI 7.5. Histidine has no UV absorption at 280 nm, and is therefore not detected as a peak. However, as shown in Figure 8-7, a dip in the baseline is observed at the histidine pI. If histidine is well resolved from sample protein peaks, this is not problematic. When the histidine pI is close to the pI of a sample peak, buffer exchange is necessary.

Some amino acids commonly used in protein buffers, such as arginine, may also be focused in cIEF. However, arginine focuses at the very basic end of the pH gradient and therefore may not interfere with most sample protein peaks.

Some carrier ampholytes may also cause baseline dips. For example, Pharmalytes pH 3-10 will quite reproducibly introduce a dip at approximately pI 6.9. In addition, some carrier ampholytes such as Servalyts cause noisier baselines (Figure 8-8) as discussed earlier. These noise effects can be diminished by using lower concentrations of Servalyts (0.5-2.0%).

Summary of sample matrix effects:

- Histidine
  - Produces a baseline dip around pI 7.5
  - If the dip is well separated from sample peaks, it will not interfere with results
  - If the dip does affect sample peaks, buffer exchange is necessary
- Other amino acids
  - Will focus at either end of the pH gradient and typically do not interfere with sample peaks
- Carrier ampholytes
  - Pharmalytes pH 3-10 will produce a dip around pI 6.9
  - Servalytes introduce baseline noise, use low concentrations (0.5%-2%) to reduce this

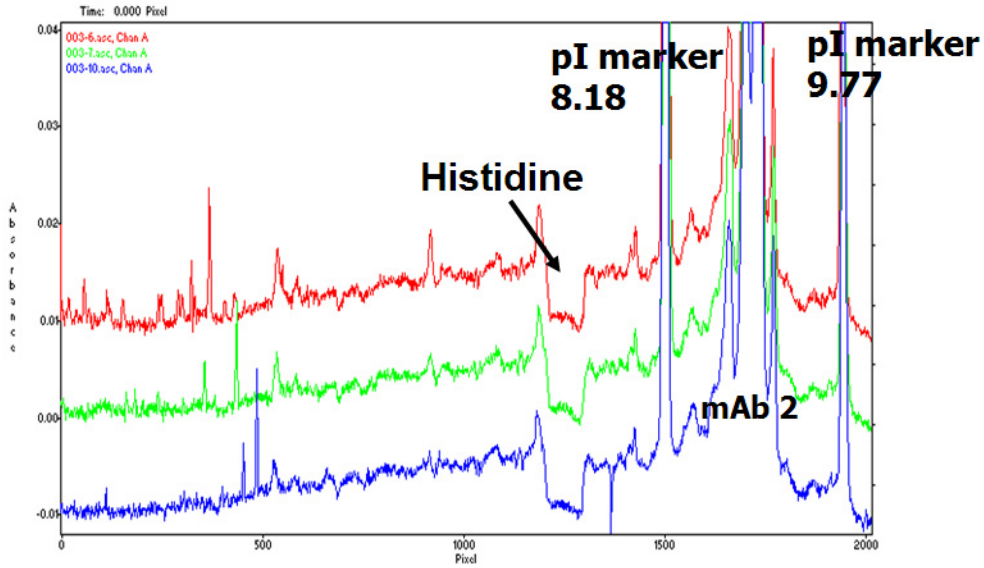

Figure 8-7: Baseline dip due to histidine.

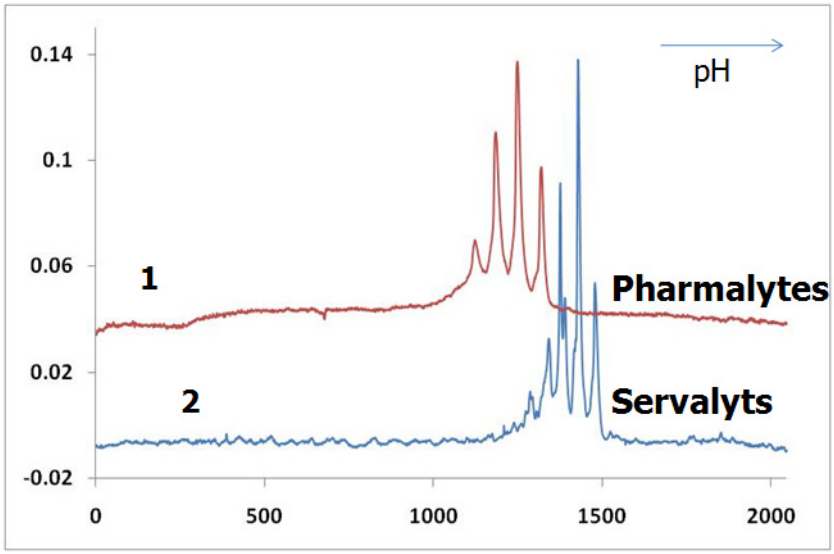

Figure 8-8: Baseline noise comparison between Servalyts and Pharmalytes.

Peak Identification and pI Calibration

Once a reproducible peak pattern is established with satisfactory resolution, pI calibration and peak identification are the next step in method development.

Use of pI Markers

Since cIEF is performed in free solutions, sample peaks will shift from run to run. Also as discussed before, salt in the sample can compress the pH gradient and the salt concentration between individual samples will vary in general. All of this can be well compensated with the use of two pI markers. Additionally with cIEF, the use of pI markers is the only way to calibrate pI values of sample peaks and identify peaks.

In Figure 8-9 (top), the absolute peak positions are not reproducible due to different salt concentrations, even thought the proteins in each sample are exactly the same. This peak shifting can be compensated by using two pI markers (Figure 8-9 bottom).

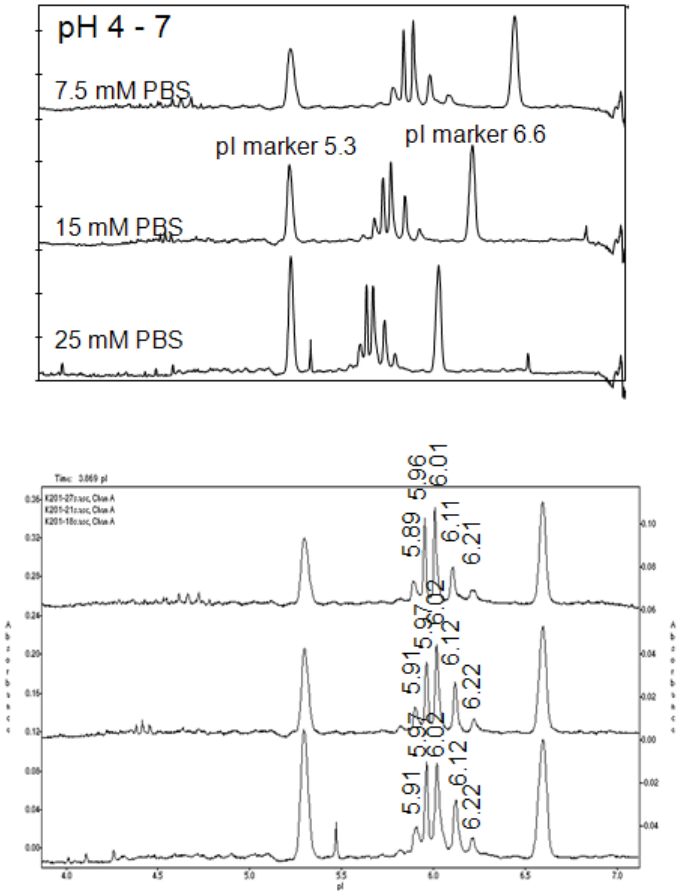

Figure 8-9: Peak pattern compressed by high salt concentration, even though sample proteins are identical (top). Salt effect compensated for by using pI markers (bottom).

Choosing pI Markers

Protein pI markers are available from all major reagent companies. However, there are many problems with commercial protein pI markers: they separate into multiple peaks in IEF, they sometimes interact with proteins in the samples, and their pI values can change in different matrices (for example in urea). Traditionally for slab gel IEF, protein pI markers must be used as protein markers and stay on the slab gel during the staining and destaining process. However, small molecules are washed away during this process.

With cIEF, it is possible to use small molecule pI markers. Their advantages include presentation of a single peak as well as high sensitivity at 280 nm (the detection wavelength for cIEF). Typically, their pI values do not vary across different matrices. ProteinSimple offers 23 small molecule pI markers ranging from pI 2.85 to pI 10.45.

Procedure for Peak Identification

Following is the procedural flow for peak identification when using two pI markers in cIEF assays.

1. Run any unknown samples without pI markers.
2. Estimate the sample peak pI values from their peak positions within the column.
3. Select two pI markers that bracket the sample peak pIs on either side.
4. Spike the pI markers into the sample. These two markers will be used as internal standards moving forward.
5. Run the sample (spiked with the two pI markers) again.
6. Sample peak pI values can now be determined using the two marker peaks with iCE CFR Software.
7. The determined pI values can be used to accurately identify sample peaks.

Chapter 9:

21 CFR Part 11 Compliance

Chapter Overview

- Using Electronic Signatures
- Audit Logs
- Administrator Features

Using Electronic Signatures

For labs that are required to be 21 CFR Part 11 compliant, iCE CFR Software provides an e-signature feature to ensure data integrity. If your administrator has turned this option on, users are required to enter their e-signature whenever system or data-related changes and events are executed. Events that require e-signatures are recorded in the audit log.

When an e-signature is required, the following window displays:

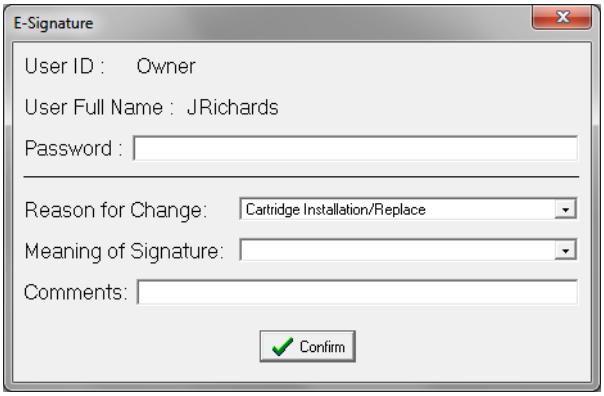

Figure 9-1: *E-signature window (cartridge installation e-signature shown).*

To enter your e-signature:

1. Enter your administrator supplied password in the **Password** box.
2. Optional. Click the down arrow in the **Meaning of Signature** box and select an option: **Approved for use**, **Approved** or **Acknowledged**.

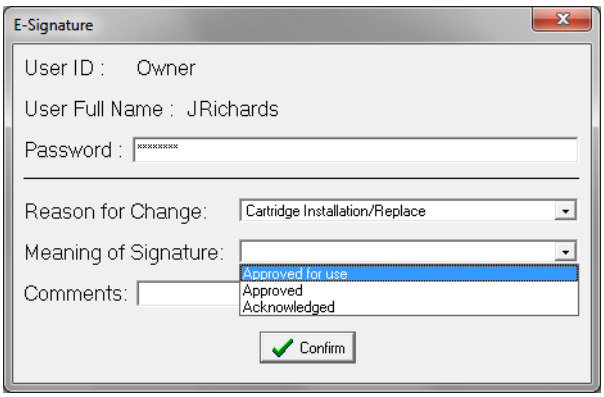

Figure 9-2: *Meaning of signature selection.*

3. Optional. Type any comments in the **Comments** box.

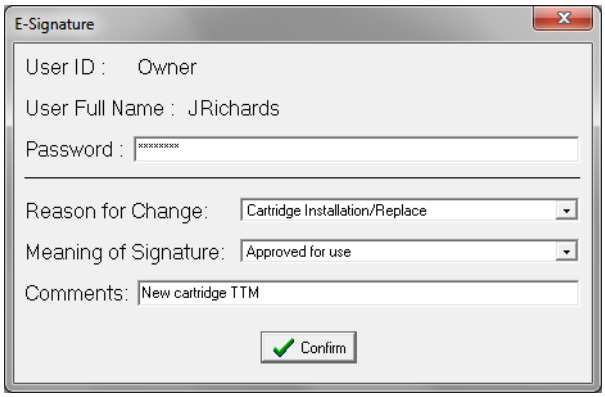

Figure 9-3: *Comments added.*

4. Click **Confirm**.

Audit Logs

To ensure system and data integrity, certain user and instrument events will create audit log entries. For instance, whenever a user logs in, processes batch data or accepts a sample time transfer measurement, an entry will be written to the audit log.

To view the audit log, select **Utility** from the main menu, then click **Security** and **Audit Logs**.

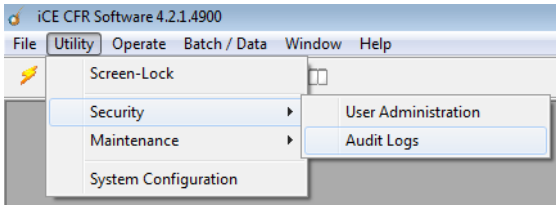

Figure 9-4: *Accessing audit logs.*

Three types of audit logs are available. To view a specific log, just click its radio button:

- **Instrument** - Shows an event log for the system hardware. For example, when cartridge calibration is performed or when an autosampler tray type is changed.

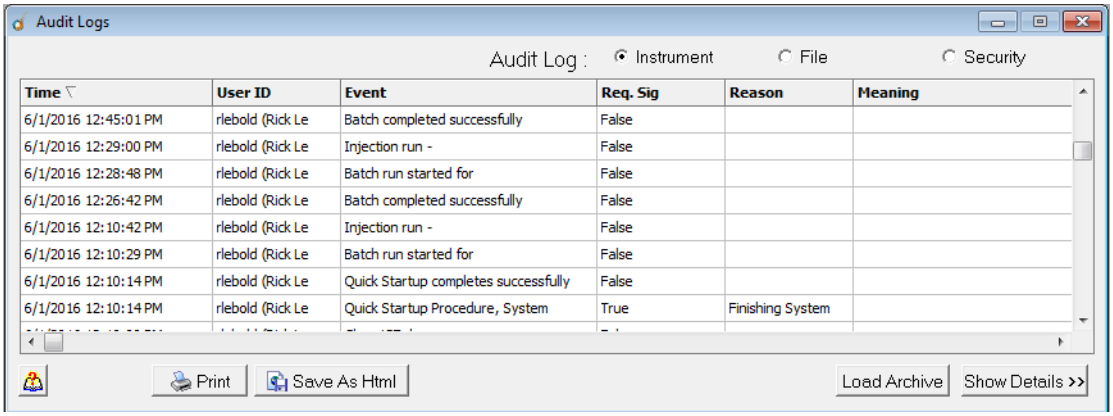

Figure 9-5: Instrument Audit Log

- **File** - Shows an event log for software files. For example, when a batch file is created or processed.

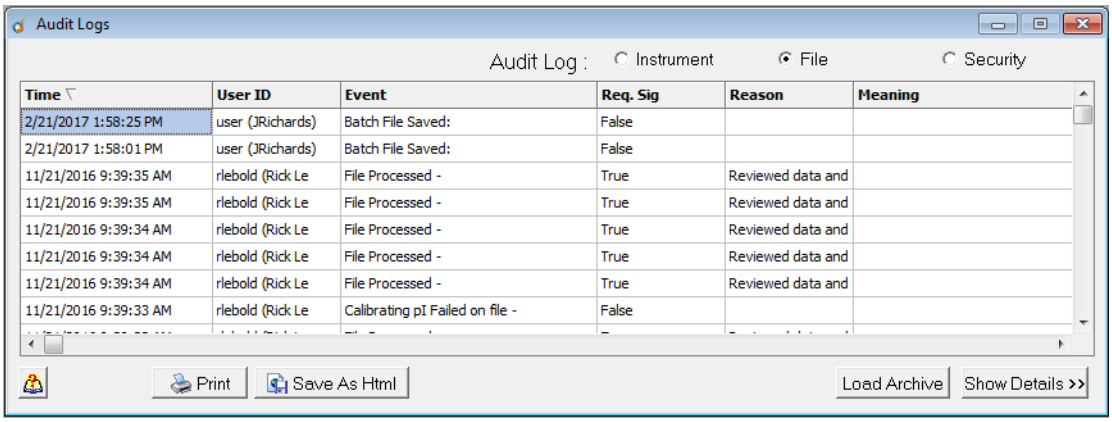

Figure 9-6: File Audit Log

- **Security** - Shows an event log of user administration events. For example, when a new user account is created, when the user type or status is modified, or when users login to iCE CFR Software.

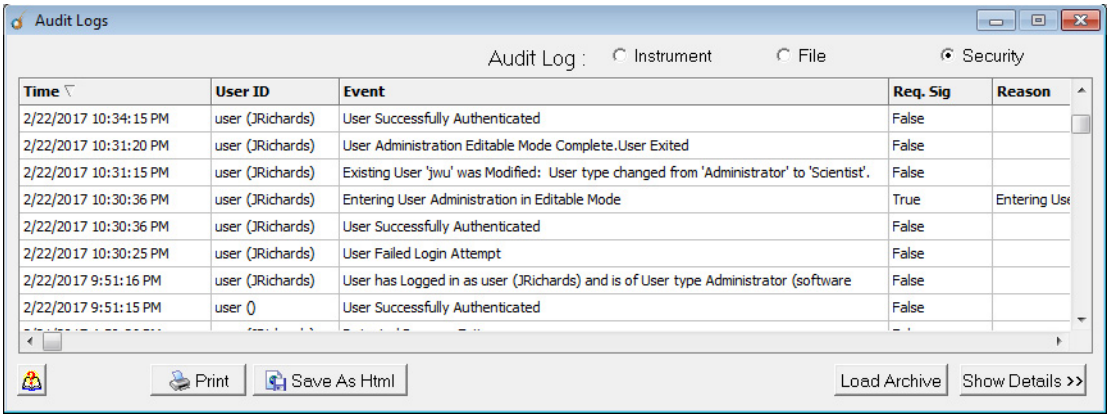

Figure 9-7: Security Audit Log

NOTE: The security audit log is only available to administrators.

Each column in the audit log contains the following event information:

- **Time** - Time and date the event was recorded
- **User ID** - User that was logged in at the time the event was recorded.
- **Event** - Description of the recorded event.
- **Req. Sig** - Describes if the event required an e-signature (true) or no e-signature required (false).
- **Reason** - Reason the e-signature was required.
- **Meaning** - Meaning of the e-signature. This is an optional selection for users in the e-signature window and will include one of three entries: approved for use, approved or acknowledged.
- **Comments** - Comments that were entered by the user in the e-signature window.

The following options are available in the audit log window:

- **Print** - Prints the audit log for the selection currently being viewed.
- **Load Archive** - Loads an archived audit log.
- **Show/Hide Details** - Shows or hides details of the currently selected event. To show details, select an event in the log table, then click **Show Details**:

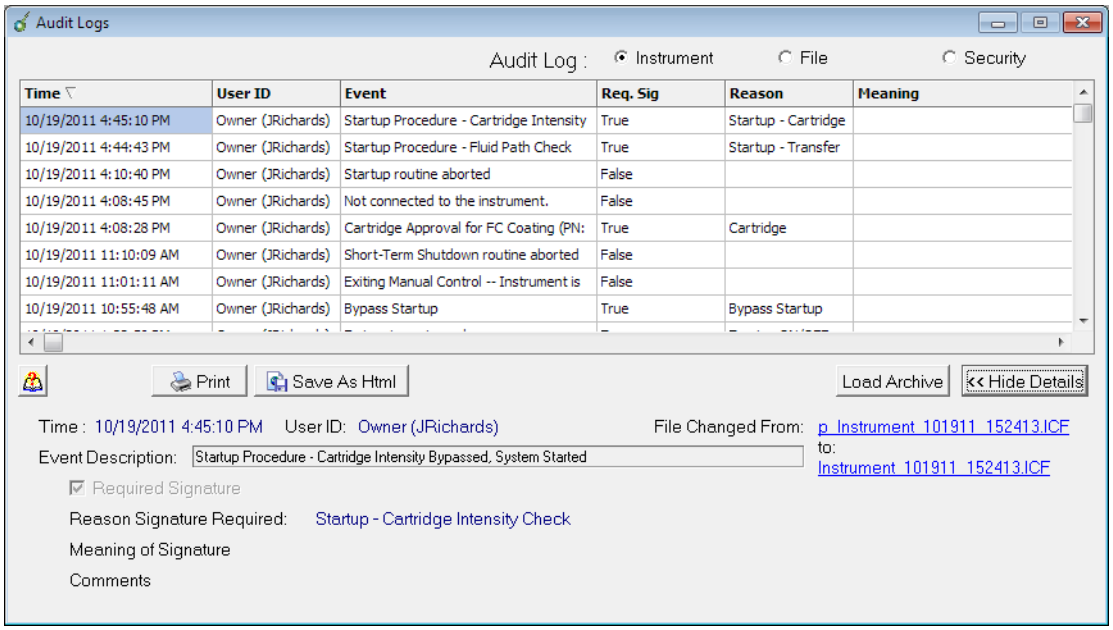

Figure 9-8: Audit Log event details shown.

Events that involved changes to .icf files will contain records of the files before and after changes were made. To hide event details, click **Hide Details**.

- **Column Sorting** - Clicking on a column header will allow you to sort events either alphabetically or chronologically depending on the information displayed.

Printing Audit Logs

Clicking **Print** lets you customize the Audit Log printout.

1. To use a custom logo or image, click **Change Picture** and select your image. Files must be 500 x 75 pixels and either in .bmp, .ico, .emf or .wmf format.
2. Customize the printout by selecting options in the Print Options box.
3. Select a time frame in the Print Range Options box.
4. Click **Print**.

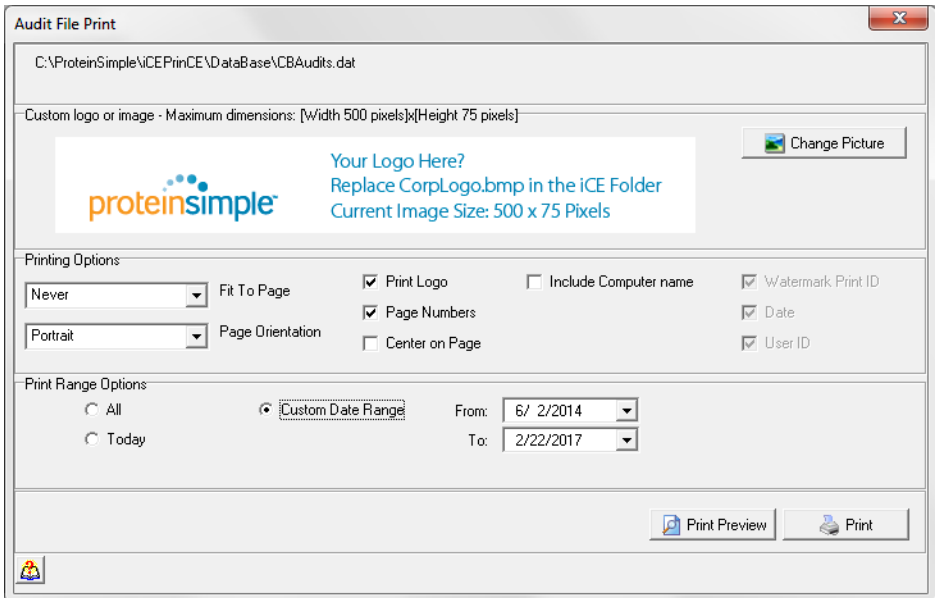

Figure 9-9: *Audit Log print options*

Administrator Features

Administrators can access iCE CFR security features to add or remove users, set user permissions or change global preferences. Both administrator and high administrator levels are available, only the high administrator has the following additional permissions:

- Perform new or initial iCE CFR Software installations
- Software reinstallation
- Software un-install
- Install software patches
- Permission to change the serial number of the iCE3 instrument or retrieve information from the autosampler in the system
- Can unlock users
- Modify privileges for all user types including administrators

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NOTE: High administrators are never locked out of the software.

To access administrator functions in the software:

1. Login to iCE CFR Software as an administrator.
2. Select **Utility** from the main menu, then click **Security** and **User Administration**.

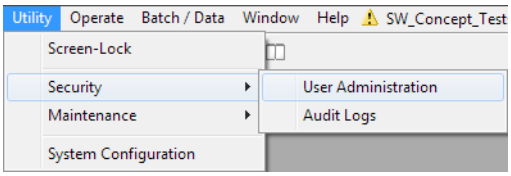


Figure 9-10: Accessing User Administration features.

3. The following message will display:

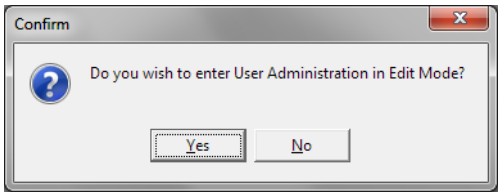


Figure 9-11: Selecting edit or read-only modes.

- Click **Yes** to enter edit mode and modify security features. Enter your e-signature and click **Confirm**.
- Click **No** to view security settings in read-only mode (no changes can be made).

After selecting a mode, the user administration screen will display:

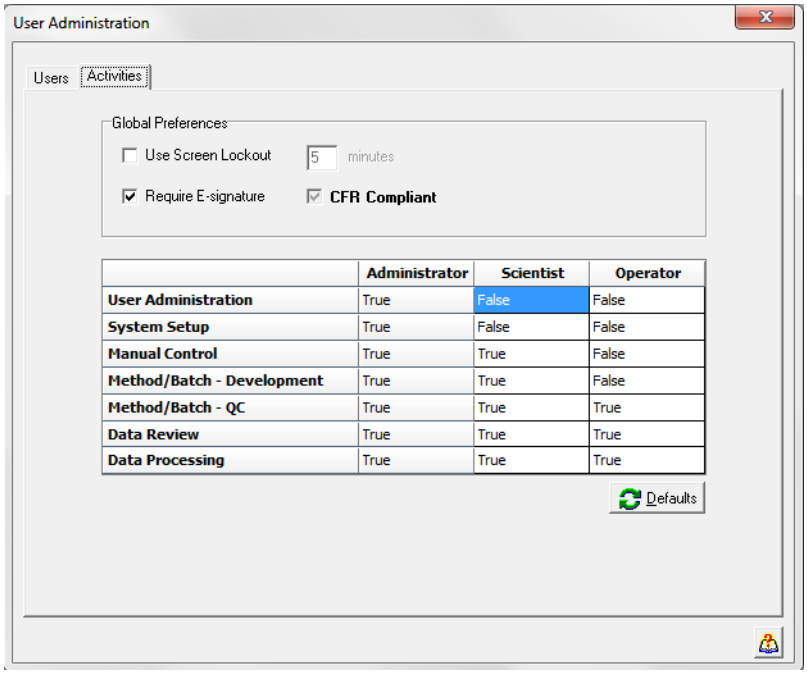


Figure 9-12: *User administration screen.*

NOTE: To switch between edit and read-only modes, you must first close the user administration screen and then re-access it.

User Administration

Click the **Users** tab to view a list of users currently able to log in to iCE CFR Software. Administrators can add, remove or modify existing users in this tab.

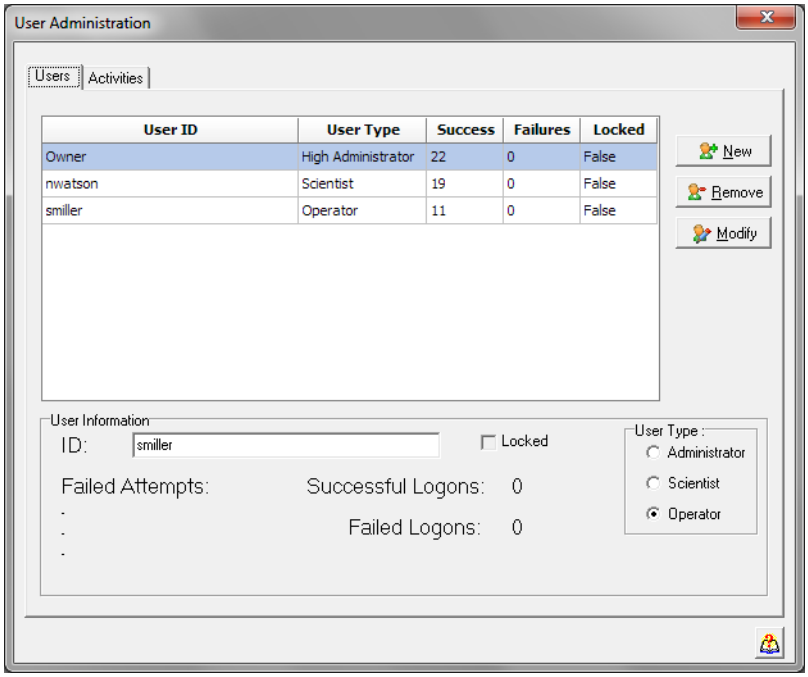


Figure 9-13: Users tab.

Adding a New User

Before adding a new user:

- Make sure the new user has a valid domain user account. This account must be verified by the domain controller for the user to login.
- Decide what access level the new user will need: Administrator, scientist or operator.
  - Administrator users should be added to the Windows local Administrators group.
  - Scientists and operator users should be put into the Windows local Users group as restricted users only.

To add a new user:

1. Select the **Users** tab.
2. Click **New User**.
3. Enter the **User ID** (the domain user account name).
4. Select a user type (access level) radio button: **Administrator**, **Scientist** or **Operator**.

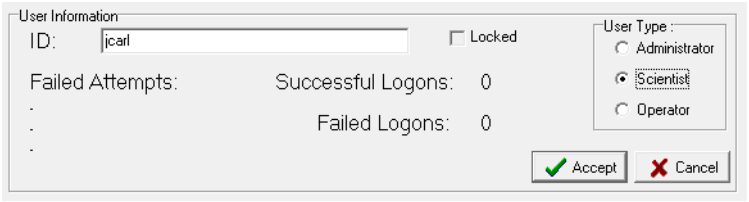


Figure 9-14: *Adding a new user.*

5. Click **Accept**. The new user will display in the table:

User ID	User Type	Success	Failures	Locked
Owner	High Administrator	22	0	False
nwatson	Scientist	19	0	False
smiller	Operator	11	0	False
jcarl	Scientist	0	0	False

Figure 9-15: *Adding a new user.*

NOTES:
Users can be added without having an actual account on the network. However, these users cannot login since they require proper server authentication.

Removing a User

1. Select the **Users** tab.
2. Click on the user in the table.
3. Click **Remove**.
4. Click **Accept**.

Modifying a User

This option allows administrators to change user types and lock or unlock users.

NOTES:
When a user has three failed login attempts their account will automatically lock, requiring the high administrator to unlock the account.

If the high administrator account name is changed, passwords for both the old and new accounts will be requested.

1. Select the **Users** tab.
2. Click on the user in the table.
3. Click **Modify**.
4. To lock a user, check the Locked box. Uncheck the box to unlock the user.
5. Select the user's access level in the User Type box.

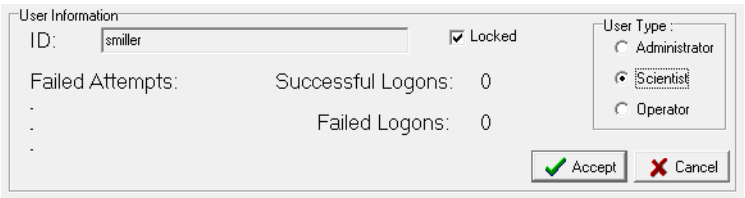


Figure 9-16: *User locked and type selection.*

6. Click **Accept**.

User Permissions and Global Preferences

Click the **Activities** tab to view a list of user types and current permissions. Administrators can set the software to lock during periods of inactivity, e-signature preferences and change user permissions in this tab:

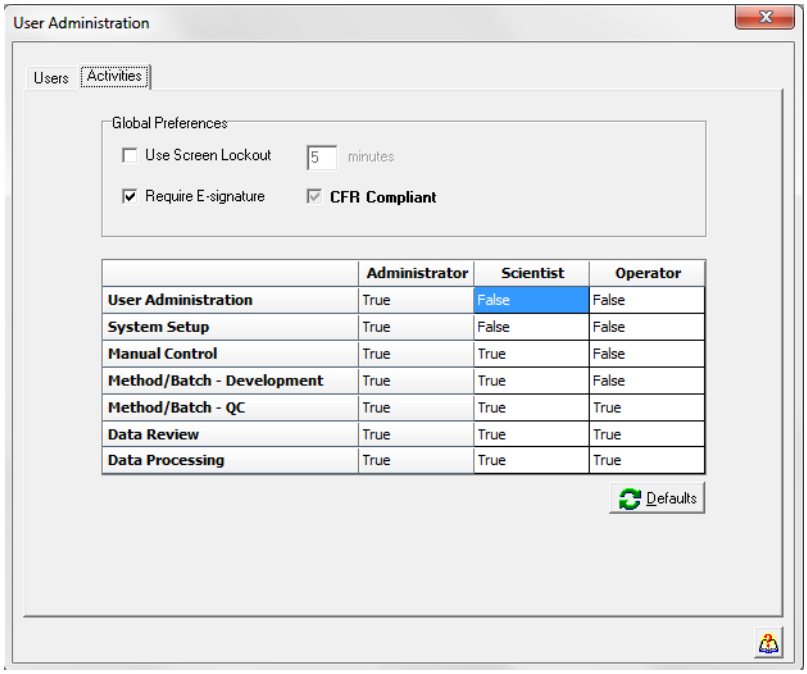


Figure 9-17: Activities tab.

Setting a Software Inactivity Lock

When this option is selected, the iCE CFR Software will automatically lock the software after a set period of inactivity. Users must login again to re-access the software. To set an inactivity lock:

1. Select the **Activities** tab.
2. Check the **Use Screen Lockout** box.
3. Enter **time** in minutes.

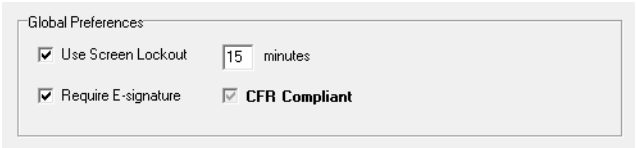


Figure 9-18: Setting inactivity lock.

Turning E-Signatures On and Off

Administrators can opt to require e-signatures in order to comply with 21 CFR Part 11 requirements. When this option is selected, the iCE CFR Software automatically prompts users for their e-signature to acknowledge system or data related changes or events to ensure data integrity. For information on using e-signatures see "Using Electronic Signatures" on page 206.

- **When e-signatures are off:**
  - iCE CFR Software **is not** 21 CFR Part 11 compliant.
  - Audit logs are inactive and cannot be viewed.
  - Information in the user administration tabs is read-only and cannot be changed.
- **When e-signatures are on:**
  - iCE CFR Software **is** 21-CFR Part 11 compliant.
  - Audit logs are active and accessible.
  - Information in the user administration tabs can be changed by administrators.

To turn e-signatures on or off:

1. Select the **Activities** tab.
2. Check the **Require E-Signatures** box to turn the option on or off:

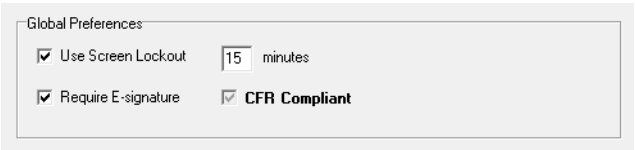


Figure 9-19: *Selecting e-signatures (option on).*

3. Enter your e-signature and click **Confirm**.
   - **If e-signatures are turned off** - The **Require E-Signatures** text will be highlighted, and the CFR Compliant checkbox is deselected automatically.

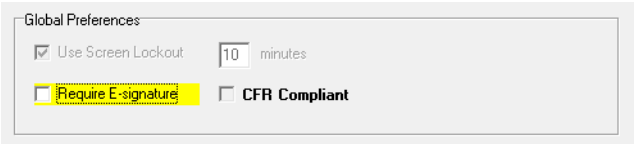


Figure 9-20: *E-signatures off.*

   - **If e-signatures are turned on** - The CFR Compliant checkbox is selected automatically. To verify data files, iCE CFR Software will require that the new cartridge installation/calibration and startup routines be performed prior to running a batch.

Figure 9-21: *E-signatures on.*

Changing User Permissions

Administrators can modify user permissions based on user type. Permission options are as follows:

Permission	Description
User Administration	Permission to access and edit user security administration (user administration screen tabs).
System Setup	Permission to modify the System Configuration (accessed from the Utility menu).
Manual Control	Permission to access major functions in the Maintenance menu (accessed from the Utility menu).
Method Batch - Development	Permission to access, create and edit Development batches.
Method Batch - QC	Permission to access, create and edit QC batches.
Data Review	Permission to review batch data.
Data Processing	Permission to process (calibrate) data.

Table 9-1: *Permission options for user types.*

To change user type permissions:

1. Click in a cell in the user type column for the permission you want to change:

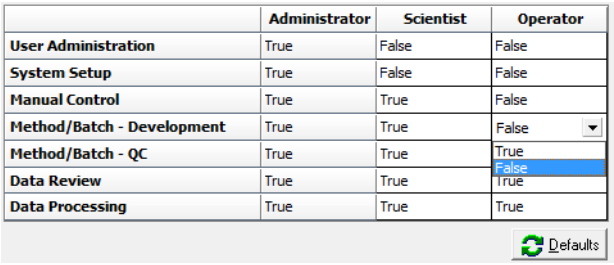


Figure 9-22: Changing permissions.

2. Click **True** to apply permissions or **False** to remove permissions for all users assigned to the user type selected.
3. Click **Defaults** to apply default iCE CFR Software permissions.

Chapter 10:

Maintenance and Troubleshooting

Chapter Overview

- Cartridge Handling and Care
- Shutdown Procedures
- Maintenance
- Troubleshooting
- Frequently Asked Questions
- Accessories and Spare Parts

Cartridge Handling and Care

Critical cIEF Cartridge Guidelines

- The surface of the cIEF Cartridge should be free from spills and particulate contamination. This ensures light will pass through the cartridge's optical cell properly.
- Lift off the cardboard cover and then remove the protective film and tank caps prior to using the cartridge.

Note: Replace the cardboard cover to protect the remaining cartridge.

- When handling the cIEF Cartridge, grasp it at either electrolyte tank only (Figure 10-1). Avoid touching other surfaces of the cartridge.

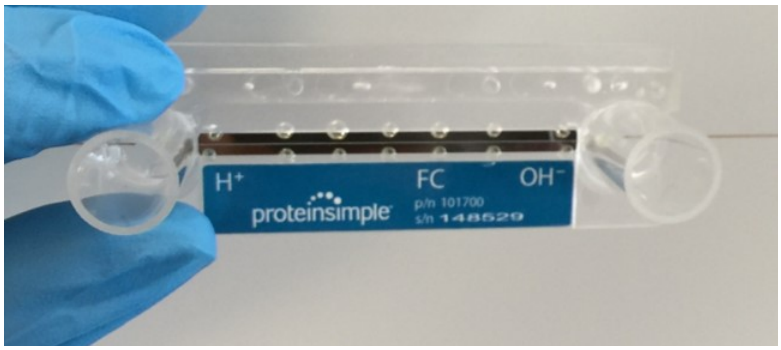


Figure 10-1: *Proper cIEF Cartridge handling.*

- Whenever a cIEF Cartridge is removed from the instrument, the electrolyte tanks should be rinsed thoroughly with HPLC-grade deionized water and allowed to dry.
- Cartridges can be stored for future use when they are still in good working condition. If cIEF Cartridges are not properly stored, any residue left in the separation column may block the capillary. Please refer to"Long Term Shutdown" on page 231 for more information.

FC Cartridge Guidelines

The FC-coated cIEF cartridge was developed and intended for use with the iCE280 and iCE3 systems. P/N 101701 contains two FC Cartridges cartridges (PN 101700). Each cartridge is individually tested and shipped with a Certificate of Analysis. The cartridges are shipped dry and should be stored free of liquid. When properly used and maintained, a single cartridge should have a lifetime of at least one hundred sample injections. Instructions and guidance for proper cartridge maintenance follow in the next sections.

Cartridge Storage

Upon receipt, store at room temperature.

Cartridge Care

The FC Cartridge must be used, washed and stored appropriately for maximum lifetime. The cartridge is designed for use with common cIEF reagents such as methyl cellulose, ampholytes, urea, anolyte and catholyte, but over exposure or high concentrations of certain components can harm the cartridge. The FC Cartridge requires the use of 0.35% methyl cellulose in the sample mixture.

Short Term Storage

The cartridge can be damaged due to over exposure to the acidic anolyte and basic catholyte reagents. Do not leave anolyte or catholyte in the cartridge tanks longer than overnight when the iCE3 system is idle. If you will be running the iCE3 system again within 7 days, either remove the cartridge and perform the Cartridge Wash Procedure on page 226 or simply use a transfer pipette to remove the anolyte and catholyte from the cartridge tanks, rinse the tanks with water three times and leave them filled with water.

Long Term Storage

If you will not be running the iCE3 system for more than 7 days it's imperative to rinse the methyl cellulose out of the cartridge by flushing first with water and then drying with air to remove all liquid. This can be done by performing the "more than 7 day shutdown" followed by the Cartridge Wash Procedure on page 226.

Sample Components

- **Methyl cellulose (MC):** The sample mix must contain 0.35% methyl cellulose. The cartridge must be flushed with 0.5% methyl cellulose between runs.
- **Solvents:** The cartridge is not compatible with solvents. Do not rinse with solvents and minimize the amount of solvent in the sample mix.
- **Salt and Surfactants:** High current can harm the internal coating in the cartridge capillary. High concentrations of salt and surfactants in the sample mix can generate high currents above 40 microamps. This high current will compress the pH gradient and also damage the cartridge. Please take care to minimize the concentration of salts and surfactants in the final sample mix to below 15 mM.

Cleaning the Outside of the Cartridge

If spikes are observed in data, the outside of the cartridge can be carefully cleaned with canned air. However, special care must be taken in order to not damage the cartridge components — specifically the metallic masking. Residue- and moisture-free canned air must be used to prevent fouling of the optical path through the separation capillary.

To clean the cartridge with canned air, first place the can's nozzle, or tube opening, 10 to 12 inches away from the capillary cartridge's top surface, then depress the aerosol actuator down halfway to generate a gentle air flow. Sweep the air stream across the entire space between the electrolyte tanks. Next, flip the capillary cartridge over and blow air on the back surface using the same technique. Finally, flip the cartridge over again and gently clean the top surface one last time before reinstalling in the iCE3.

HT Cartridge Guidelines

The HT cIEF Cartridge was developed and validated for use with the iCE3 system with the updated electrode assembly and iCE CFR Software version 4.0 and higher. P/N P-0000035-00 contains two HT cIEF cartridges (PN A-0000241-00). Each cartridge is individually tested and shipped with a Certificate of Analysis. The cartridges are shipped dry and should be stored free of liquid. When properly used and maintained, a single cartridge should have a lifetime of at least one hundred sample injections. The low sample viscosity used with the HT Cartridge makes control of hydrodynamic flow critical for resolution and reproducibility. The iCE3 has a closed fluid path which eliminates hydrodynamic flow. Instructions and guidance for proper cartridge maintenance follow in the next sections.

Cartridge Storage

Upon receipt, store at room temperature.

Cartridge Care

The HT Cartridge must be used, washed and stored appropriately for maximum lifetime. The cartridge is designed for use with common cIEF reagents such as methyl cellulose, ampholytes, urea, anolyte and catholyte, but over exposure or high concentrations of certain components can harm the cartridge. The HT Cartridge does not require the use of 0.35% methyl cellulose in the sample mixture, but for best results we recommended rinsing the cartridge with 0.5% methyl cellulose.

Short Term Storage

The HT Cartridge is designed for rapid, continual analysis but it can be damaged due to over exposure to the acidic anolyte and basic catholyte reagents. Do not leave anolyte or catholyte in the cartridge tanks longer than 4 hours when the iCE3 is idle. If you will be running the iCE3 system again within 7 days, either remove the cartridge and perform the Cartridge Wash Procedure on page 226 or simply use a transfer pipette to remove the anolyte and catholyte from the cartridge tanks, rinse the tanks with water three times and leave them filled with water.

Long Term Storage

The HT Cartridge is designed for rapid, continual analysis and it can be damaged due to overexposure to the acidic anolyte and the basic catholyte reagents. Do not leave the anolyte or catholyte in the cartridge tanks longer than 4 hours when the iCE3 is idle. If you will not be running the iCE3 system for more than 7 days it's imperative to rinse the methyl cellulose out of the cartridge by flushing first with water and then drying with air to remove all liquid from the capillary. This can be achieved by performing the "more than 7 day shut-down" followed by the Cartridge Wash Procedure on page 226.

Sample Components

- **Methyl cellulose (MC):**
  - **Sample Mix:** For maximum analysis speed on the HT Cartridge, simply replace the volume of the methyl cellulose solution in the sample mix with DI water. The concentration of the remaining components will remain unchanged. Methyl cellulose in the sample mix is safe to use with the HT Cartridge but there will be no reduction in analysis time.
  - **Rinse Solution:** For the best reproducibility and consistency, we highly suggest flushing with 0.5% methyl cellulose between runs.
- **Solvents:** The cartridge is not compatible with solvents. Do not rinse with solvents and minimize the amount of solvent in the sample mix.
- **Salt and Surfactants:** High current can harm the internal coating in the cartridge capillary. High concentrations of salt and surfactants in the sample mix can generate high currents above 40 micro-amps. This high current will compress the pH gradient and also damage the cartridge. Please take care to minimize the concentration of salts and surfactants in the final sample mix to below 15 mM.

Cleaning the Outside of the Cartridge

If spikes are observed in data, the outside of the cartridge can be carefully cleaned with canned air. However, special care must be taken in order to not damage the cartridge components- specifically the metallic masking. Residue- and moisture-free canned air must be used to prevent fouling of the optical path through the separation capillary.

To clean the cartridge with canned air, first place the can's nozzle, or tube opening, 10 to 12 inches away from the capillary cartridge's top surface, then depress the aerosol actuator down halfway to generate a gentle air flow. Sweep the air stream across the entire space between the electrolyte tanks. Next, flip the capillary cartridge over and blow air on the back surface using the same technique. Finally, flip the cartridge over again and gently clean the top surface one last time before reinstalling on the iCE3.

Cartridge Wash Procedure

NOTE: With iCE CFR Software version 5.0.0, you can choose to wash the cartridge using either a manual or an automated process.

Manual Cartridge Wash

Locate the following items that were provided with the autosampler accessory kit (Figure 10-2):

NOTE: If you need to replace these items you can order a Cartridge Wash Kit (P/N 045-207). This will also come with the cartridge wash procedure.

- Purge Kit (P/N 045-098) Includes:
- 5 mL disposable syringe with luer lock
- Adapter, female luer to tapered thread
- Purge line
- Cartridge inlet sleeve fitting (P/N 045-070)

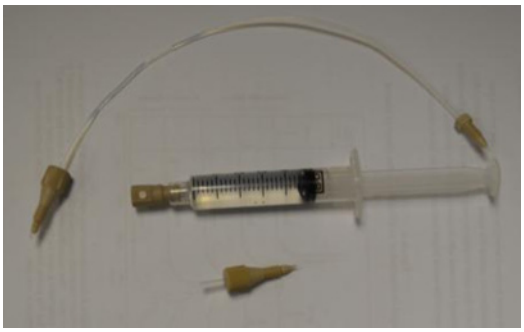


Figure 10-2: *Parts needed for cIEF Cartridge cleaning.*

1. Disconnect the cIEF cartridge from port 2 of the iCE valve.
2. Aspirate the anolyte and catholyte solutions in the cIEF cartridge tanks, or empty the contents into a waste container.
3. Wash each tank with HPLC-grade deionized water and aspirate. Repeat 2 more times for a total of 3 washes.
4. Add HPLC-grade deionized water to each electrolyte tank so both are filled approximately half way.

5. Attach the luer adapter to the end of the syringe.
6. Aspirate 1 mL of air into the syringe. Then with the air in the syringe, aspirate 2 mL of HPLC-grade deionized water.
7. Loosely attach the cartridge inlet sleeve fitting to the luer adapter.
8. Thread the cartridge inlet capillary (right side) through the cartridge inlet sleeve until it reaches a hard stop.
9. While holding the capillary at its stopped position, tighten the fitting finger-tight. Gently tug on the capillary to make sure its secured in place.
10. Hold the syringe vertically with the tip facing up. Depress the plunger until it reaches the 2 mL mark and hold it in this position. This applies the pressure needed to push the water through the cartridge.
11. Watch for droplets at the cartridge outlet capillary. Continue to apply pressure until 5 large droplets have exited the capillary.
12. Release the syringe plunger.
13. Dispense the remaining water and aspirate 3 mL of air.
14. Reattach the syringe to the luer adapter.
15. Gently depress the plunger to apply the pressure needed to push the air into the cartridge (Figure 10-3). Droplets will exit the cartridge outlet capillary. Continue to apply pressure until the water stops exiting the capillary. Refill the syringe with air if necessary.

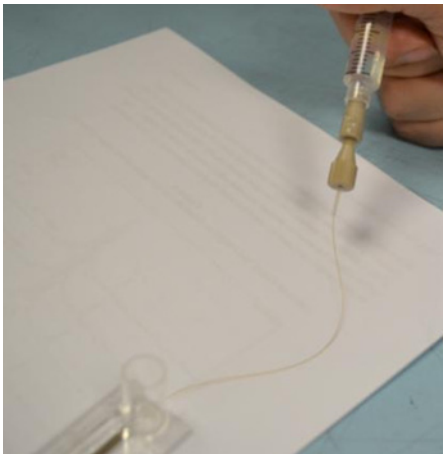


Figure 10-3: *Syringe connected to cartridge.*

16. Loosen the cartridge inlet sleeve fitting and carefully remove the capillary.
17. Aspirate the water in the cartridge tanks or empty the tank contents into a waste container.

18. Store the cIEF Cartridge in its original shipping container at room temperature.

Automated Cartridge Wash

In iCE CFR Software, select **Utility** from the main menu, click **Maintenance** and then **Cartridge Wash**. The cartridge wash control screen will display (Figure 10-4). Follow the steps on the screen.

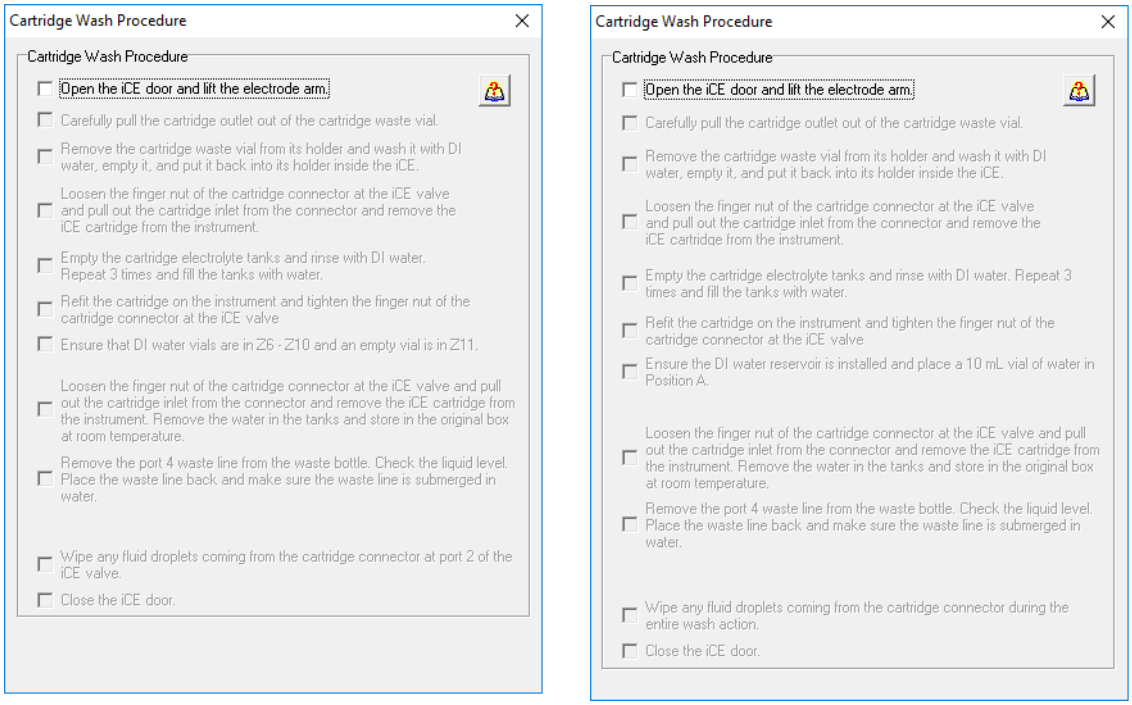


Figure 10-4: *Automated Cartridge Wash control screen for PrinCE Next (left) and Alcott 720NV (right).*

Shutdown Procedures

ProteinSimple recommends that you perform a shutdown procedure after operating the system. This will make sure the system is left in a safe state and prevents any damage to the cartridge and system.

If you will be running the system within the next 7 days, perform a short term shutdown using the procedure that follows. If you will not use the system within the next 7 days or you are unsure when you will use it next, perform the Long Term Shutdown on page 231. To maximize cartridge lifetime, we recommend the cartridge be removed from the system, washed and stored dry using the Cartridge Wash Procedure on page 226.

NOTE: For iCE3 systems with PrinCE Next autosamplers, the system should be left with the transfer line stored in water and the end of the transfer line in a water vial (Z6). If you open the autosampler door to remove reagent and sample vials, you may inadvertently leave the transfer line exposed to air and it will likely result in a blockage of the transfer line. To prevent a blockage from occurring, please go to Manual Control and manually move the transfer line back to a water vial (Z6).

Short Term Shutdown

The short term shutdown will leave the cartridge in methyl cellulose and rinse the transfer line and waste line with water. If the cartridge is allowed to dry out, the methyl cellulose will dry out and cause a blockage in the cartridge.

NOTE: Please make sure to check the water level in the cartridge waste vial and the electrolyte tanks after 3 days and add more water as needed.

1. In iCE CFR Software, select **Operate** from the main menu, then click **Shutdown** and **Less than 7 days**. The short term protocol checklist will display (Figure 10-5):

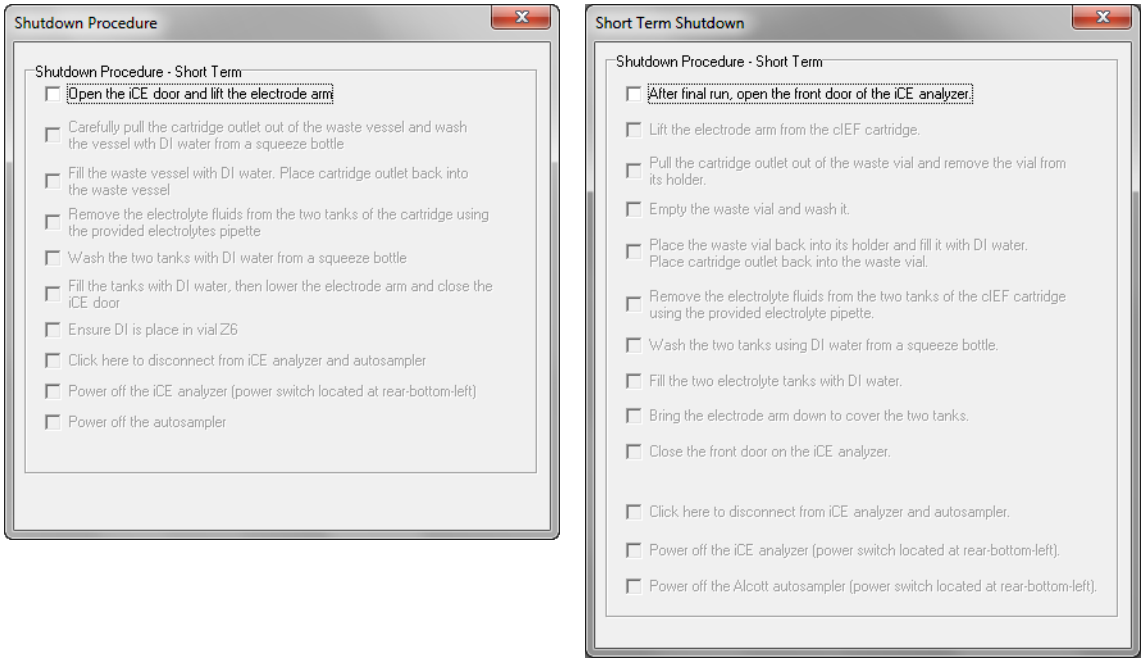


Figure 10-5: *Short term shutdown protocol in iCE CFR Software for PrinCE Next (left) and Alcott 720NV (right).*

NOTES:
Before starting the procedure, for the PrinCE Next autosampler make sure make sure a full vial containing DI water is in position Z6. For the Alcott 720NV autosampler, make sure the water reservoir is connected and the fluid level is adequate.

NOTE: The software will step you through the procedure, but software screens may differ depending on the version of iCE CFR software and the autosampler you are using. Click the checkbox as you complete each step to proceed to the next step.

---

2. Perform the steps in the screen as described in the software:
   a. Open the iCE3 system door and lift the electrode arm.
   b. Carefully remove the cIEF cartridge capillary outlet out of the waste vial.
   c. Remove the waste vial and empty its contents. Rinse the vial with HPLC-grade deionized water.
   d. Fill the waste vial with water then reinstall it in the instrument. Insert the cIEF cartridge capillary outlet back into the waste vial.

e. Aspirate the anolyte and catholyte solutions in the cIEF cartridge tanks using the provided electrolyte pipette.

f. Wash each electrolyte tank with HPLC-grade deionized water and aspirate. Repeat 2 more times for a total of at least 3 washes.

g. Fill each tank with HPLC-grade deionized water. Lower the electrode arm and close the system door.

h. Wait for the autosampler to finish washing the transfer line.

i. Disconnect the iCE3 instrument and autosampler.

j. Power off the iCE3 instrument and autosampler.

k. Check the system after 3 days and add additional water to both the electrolyte tanks and the cartridge waste vial as needed to keep the cartridge from drying out. The cartridge is stored in methyl cellulose at the end of the short term shutdown, so if it is exposed to air the methyl cellulose will dry out and cause a blockage in the cartridge.

Long Term Shutdown

ProteinSimple recommends that users perform long term shutdown procedures whenever the iCE3 system will not be used for more than a 7-day period (one week). These procedures leave the system and cartridge in a safe state. The long term shutdown procedure has been modified in iCE CFR software version 4.1and higher to provide additional water rinses of the valve and to remove the drying step of the transfer line to minimize residual methyl cellulose.

Long Term Shutdown iCE CFR Software Versions 3.0 and 4.0

1. In iCE CFR Software, select **Operate** from the main menu, then click **Shutdown** and **More than 7 days**. The long term protocol checklist will display (Figure 10-6):

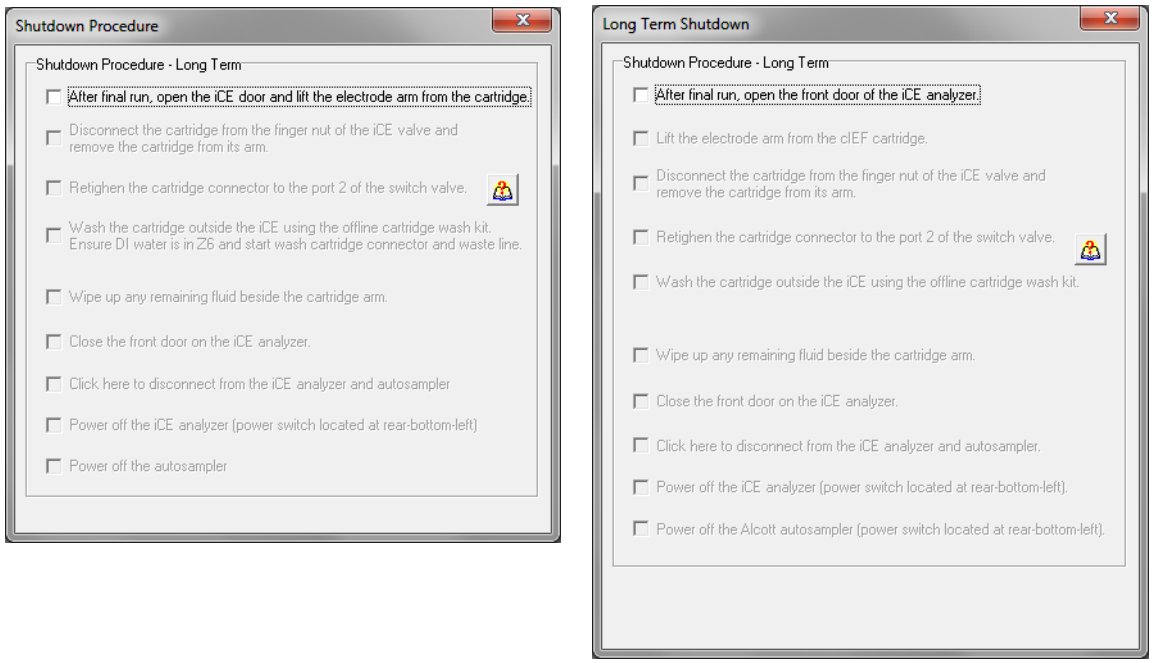


Figure 10-6: *Long term shutdown protocol in iCE CFR Software for PrinCE Next (left) and Alcott 720NV (right) in versions 3.0 and 4.0.*

NOTES:
Before starting the procedure, for the PrinCE Next autosampler make sure make sure a vial containing DI water is in position Z6. For the Alcott 720NV autosampler, make sure the water reservoir is connected and the fluid level is adequate.

NOTE: The software will step you through the procedure. Click the checkbox as you complete each step to proceed to the next step.

---

2. Perform the steps in the screen as described in the software:
   a. Open the iCE3 door and lift the electrode arm.
   b. Disconnect the cIEF Cartridge from the iCE valve and remove the outlet capillary from the waste vial. Remove the cartridge from the holder.
   c. Wash the cartridge with the Cartridge Wash Procedure on page 226. The cartridge must be washed with water to remove the methyl cellulose and electrolytes and then dried with air and stored dry to maximize cartridge lifetime.

d. Wait until the autosampler finishes washing the transfer and waste lines and the cartridge connector.

e. Disconnect the iCE3 instrument and autosampler.

f. Power off the iCE3 instrument and autosampler.

Long Term Shutdown iCE CFR Software Versions 4.1, 4.2 and 4.3

1. In iCE CFR Software, select **Operate** from the main menu, then click **Shutdown** and **More than 7 days**. The long term protocol checklist will display (Figure 10-6):

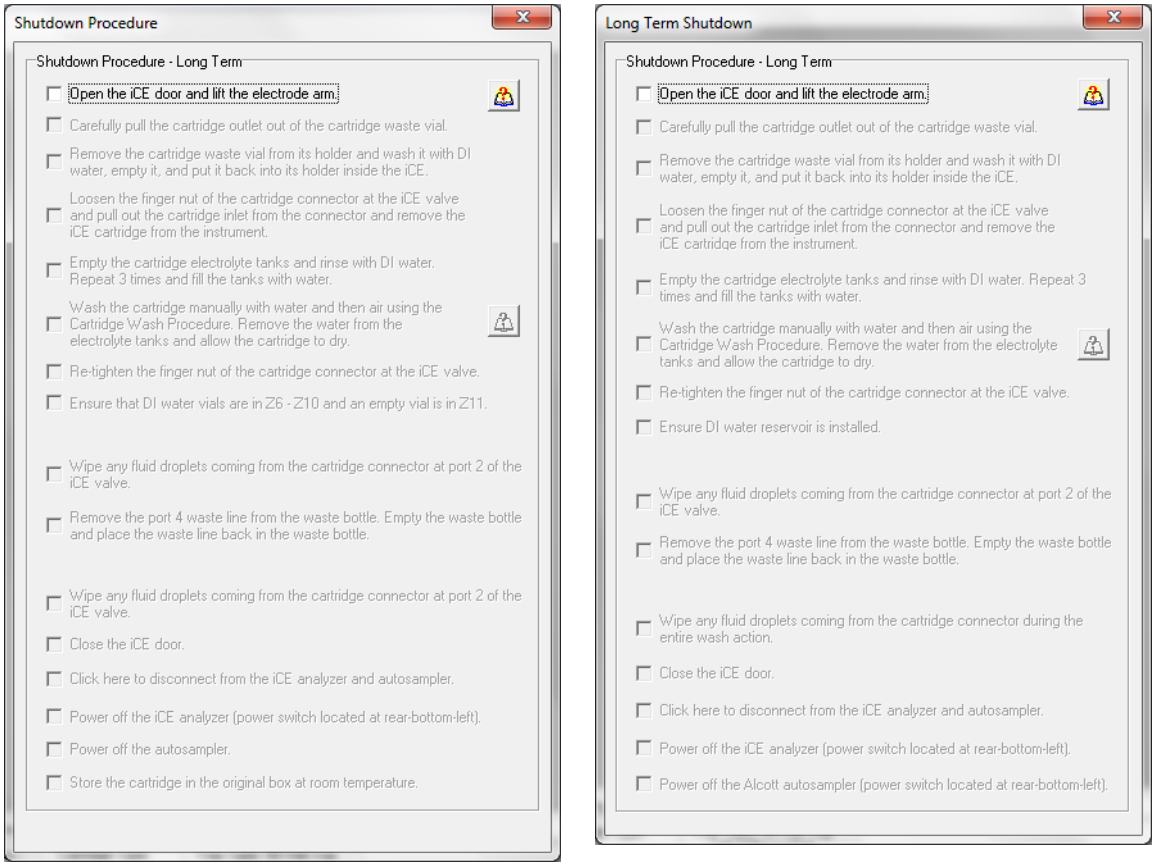


Figure 10-7: *Long term shutdown protocol in iCE CFR Software for PrinCE Next (left) and Alcott 720NV (right) in version 4.x.*

2. Perform the steps in the screen as described in the software:

   a. Open the iCE3 door and lift the electrode arm.

b. Remove the outlet capillary from the waste vial, remove the cartridge waste vial and wash it with water and replace back in the instrument.

c. Disconnect the cIEF Cartridge from the switch valve and remove the cartridge from the holder.

d. Wash the cartridge using the Cartridge Wash procedure on page 226. The cartridge must be washed with water to remove the methyl cellulose and electrolytes and then dried with air and stored dry to maximize cartridge lifetime.

e. Re-tighten the cartridge inlet sleeve in port 2.

f. The autosampler will then wash the iCE valve. Wipe any drops from port 2 of the iCE valve.

g. Empty the waste bottle connected to the port 4 waste line and place the waste line back in the empty waste bottle.

h. The autosampler will then perform a second valve wash. Wipe any drops from port 2 of the iCE valve.

i. Close the iCE3 door.

j. Disconnect the iCE3 instrument and autosampler.

k. Power off the iCE3 instrument and autosampler.

Long Term Shutdown iCE CFR Software Version 5.0

Manual Cartridge Rinse

1. In iCE CFR Software, select **Operate** from the main menu, then click **Shutdown** and **More than 7 days**. The long term protocol checklist will display with options for manual or automated Cartridge Rinse options (Figure 10-8).
2. Select **Manual Wash**.

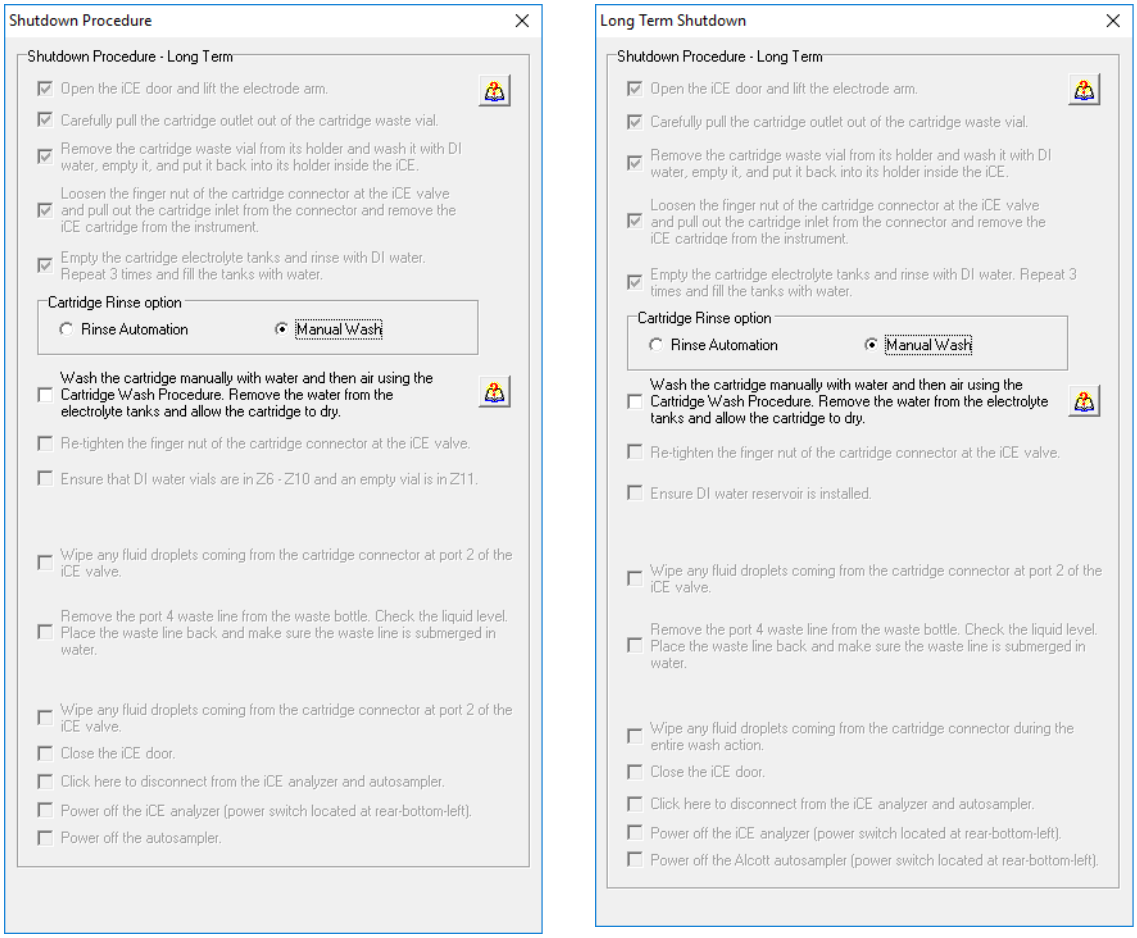


Figure 10-8: *Long term shutdown protocol using the Manual Wash Cartridge Rinse option in iCE CFR Software for PrinCE Next (left) and Alcott 720NV (right) in version 5.0.*

3. Perform the steps in the screen as described in the software:
   a. Open the iCE3 door and lift the electrode arm.
   b. Remove the outlet capillary from the waste vial, remove the cartridge waste vial and wash it with water and replace back in the instrument.
   c. Disconnect the cIEF Cartridge from the switch valve and remove the cartridge from the holder.
   d. Wash the cartridge using the Cartridge Wash procedure on page 226. The cartridge must be washed with water to remove the methyl cellulose and electrolytes and then dried with air and stored dry to maximize cartridge lifetime.
   e. Re-tighten the cartridge inlet sleeve in port 2.

f. The autosampler will then wash the iCE valve. Wipe any drops from port 2 of the iCE valve.

g. Empty the waste bottle connected to the port 4 waste line and place the waste line back in the empty waste bottle.

h. The autosampler will then perform a second valve wash. Wipe any drops from port 2 of the iCE valve.

i. Close the iCE3 door.

j. Disconnect the iCE3 instrument and autosampler.

k. Power off the iCE3 instrument and autosampler.

Automated Cartridge Rinse

1. In iCE CFR Software, select **Operate** from the main menu, then click **Shutdown** and **More than 7 days**. The long term protocol checklist will display with options for manual or automated Cartridge Rinse options (Figure 10-9).
2. Select **Rinse Automation**.

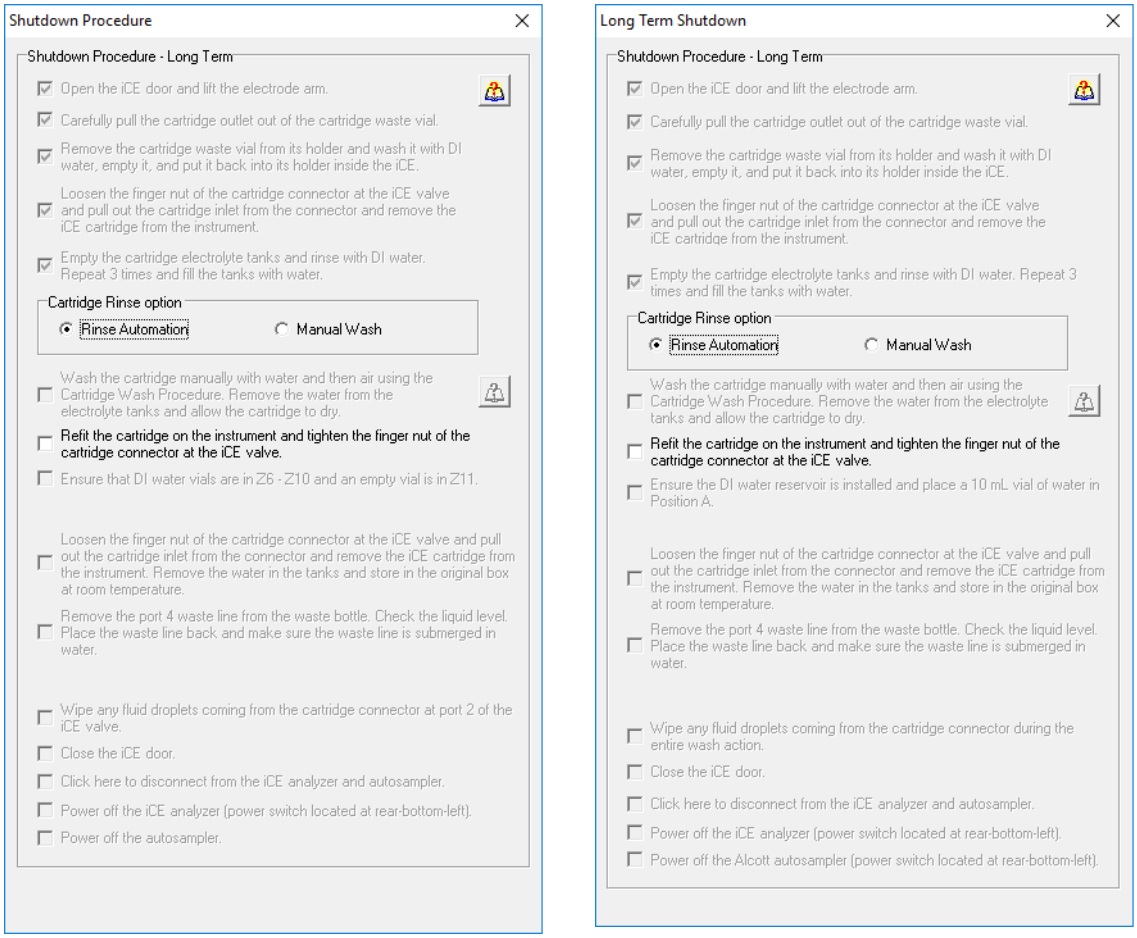


Figure 10-9: *Long term shutdown protocol using the Rinse Automation Cartridge Rinse option in iCE CFR Software for PrinCE Next (left) and Alcott 720NV (right) in version 5.0.*

3. Perform the steps in the screen as described in the software:
   a. Open the iCE3 door and lift the electrode arm.
   b. Remove the outlet capillary from the waste vial, remove the cartridge waste vial and wash it with water and replace back in the instrument.
   c. Disconnect the cIEF Cartridge from the switch valve and remove the cartridge from the holder.
   d. Empty the cartridge electrolyte tanks, rinse with DI water three times, and fill the tanks with water.
   e. Refit the cartridge on the instrument and tighten the finger nut of the cartridge connector at the iCE valve.

f. Ensure the DI water reservoir is installed. For the Alcott 720NV, place a 10 mL vial of water in Position A. For the PrinCE Next, place DI water vials in positions Z6-Z10 and an empty vial in Z11. Once the vials are placed, the automated cartridge rinse will start.

g. After the rinse completes, disconnect the cIEF Cartridge from the switch valve and remove the cartridge from the holder. Remove the water in the tanks and store the cartridge at the room temperature.

h. Empty the waste bottle connected to the port 4 waste line and place the waste line back in the empty waste bottle.

i. The autosampler will then perform a valve wash. Wipe any drops from port 2 of the iCE valve.

j. Close the iCE3 door.

k. Disconnect the iCE3 instrument and autosampler.

l. Power off the iCE3 instrument and autosampler.

Maintenance

Flow Path Maintenance

Periodically check all flow path connections to ensure no leaks are present.

Valve Maintenance and Cleaning

The valve on the iCE3 prevents hydrodynamic flow and eliminates the need to balance the system, but to remain in good operational order it is important to perform regular maintenance on the valve. The following information provides guidance on checking that the valve is correctly plumbed and valve cleaning.

Valve Plumbing

The correct plumbing of fluid lines to and from the valve is shown in Figure 10-10. It's important that all fluid connections be made correctly and with the correct parts. The plug, transfer line sleeve and cartridge sleeve connections are specific to each port location, do not substitute different connectors.

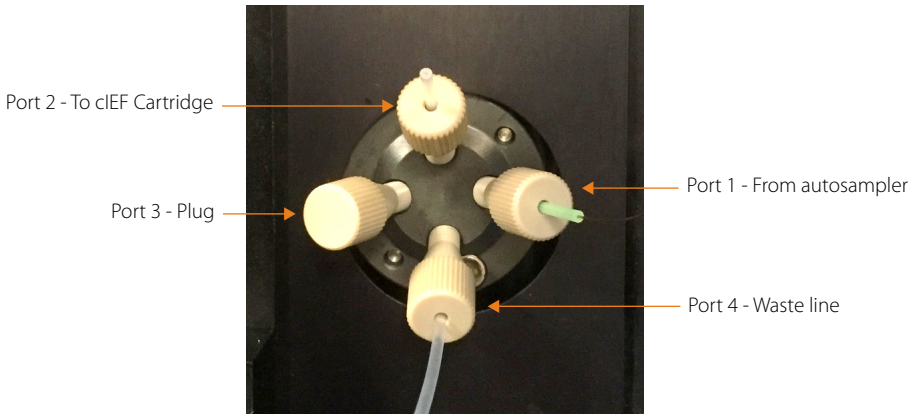


Figure 10-10: *iCE valve plumbing.*

Please see "Making Fluid Connections" on page 55 for iCE valve plumbing with the PrinCE Next autosampler or "Making Fluid Connections" on page 68 for iCE valve plumbing with the Alcott 720 NV autosampler.

Washing the iCE3 Valve

iCE CFR software versions 4.0 and higher provide additional valve washing between sample injections. But if the system is analyzing samples containing high concentrations of additives, the transfer capillary and valve may require additional cleaning. As a precautionary measure, we recommend you perform a manual iCE valve wash both before and after a Batch, and both before and after a Long Term Shutdown. To do this:

1. In iCE CFR software, select **Utility** from the main menu, click **Maintenance** and then **Manual Control**.
2. Select **Connect** under iCE3.
3. Select **Connect** under Autosampler.
4. Open the iCE door.
5. Disconnect the cartridge inlet from its connector in port 2 of the iCE valve.

   For iCE3 systems with a PrinCE Next autosampler:

   a. Select buffer vial **Z6** and set to **water**.

   b. Set the pressure to **2000 mbar.**

   c. Wait 3 minutes and make sure you observe drops coming out of the waste line (port 4).

   d. If no droplets are observed, please refer to "Troubleshooting Fluid Path and Flow Issues" on page 243.

   e. Select **Set Switch to Inject**.

f. Wait 3 minutes and make sure you observe drops from the cartridge inlet (port 2).

g. If no droplets are observed, please refer to "Troubleshooting Fluid Path and Flow Issues" on page 243.

h. Set the pressure to **0 mbar**.

i. Select **Set Switch to Load**.

j. If flow is passing through the valve as expected, exit Manual Control and start the Cartridge Installation procedure.

For iCE3 systems with an Alcott720NV autosampler:

a. Verify the water bottle is in place and filled with water.

b. Click **Perform Rinse**. After performing 3-4 rinses, you should observe droplets from the port 4 waste line.

c. If no droplets are observed, please refer to "Troubleshooting Fluid Path and Flow Issues" on page 243.

d. Select **Set Switch to Inject**.

e. Click **Perform Rinse**. After performing 3-4 rinses, you should observe droplets from the port 2 cartridge inlet.

f. If no droplets are observed, please refer to "Troubleshooting Fluid Path and Flow Issues" on page 243.

g. Select **Set Switch to Load**.

Cleaning the Air Filters

Inspect the air filters every two months. If the filters are dirty, they can be cleaned as follows:

1. Remove the plastic filter covers located on the rear panel of the iCE3 instrument (Figure 10-11). A tool is not required.

Figure 10-11: *Rear panel.*

2. Wash the filters with water then pat dry.
3. Replace the filters and snap the covers back into place.

Changing the Fuse

1. Power down the iCE3 instrument and unplug the power cord from the system.
2. Locate the fuse holder on the rear panel of the instrument:

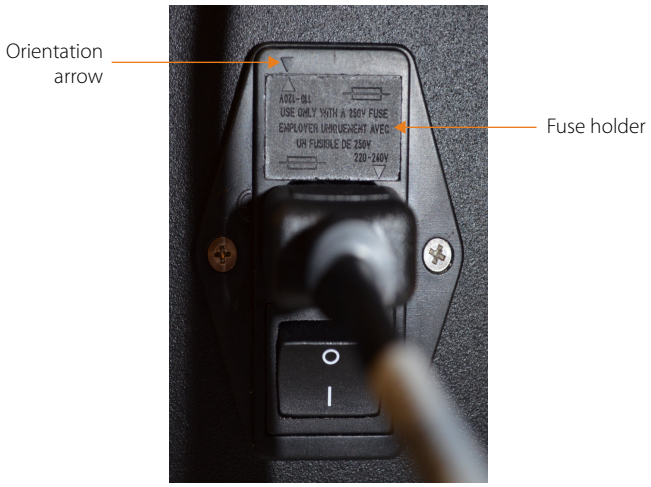


Figure 10-12: *Fuse holder in power entry module.*

3. Use a flat-head screwdriver to gently pry the fuse holder open. Remove the fuse holder.
4. Remove the old fuse.
5. There are two fuse positions in the fuse box. Insert a new 2A time lag high capacity 250 VAC fuse (P/N 011-800), making sure to insert it in the correct position for your laboratory voltage as shown in Figure 10-13.

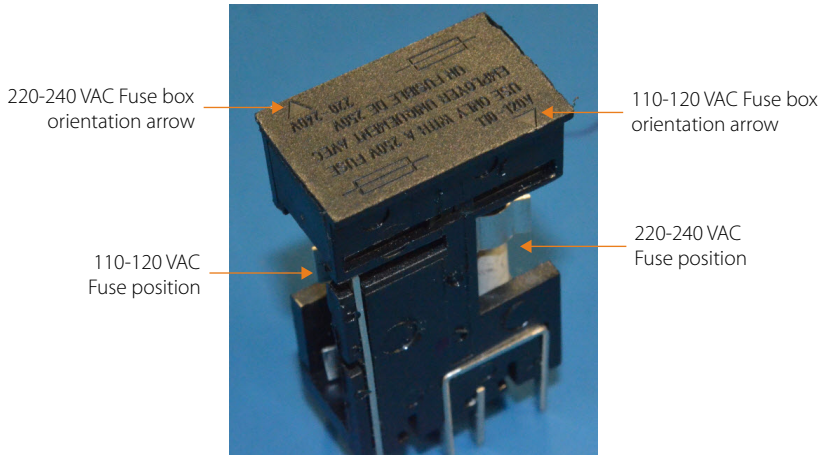


Figure 10-13: *Fuse position in fuse box.*

6. Reinsert the fuse holder, making sure to align the arrow on the fuse box associated with the correct voltage for your laboratory (110-120 VAC or 220-240 VAC, shown in Figure 10-13) with the orientation arrow on the power entry module (Figure 10-12).
7. Plug the instrument power cord back into the system and turn the power on.

Troubleshooting

Please contact ProteinSimple Technical Support at (888) 607-9692 or support@proteinsimple.com for additional troubleshooting, service or support-related questions.

Troubleshooting Fluid Path and Flow Issues

If there are no droplets or liquid flow observed during the cartridge installation procedure, you will need to correct this prior to continuing with the Cartridge Installation process.

1. Check all fluid connections at the iCE valve for leaks. If valve connections are too loose or too tight this may result in no flow through the valve. To fix this issue, simply remove the cartridge sleeve or transfer line sleeve and re-seat as described in "Making Fluid Connections" on page 55 for iCE3 systems with the PrinCE Next autosampler or on page 68 for iCE3 systems with the Alcott 720NV autosampler.
2. Check that the reagent vials are in the correct location and filled adequately.

3. If using the iCE3 with the PrinCE Next autosampler, confirm the system is holding pressure. Check that the pressure is at 2000 mBar. If the system isn't holding pressure, confirm all vials are in the correct position. If the vials are in the correct location and the system still can't maintain pressure, please contact Technical Support.

If the above doesn't resolve the source of the flow problem, it's possible there's a blockage in the fluid path. The iCE3 system uses methyl cellulose to rinse the capillary and minimize electroosmotic flow within the capillary. If the methyl cellulose is allowed to dry out in the system it can precipitate and block the transfer lines, valve and cartridge. The instructions that follow will allow you to determine if you have a blockage and its location. Please follow the instructions for your specific instrument configuration.

iCE3 System with PrinCE Next Autosampler

1. Disconnect the transfer line from port 1 of the iCE valve.
2. In iCE CFR software, select **Utility** from the main menu, click **Maintenance** and then **Manual Control**.
3. The Manual Instrument Control screen will display (Figure 10-14):

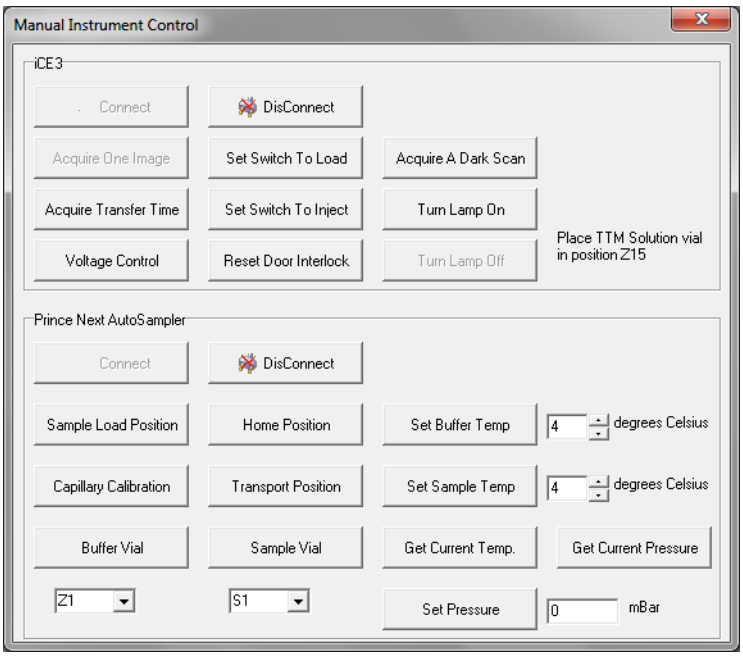


Figure 10-14: *PrinCE Next autosampler manual control screen.*

4. Select **Sample Load Position**. Open the autosampler door and place a water vial in position Z6. Close the door.

5. Using the manual controls, set the buffer vial to **Z6** and select **Buffer Vial**.
6. In the pressure field, enter **2000 mbar** and click **Set Pressure**.
7. Observe the pressure reading in the lower right hand corner of the main software window. Check that the system is holding 2000 mbar of pressure and wait approximately 30 seconds, you should observe water droplets from the end of the transfer line. Enter **0 mbar** in the pressure field and click **Set Pressure** to stop the pressure test.
8. If no droplets appear, your transfer line is most likely blocked. Replace the transfer line and reapply pressure. If you still do not see droplets, please contact Technical Support.
9. If you see droplets from the transfer line, reconnect this line to the valve as described in "Making Fluid Connections" on page 55.
10. Using the manual controls, select **Set Switch to Load**, then make sure the buffer vial is still set at **Z6**. Enter **2000 mbar** in the pressure field and select **Set Pressure**.
11. Pull the waste line connected to port 3 from the waste container. After 30 to 40 seconds, droplets should be observed from the waste line.
12. If droplets are observed, the waste line and valve are not blocked. Skip to step 14.
13. If no droplets are observed at the waste line, remove the waste line from port 3 and reapply 2000 mbar pressure. If droplets are observed coming from port 3 of the valve, the waste line is blocked and creating the flow problem. If no droplets are observed coming from port 3, the valve is possibly blocked. Please see "Removing Valve Blockages" on page 247.
14. Make sure the cartridge is disconnected from port 2. While still in the Manual Control screen, select **Set Switch to Inject** and apply **2000 mbar** pressure. You should see water droplets coming from port 2. If no droplets are seen, the valve is the source of the issue. Please see "Removing Valve Blockages" on page 247.
15. If droplets are observed at port 2, connect the cartridge to port 2 following the instructions on "Installing the Transfer Capillary" on page 56. Reapply **2000 mbar** of pressure and wait approximately 30 seconds. Drops should be observed coming from the cartridge outlet. If no droplets are observed, the cartridge may be blocked. Clean the cartridge using the "Cartridge Wash Procedure" on page 226 with the Cartridge Wash Kit to remove any small blockages. If the cartridge is completely blocked then replace the cartridge.
16. If you still can't obtain flow through the iCE3 system, please contact Technical Support.

iCE3 System with Alcott 720NV Autosampler

1. Verify water bottle is in place and filled with water.
2. Disconnect the transfer line from port 1 of the iCE valve.
3. In iCE CFR Software, select **Utility** from the main menu, click **Maintenance** and then **Manual Control**.

4. The Manual Instrument Control screen will display (Figure 10-15):

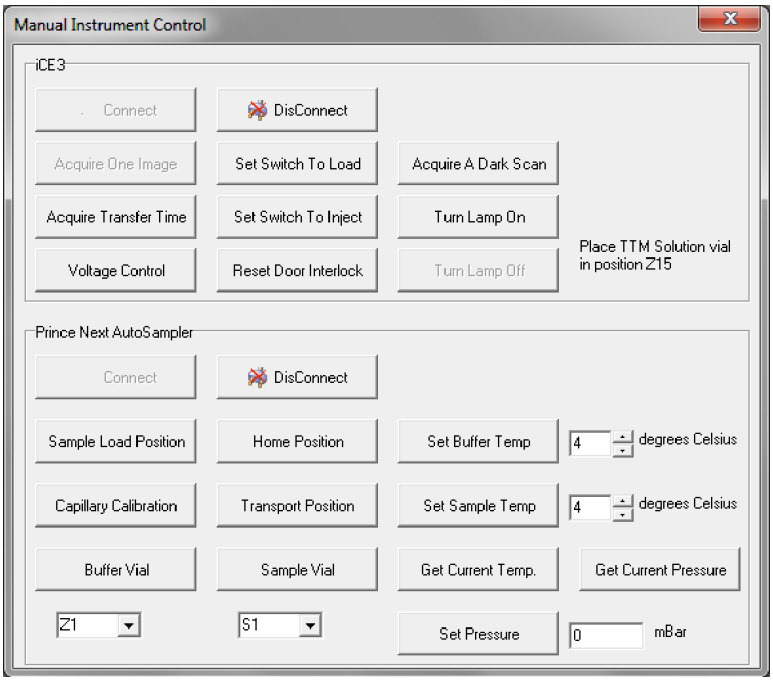


Figure 10-15: *Alcott 720NV autosampler manual control.*

5. In the Manual Control screen select **Set Switch to Load** and then **Perform Rinse**. After performing 3-4 rinses, you should observe droplets coming from the transfer waste line. If droplets are observed, reconnect this line to the valve as described in "Making Fluid Connections" on page 68 and skip to step 6. If no droplets are observed, you may have a blocked transfer line. Please contact Technical Support.
6. Pull the waste line connected to port 3 out of the waste container, then in the Manual Control screen select **Perform Rinse**. After performing 3-4 rinses, you should observe water droplets coming from the waste line. If droplets are observed, the waste line and valve are not blocked. Skip to step 10.
7. If no droplets are observed, disconnect the waste line from port 3 and perform 3-4 rinses. If droplets are observed coming from port 4 of the valve, the waste line is blocked and creating the flow problem. Replace the waste line. If no droplets are observed coming from port 4, the valve is possibly blocked and needs to be washed. Please "Washing the iCE3 Valve" on page 239.
8. Make sure the cartridge is disconnected from port 2. While still in the Manual Control screen, select **Set Switch to Inject** and then **Perform Rinse**. After performing 3-4 rinses, you should see water droplets coming from port 2. If no droplets are observed, it's possible the valve is the source of the issue and needs to be washed. Please "Washing the iCE3 Valve" on page 239.

9. If droplets are observed in step 8, connect the cartridge to port 2 following the instructions on page 127. Perform 3-4 rinses. Drops should be observed coming from the cartridge outlet. If no droplets are observed, the cartridge may be blocked. Clean the cartridge using the "Cartridge Wash Procedure" on page 226 with the Cartridge Wash Kit to remove any small blockages. If the cartridge is completely blocked then replace the cartridge.
10. If you still can't obtain flow through the iCE3 system, please contact Technical Support.

Removing Valve Blockages

If there is no liquid flow through the iCE valve you can attempt to clear it using the following procedure. If the procedure below does not restore liquid flow please contact Technical Support.

1. Locate the Purge Kit (P/N 045-098) that is shipped with the instrument. It includes:
   - 5 mL disposable syringe with luer lock
   - Adapter, female luer to tapered thread
   - Purge line
   - Cartridge inlet sleeve fitting (P/N 045-070)

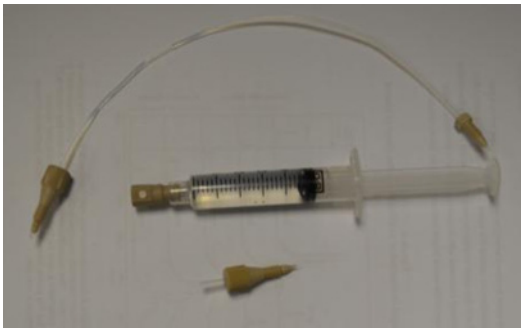


Figure 10-16: *Parts needed for iCE valve cleaning.*

2. Disconnect all connections from the iCE valve.
3. Connect the purge line to port 1 on the iCE valve.
4. Fill the syringe with 3 mL of distilled, deionized water.
5. Connect the syringe to the adapter and connect the adapter to the purge line.
6. In iCE CFR software, select **Utility** from the main menu, click **Maintenance** and then **Manual Control**.
7. Select to **Connect** under iCE3.
8. Select **Connect** under Autosampler.
9. Select **Set Switch to Load**.

10. Press down on the syringe to push water through port 1 until droplets are observed.
11. Remove purge line and connect to port 4. Refill the syringe with distilled, deionized water if necessary. Press down on the syringe to push water through port 4 until droplets are seen coming from port 1.
12. Select **Set Switch to Inject**. Repeat the steps 10 and 11 for ports 2 and 3.
13. If flow can't be restored using this procedure, please contact Technical Support.

Abnormal Focusing

Problem	Solution
Incorrect transfer time When the transfer time is not optimal, the separation column may not be completely filled with sample during focusing.	Perform Transfer Time Measure.
cIEF Cartridge tank level low If the anolyte or catholyte tank fluid level is not high enough to make good contact with the electrodes, the current will drop to < 2 µA.	Add anolyte or catholyte to the cIEF Cartridge tanks to bring the solution level up as needed.
Electrolyte contamination	Replace the anolyte and catholyte solutions in the cIEF Cartridge tanks.

Artificial Peaks

Problem	Solution
cIEF Cartridge surface contamination	• When installed on the holder, compressed air or nitrogen can be used to gently clean the optical slit on the cartridge. • If the above does not rectify the problem, remove the cartridge from the holder. Rinse with a squeeze bottle containing HPLC-grade deionized water. Let the cartridge dry for 10 minutes, then reinstall.

Problem	Solution
Dust or particulates on the illumination lens	Remove the cartridge. Use compressed air or nitrogen to gently clean the illumination lens, then reinstall the cartridge.
Particles or precipitate in sample	Use an aqueous additive to stabilize the sample solution.
Air bubbles in sample	Centrifuge samples to remove air.

Shifting Electropherogram

Problem	Solution
Electrolyte contamination	Replace the anolyte and catholyte solutions in the cIEF Cartridge tanks.

Uneven Light Intensity

Problem	Solution
cIEF Cartridge surface contamination	Remove the cartridge from the holder. Rinse with a squeeze bottle containing HPLC-grade deionized water. Let the cartridge dry for 10 minutes, then reinstall.
cIEF Cartridge not installed properly	Remove the cIEF Cartridge and reinstall, making sure it sits properly in the correct position.
UV light unstable	• The UV lamp is at or near its maximum lifetime. Replace the lamp. *NOTE: The UV light bulb has a lifetime of approximately 2000 hours. If the bulb is close to the end of its life, contact a ProteinSimple Service Representative.*

Frequently Asked Questions

NOTE: Please refer to Chapter 8, "Method Development" for more information on initial development conditions, carrier ampholytes, minimizing sample matrix affects and recommended additives.

I have a new protein sample to analyze. What starting conditions should I use?

Begin with the following initial sample conditions:

- Carrier ampholytes: pH 3-10 Pharmalytes (4%)
- Additive: 0.35% methyl cellulose
- Sample analyte: 0.1 mg/mL concentration in final solution. The balance of the solution should be HPLC-grade deionized water.

NOTE: ProteinSimple provides a 1% methyl cellulose solution (P/N 101876).

Another way to start is to simply use the same sample conditions used if you were successful in running this sample on slab gel IEF. Use the same carrier ampholytes and additives for iCE3 system analysis.

What carrier ampholytes are commercially available, and which one is best for my sample?

At present, carrier ampholytes are commercially available from four different manufacturers under the following brand names:

- Pharmalytes
- Servalyts
- Biolytes

Other carrier ampholytes exist, however, they are all repackaged and resold using one of the products listed above. Pharmalytes are available from Sigma. Servalyts are distributed in North America by Crescent Chemical, NY and Biolytes are sold by Bio-Rad Laboratories.

Each brand may give slightly different separation resolution due to slight differences in ampholytic compositions. Identification of the optimal carrier ampholytes for a given protein sample is best determined experimentally.

The iCE3 system uses whole column, UV absorption detection at 280 nm. All carrier ampholytes exhibit some degree of absorption at this wavelength, which causes some baseline noise. Pharmalytes have low and uniform UV absorption along the entire pH range, and because of this Pharmalytes are recommended for initial sample conditions.

Does my sample matrix affect my results?

Yes. However, the sample is diluted 20X in carrier ampholytes, methyl cellulose and HPLC-grade deionized water, which minimizes matrix effects. For example, if the concentration of your sample stock solution is 2 mg/mL, 10 mL of the sample can be directly diluted by adding 112 mL of HPLC-grade deionized water, 8 mL of pH 3-10 Pharmalytes and 70 mL of 1% methyl cellulose. The final solution is 200 mL with a sample concentration of 0.1 mg/mL. In this example, the original sample matrix will not affect analysis.

If the original stock sample concentration is >2 mg/mL and contains high salt concentrations, then desalting may be necessary.

I cannot get reproducible peaks due to sample precipitation, what should I do?

Many additives may be used to increase protein solubility. The following additives have been successfully tested with the iCE3 system and should help stabilize proteins during analysis:

- Up to 25% sorbitol
- Up to 25% sucrose
- Up to 25% glycerol
- < 4 M urea
- Denaturing conditions, such as 8 M urea

In rare cases, sample precipitation may be caused by the carrier ampholytes. To avoid this problem, try using a different brand of carrier ampholytes. If additive conditions for stable sample runs have been established for gel IEF, then these additive conditions can often be successfully used for cIEF analysis on the iCE3 system.

NOTE: All additives may change the pI value of the protein slightly.

How do I prepare sample solutions in 8 M Urea?

For a 200 µL final sample solution:

- Weigh 96 mg of urea powder in a 1.5 mL centrifuge tube.
- Add 32 µL HPLC-grade deionized water, 70 µL of 1% methyl cellulose, 8 µL of carrier ampholytes and 10 µL of sample to the urea powder in the centrifuge tube.

This will make a final volume of 200 µL (96 mg urea powder and 120 µL liquid). If more than 10 µL or less than 10 µL of sample is added, the volume of water should be adjusted to ensure a final volume of 120 µL.

When running samples in 8 M urea, the focusing time should be increased 1-2 minutes compared to normal conditions. This is due to the higher viscosity of the urea solution.

How can I identify peaks in different runs and different samples?

A reliable way to identify peaks in electropherograms is to use internal pI Markers. First run the sample without internal pI Markers. The pI values of sample peaks can be estimated from their peak positions relative to the full pI range of the carrier ampholytes.

On the iCE3 system, the left side of the electropherogram is the anodic end of the column (acidic) and the right side is the cathodic end (basic). For example, if pH 3-10 Pharmalytes are used as the carrier ampholytes, the x-axis of the electropherogram represents pI 3 to pI 10 from left to right. The pI value of a peak at the middle of the trace should be about 6.5.

Two pI Markers are mixed into the sample solution. Ideally, the peaks of the two markers should bracket the sample peaks and the two marker peaks should be as close as possible in order to achieve good precision in peak identification.

The electropherograms of the sample mixed with pI Markers are processed using iCE CFR Software for pI calibration. The software calibrates the pI value between the two pI Marker peaks using the pI values of the two Markers and transforms the x-axis of the e-grams from peak position into pI value.

In this way, the sample peaks are identified by their measured pI values. The precision of peak identification by measuring the pI values using the iCE3 system is less than $\pm$0.03 pH units.

Since the measured pI value of a protein is affected by many factors such as sample matrix and the type of carrier ampholytes used, to correctly identify peaks in different samples or different runs, all runs should be done under the exact same conditions.

What kind of pI markers can I use?

ProteinSimple recommends using low molecular weight amphoteric compounds with well defined isoelectric points and strong UV absorbance when using the iCE3 system. Conversely, we do not recommend using protein pI markers since they often produce multiple isoelectric points and, on occasion, may interact with the sample analyte.

ProteinSimple offers a broad range of synthetic, small molecule compounds specifically designed for use as internal pI Markers. These include markers at pI 2.85, 3.21, 3.59, 4.22, 4.65, 5.12, 5.85, 6.14, 7.05, 7.40, 7.96, 8.40, 8.79, 9.22, 9.50, 9.77, 10.10 and 10.45 and all are available individually.

The distance between the two pI Markers in my sample electropherograms is different from run to run even though I use the same pI Markers and carrier ampholytes. What is the reason for this?

Usually this is caused by different salt concentrations in the sample solutions. Salt can compress the pH gradient created by the carrier ampholytes. So, the higher the salt concentration, the shorter the distance between the two pI Markers.

However, since the whole pH gradient is compressed by the salt, this will not affect peak identification results as long as two pI Markers are used and their peaks bracket the sample peaks.

Can I use narrow pH range carrier ampholytes to improve the resolution for my sample?

Yes. The most efficient way to do this is to use a mixture of narrow pH range carrier ampholytes and wide pH range carrier ampholytes. The proportion of carrier ampholytes can be from 1:1 (narrow range: wide range) up to 5:1 depending on the resolution requirement. Focusing time should be increased with the increasing proportion of the narrow pH range carrier ampholytes, from 6 to 12 minutes.

The measured pI value of my sample peak is slightly different when I use different pI Markers or different carrier ampholytes with the same pI markers. What is the reason for this?

When using different pI Markers, the small difference in the measured pI value is due to the slight non-linearity of the pH gradient established by the carrier ampholytes along the separation column. The iCE CFR Software pI calibration program assumes that the pH gradient is perfectly linear between the two pI Markers. In reality, carrier ampholytes are not perfectly linear throughout their pH gradient.

When different carrier ampholytes are used, their pH gradients may be slightly different causing a small difference in measured pI value. This effect is most obvious when using a carrier ampholyte mixture (i.e. narrow and wide pH range carrier ampholytes). Under these conditions, the pH gradient will not be linear at the edges of the overlapping pH regions of the different carrier ampholytes. Changing the ratio of the different carrier ampholytes in the mixture will affect the measured pI values of a protein.

In conclusion, only measured pI values obtained using the same carrier ampholytes and the same pI markers can be compared. Also, as long as the run conditions are the same, the measured pI values can be used to identify protein peaks.

Accessories and Spare Parts

A set of common spare parts, accessories and service information is listed below. To place an order, please call (888) 607-9692.

Part Description	Part Number
Cartridge Inlet Sleeve, 200 µm	045-070
Cartridge Inlet Sleeve and Tool	045-284
Cartridge Inlet Sleeve Tool	045-285
Transfer Capillary, PrinCE Next	045-074
iCE3 Waste Line Tubing assembly	045-089
Vial Pack, 2 mL glass (qty 100)	045-132
Vial Pack, 300 µL PP (qty 100)	045-133
Septa Pack, iCE3 (qty 100)	045-134
Vial Insert Pack, 300 µL, PP (qty 100)	045-135
Vial Pack, 10 mL, glass, amber (qty 10)	045-139
Platinum Wire Electrodes	101454
Electrolyte Pipette	101788
Lens Paper Tissue	101862
4 mL Vial Level Line assembly (qty 2)	102142
Waste Vial Cap	102531
Waste Vial, Constant Level Waste	102555
Fitting, Finger Tight Plug 1/16, 10-32 PEEK	102677
Fuse, T2AH, 250 V AC	011-800
Network Cable, 5 feet, PrinCE Next	011-661
Waste Bottle	011-140
Balancing Line	045-076

Appendix A:

Using Chrom Perfect

Chapter Overview

- Chrom Perfect Overview
- Data Processing in Chrom Perfect
- Some General Features of Chrom Perfect

Chrom Perfect Overview

This purpose of this guide is provide users with basic information concerning the installation of Chrom Perfect and how to use it to quantitatively analyze and process data generated by the iCE system. Chrom Perfect software also has powerful report generation capabilities.

Key to this Guide is the Data Processing Tutorial that uses real, iCE data files to teach users Chrom Perfect through a 'hands on' learning experience.

This document presents instructions in a step-by-step manner for the entire data import, data processing, and report writing process. It only covers the basic steps. However users can use other advanced functions in Chrom Perfect after they are familiar with all the steps presented here.

The iCE CFR Software Help files provide detailed information to convert .IEF files to a standard ANDI format suitable for Chrom Perfect.

Starting Chrom Perfect Software

As initially installed, Chrom Perfect comes up in a very simple configuration. The security features are inactive. It is completely unaware of the location of the users' directories and of any hardware that may be installed. Several issues must be addressed before Chrom Perfect will be ready for use. This is only a summary. In all cases, the detailed instructions are to be found in the Main Manual.

- To enable the security features, you must run the System Manager, and assign users, groups, passwords and system defaults. This process is summarized later in this chapter.
- To specify the paths that the data system will search for Method, Calibration, and other files used by the system, run the Profile Editor. If the security features are inactive, then each user must perform this task. Otherwise, it need be done only once per station.
- If you have files from an earlier version of Chrom Perfect, then you may wish to archive your old files and then import them into Chrom Perfect. This process is summarized later in this chapter.
- To install the hardware drivers, configure the instruments, and assign channels of data, run the Acquisition program. It is not necessary to do this until such time as the hardware is installed and the instruments are connected.

Many users will wish to make other changes, all of which can easily be deferred to another day. For example, the Main Menu can be configured to hide certain buttons, or to show additional buttons that launch your own programs.

Each of these topics is discussed in detail in the on-line help files that have now been installed.

Security Features

As installed, the software has all security features disabled, and is open to modification by any user. You may wish to enable the security features immediately, or to wait until users become fully acquainted with the software.

When first installed, Chrom Perfect comes up in "Single-user" mode. No user log-on dialog is conducted, so there are no user names or passwords. Logging is disabled. Users have total power, as is appropriate in a single-user environment. users may remain completely unaware of the security features and the System Manager for some time. Sooner or later, however, the security features will become desirable, in whole or in part.

The opposite extreme is a multi-user and/or networked environment, in which users must be restricted so that they do not inadvertently disturb one another. In this environment, it will make sense to define a number of groups and give the several groups different privileges. For example, the following groups might be desirable:

Operator group users…

- have control over their own search directory structure
- may not modify Method, Format files

Administrator group

- has complete power

Finally, the Administrator may determine what appears in the top, bottom, and left margins of reports generated by Chrom Perfect. However, it is not necessary to do this until such time as "real" reports are generated.

Main Menu

After you start Chrom Perfect, a Main Menu is presented with six big push buttons representing the Chrom Perfect program "applications". When you push one of the buttons, an application window will appear where you can perform tasks relative to that operation. The six main menu items are Analysis, File Editor, Profile Editor, Audit History, System Manager, and Where is. The user can always check the manual under the default installation folder **C:\CPData\Manuals**, and also get the help information by clicking Help in the application.

Figure A-1: *Chrom Perfect Main Menu.*

The primary purpose of the Main Menu program is to launch the other Chrom Perfect programs. There are six of these programs and a large button on the Menu for each.

- The **Analysis** program inputs ANDI CDF files, integrates Raw files, generates reports and plots.

- The **File Editor** program creates, edits, and updates Method and other files. The Profile Editor program maintains the user search directories.
- The **Audit History** program displays contents of the data, error, and alarm-log files.
- The **Where is**… program searches directories for Chrom Perfect files that match specified criteria.
- The **System Manager** program maintains system-wide security and audit settings, user passwords, etc. The System Manager button will be inactive unless the user has logged on as the Administrator.

The **Main Menu** program is not strictly necessary, because all of these Chrom Perfect programs can be launched directly, for example, by clicking on their icons from within the Windows Explorer. However, if passwords are enabled and the Main Menu program is not running, then the user will be forced to log-on each and every time a Chrom Perfect program is launched. By leaving the Main Menu running, the user is spared the trouble of repeated log-on requests. The Administrator may limit the amount of time that an unused Main Menu will remain running.

Data Processing in Chrom Perfect

The following shows basic steps in using Chrom Perfect to process pI calibrated data files generated by the iCE system. More detailed description of Chrom Perfect can be found in it manual.

Input the ANDI CDF Data File in Chrom Perfect

1. Launch Chrom Perfect software, and then select the button **Analysis** in the main menu.

   Chrom Perfect Analysis window is open.

2. If this is the first time this window is open, file name inquiry window will be automatically open, otherwise, select **File** > **Open** to open data files as shown in Figure A-2.

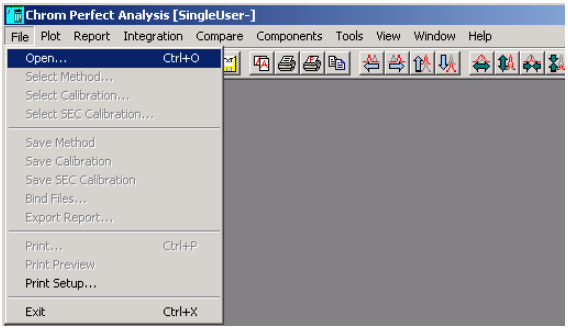


Figure A-2: *Opening Data Files*

3. Click the ellipsis button (**...**) at the right side of the window to browse from other directories as shown in Figure A-3.

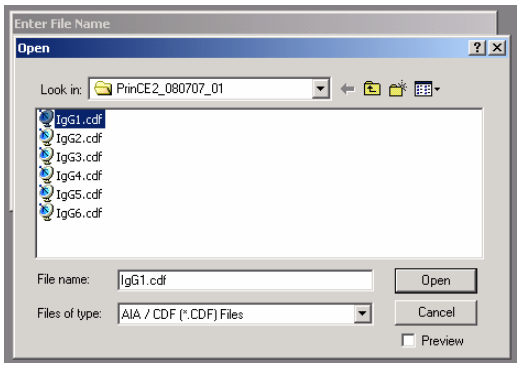


Figure A-3: *Browse Directories for Data Files*

4. Open a data file by highlighting it and click **Open** button. Or use the drop-down button to select a previous opened data file in the file name inquiry window (Figure A-4).

   This data file will be plot in the window. In Figure A-3, the data file IgG1 is opened.

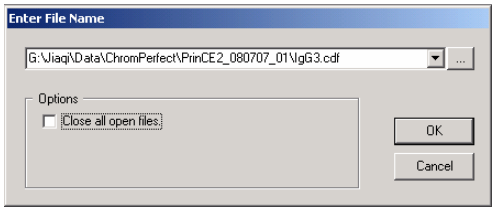


Figure A-4: *Previously Opened Data File*

Set Integration Parameters and Events

Set Integration Parameters

As shown in Figure A-5, click **Integration** > **Auto** to open Integ Options window (Figure A-6).

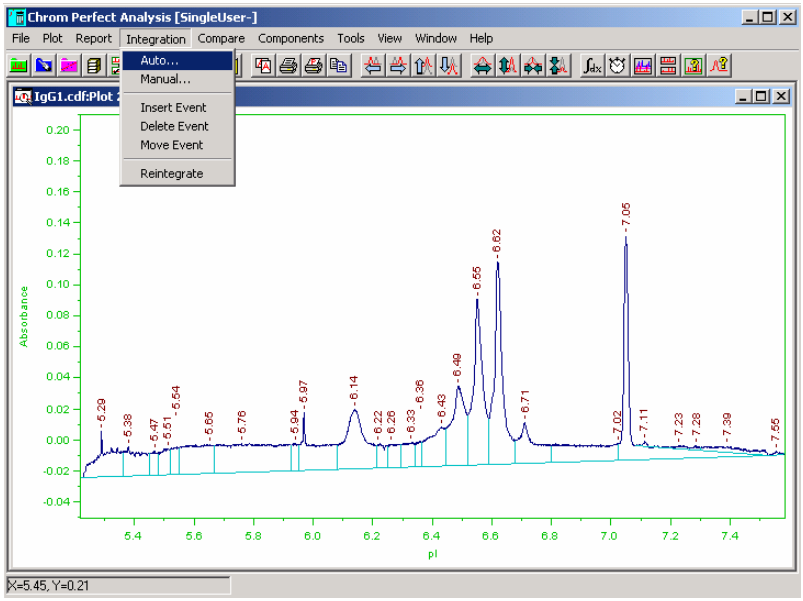


Figure A-5: *Integration > Auto Menu*

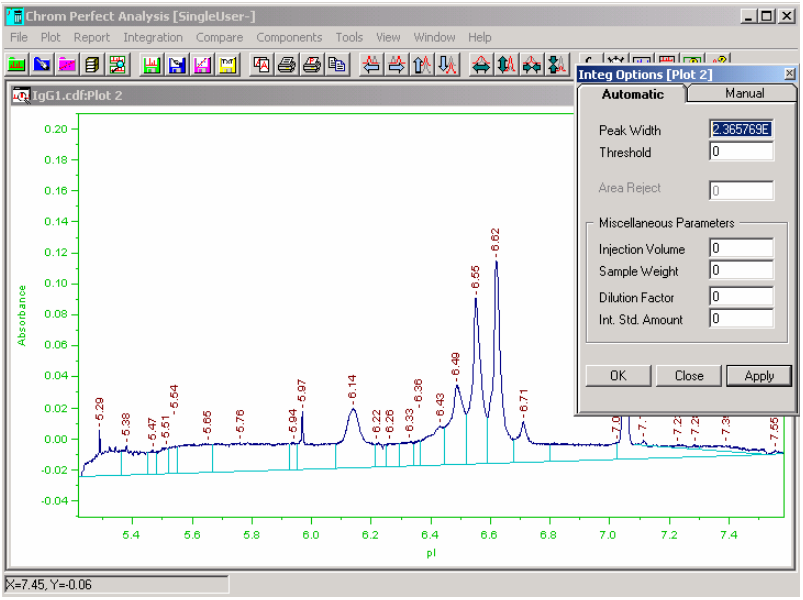


Figure A-6: *Integration Options (Automatic and Manual)*

5. Adjust parameters of **Peak Width** and **Threshold** and then, click **Apply** button until satisfactory result is achieved (the IgG1 example is shown in Figure A-7).
6. Turn off integration in some regions. This can be done by using **Integration** > **Insert Event** command as shown in Figure A-7 to open Select Event window.

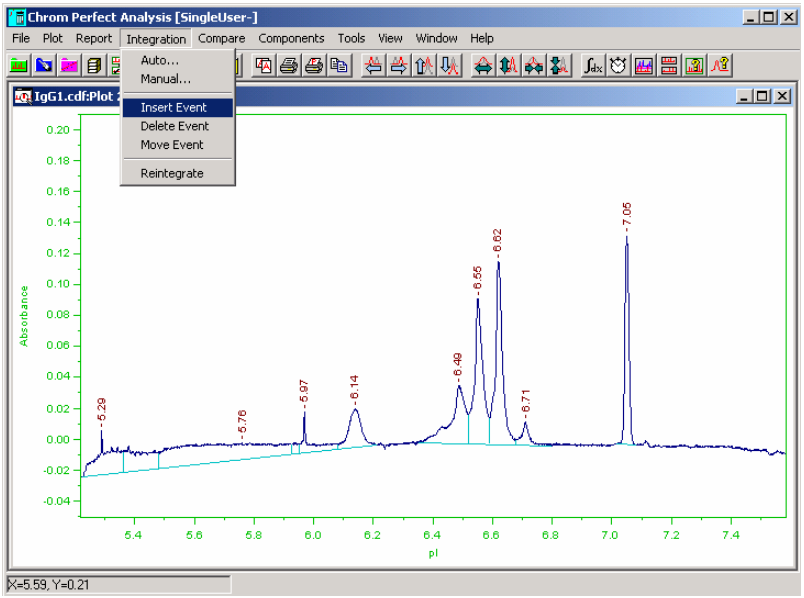


Figure A-7: *Inserting an event*

Setting Integration Events

1. First, turn off integration from the beginning to the position after the pI 6.14 marker peak.

   This action needs two steps. (Figure A-8 and Figure A-9 show the two steps.)

   a. In the Select Event window, click **INT-**, then click **OK** button to close the table.

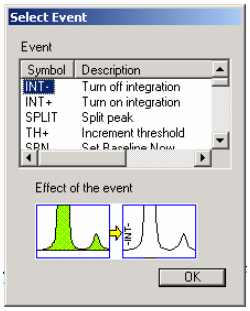


Figure A-8: *Select Event Window*

   b. Click the start point of "turn off integration" on the display, then right-click to apply the "turn off". In this example, it is at the beginning of the plot, as shown in Figure A-9, Figure A-10, and Figure A-11.

Now the integration is turned off from the beginning of the display.

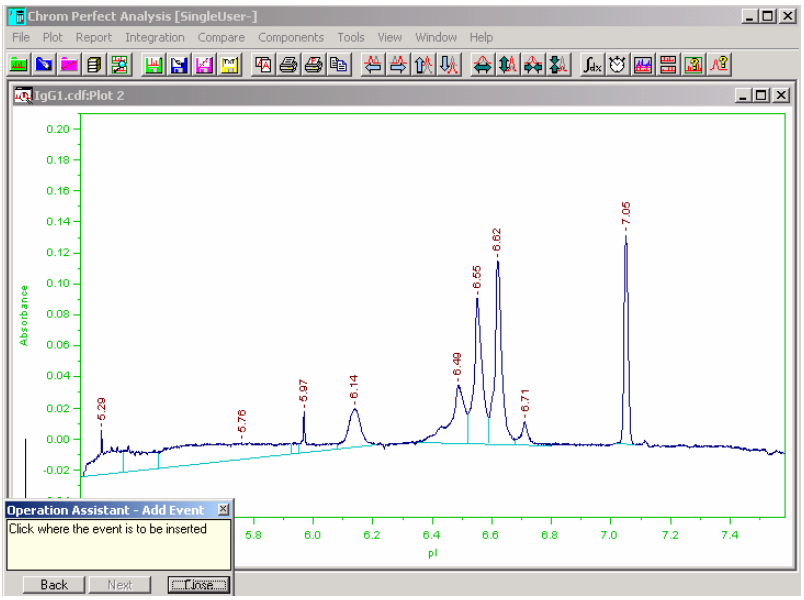


Figure A-9: Turning integration off

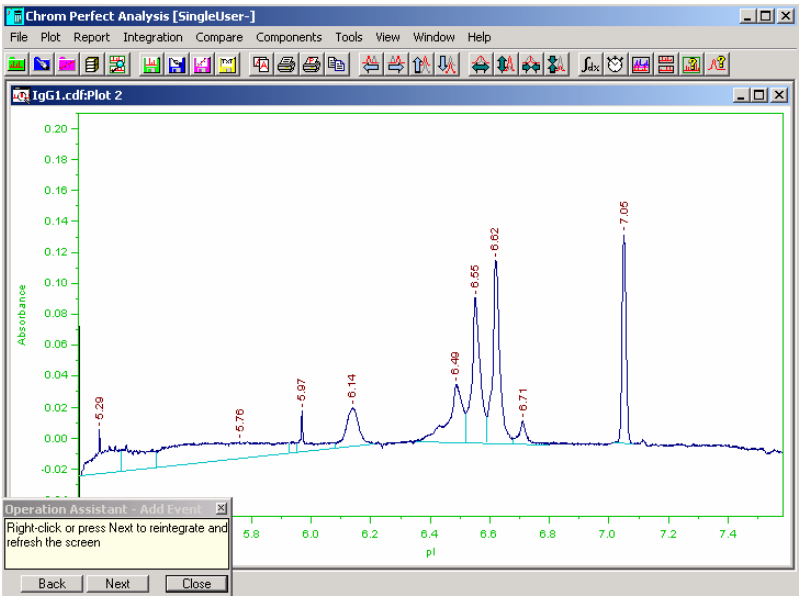


Figure A-10:

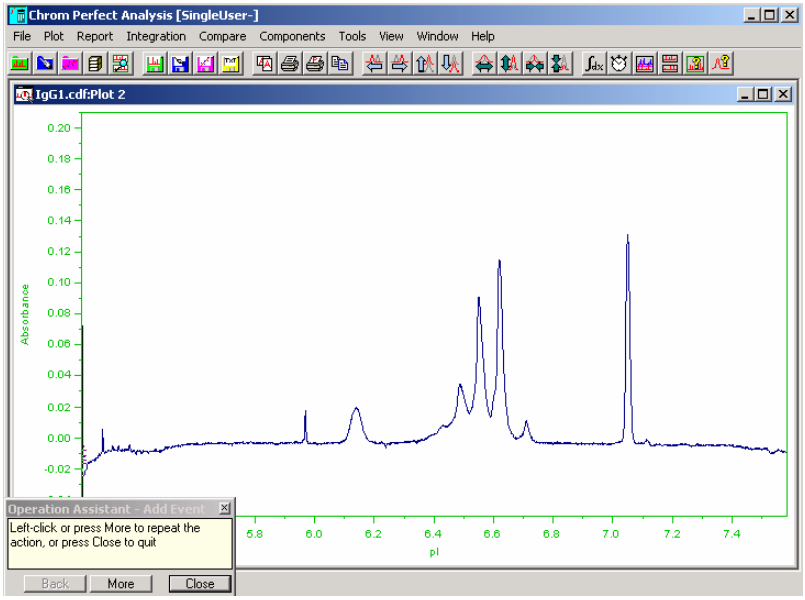


Figure A-11:

c. Second, turn on integration from the left side of the first pI marker (pI 6.14 marker) peak.

d. As shown in the Figure A-12 and Figure A-13, first click **More** button in the Operation Assistant—Add Event window (as shown in Figure A-11), select **INT+** (turn on integration) in the Select Event window and click **OK** button in the window.

e. Then, click on the display at a position just right of the pI marker peak.

f. Finally, right click to apply.

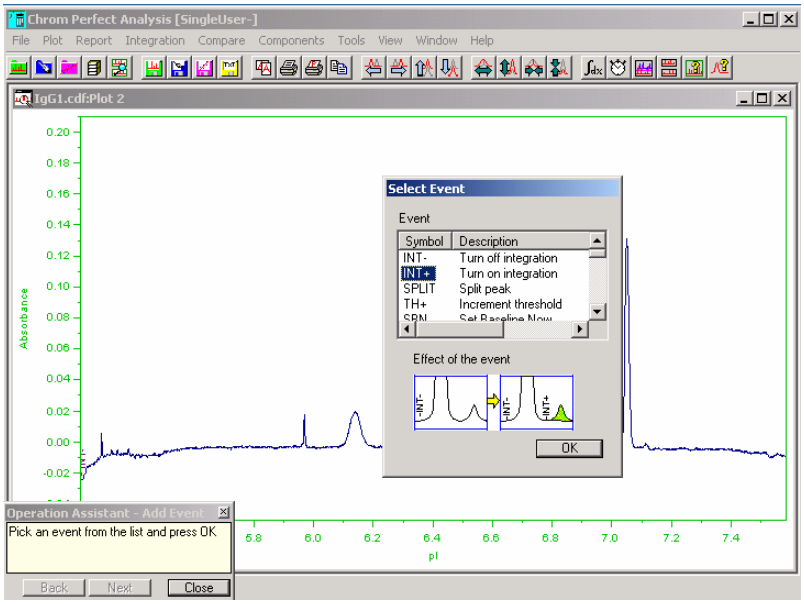


Figure A-12:

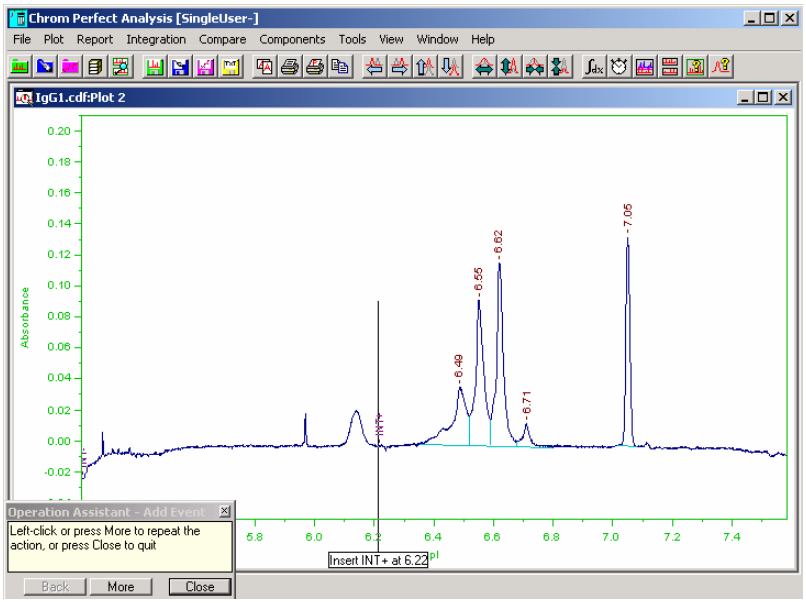


Figure A-13:

g. Finally, repeat to turn of integration from left side of the second pI marker (pI 7.05) peak.

The steps are shown in Figure A-14 and Figure A-15.

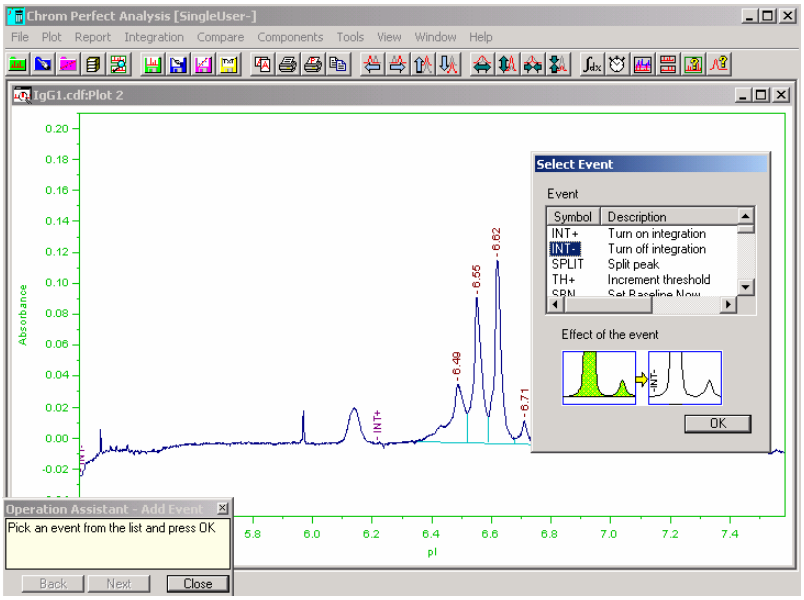


Figure A-14:

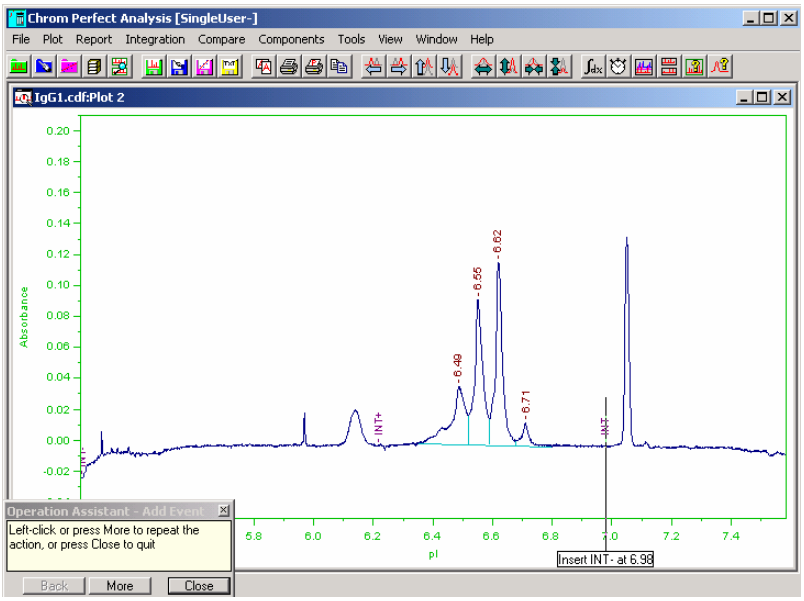


Figure A-15:

Modify Display

1. Zoom in on the display to focus on the region of sample peaks (as shown in Figure A-16 and Figure A-17). Use mouse to draw a box on the display, then, right click.

   The box will be the boundary of the new display (to return to previous boundary, press **Backspace** key).

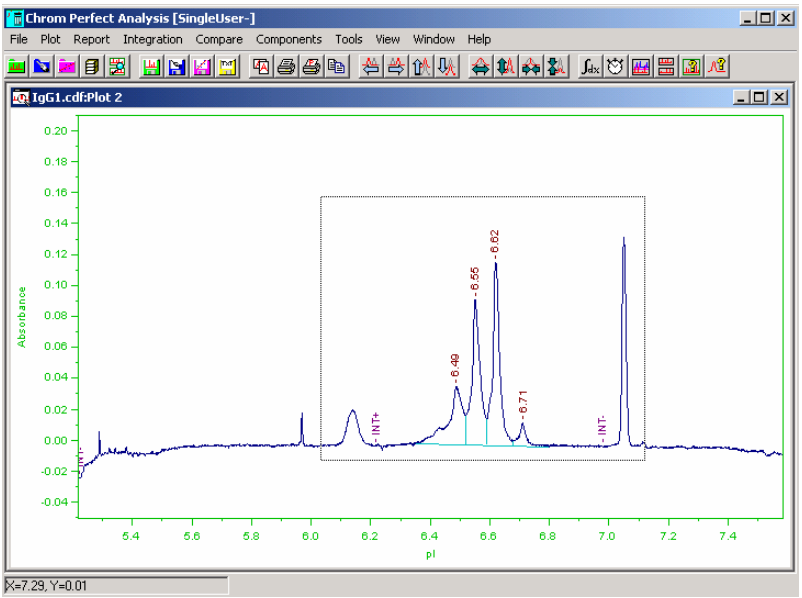


Figure A-16: *Displaying Focused Peaks (A)*

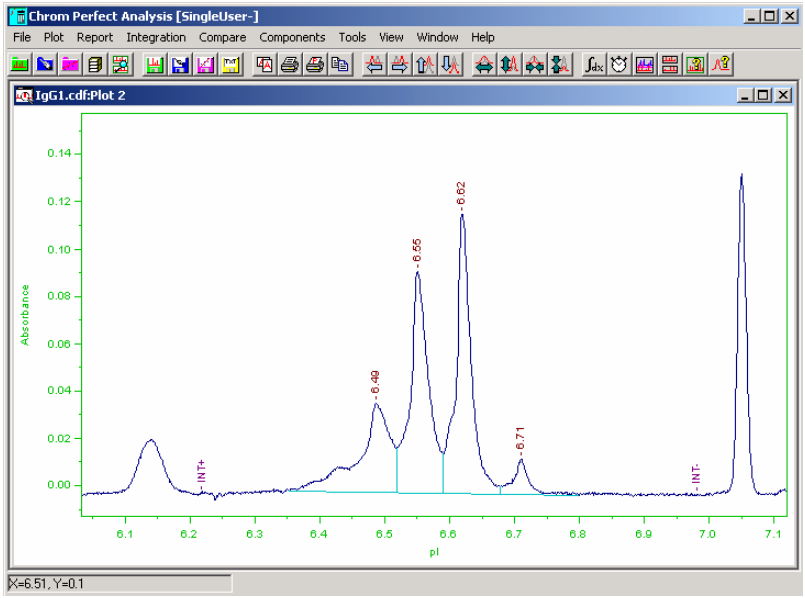


Figure A-17: *Displaying Focused Peaks (B)*

The labels of the peaks in the display can be changed.

2. To do this, as shown in Figure A-18, Figure A-19, and Figure A-20, right click one the plot to select **Properties**… to open Plot Properties window.

   In the window, display settings and labels can be selected.

3. Finally, click **OK** to close the window and apply the settings, or click **Apply** to keep the window open but apply the settings.

   In this example, peak number is added in the peak labeling.

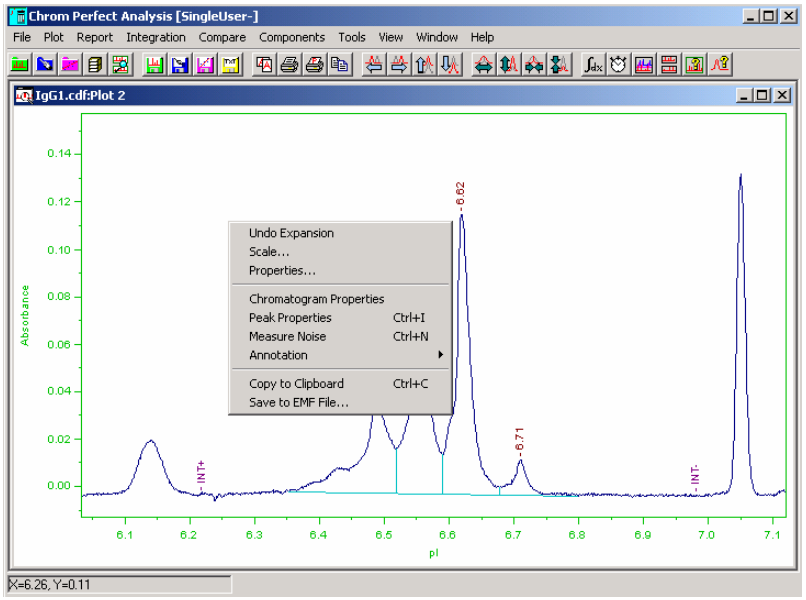


Figure A-18: Plot Properties (A)

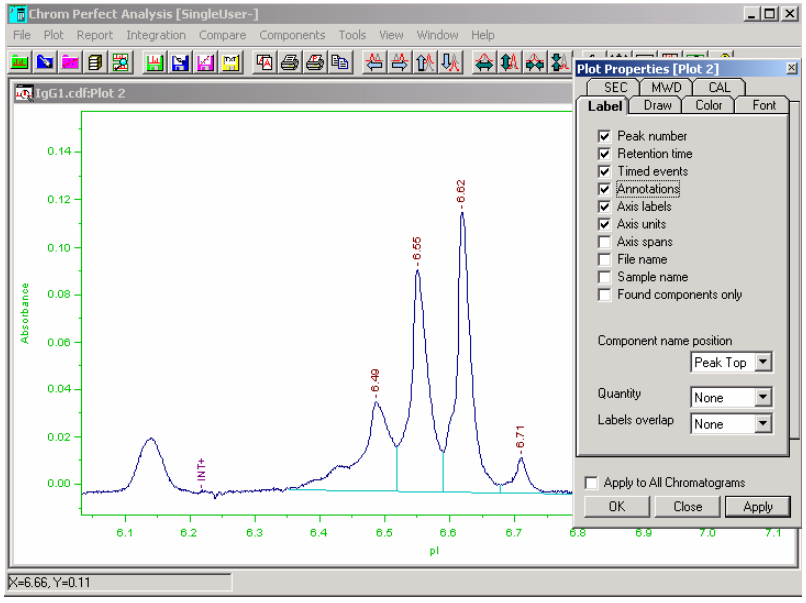


Figure A-19: Plot Properties (B)

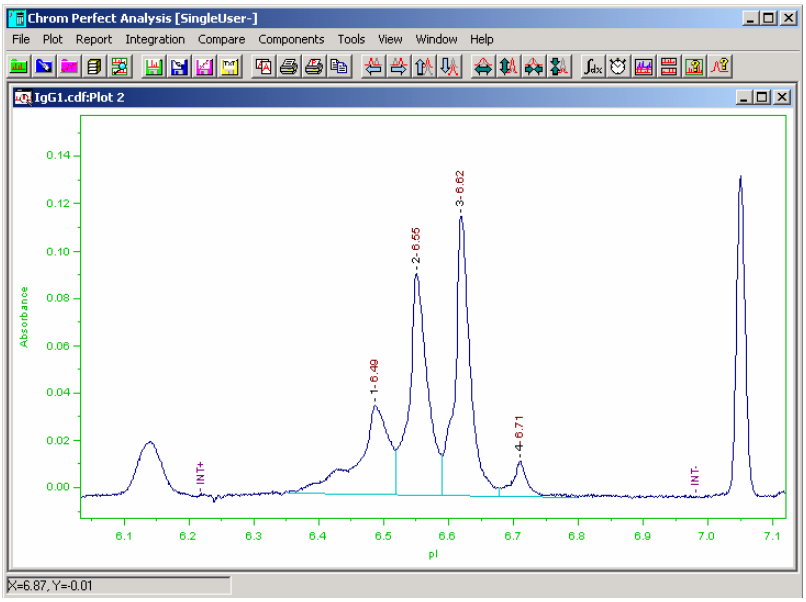


Figure A-20: *Plot Properties (C)*

Save All Settings into a Method

All integration parameters, events and display settings can be saved into a single method.

1. Click **File** > **Save Method** to open the file name inquiry window as shown in Figure A-21 and Figure A-22.

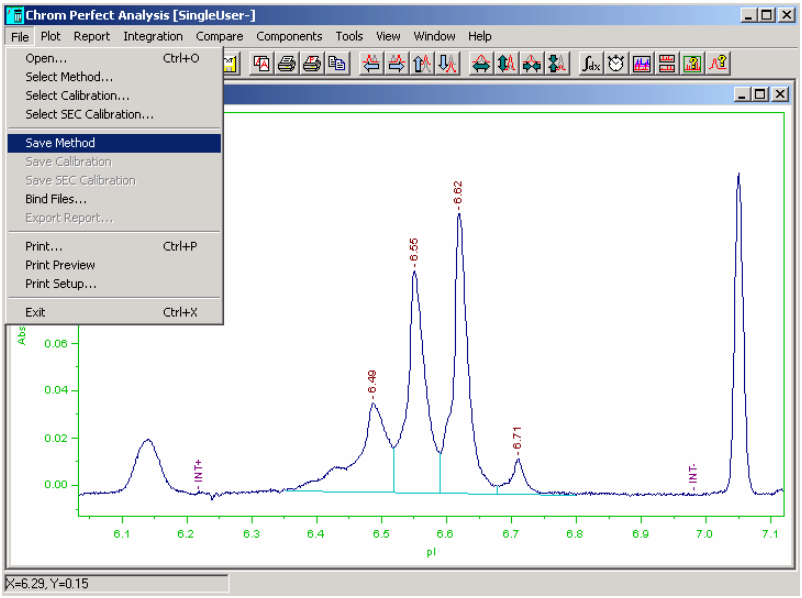


Figure A-21: *File > Save Method Menu*

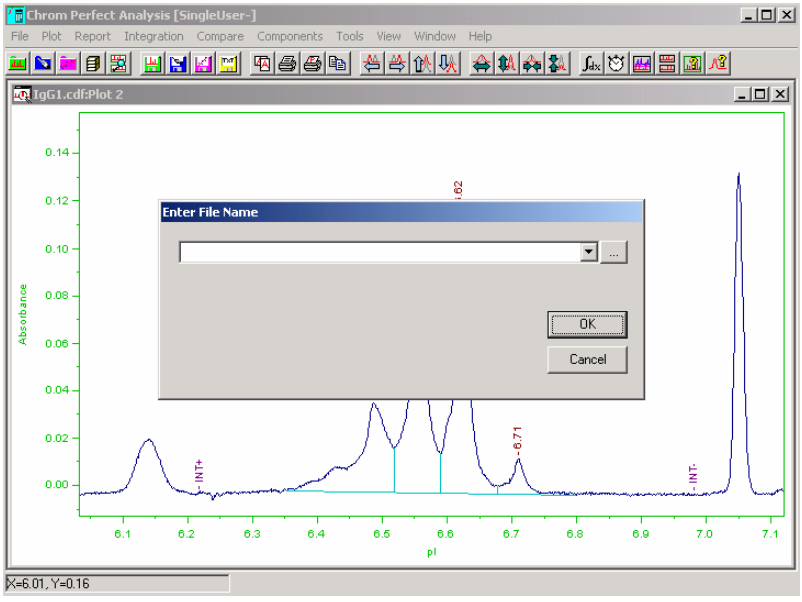


Figure A-22: *Enter File Name*

2. Click the ellipsis (**…**) button at the right side of the file name inquiry window to browse the folder for saving the method.

   In this example, the name for the method is **Test1**.

3. Enter the name of the method and click **Save As** button (Figure A-23).

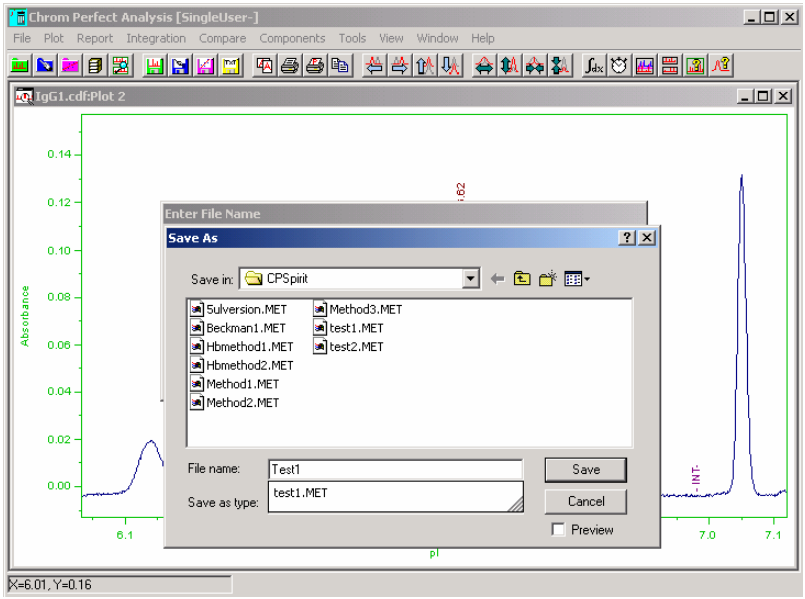


Figure A-23: Save As

Edit Customer Report

Edit Formatted Report File

1. Go back to the main menu of the Chrom Perfect, click **File Editor** button.

   The Chrom Perfect File Editor window will be open (Figure A-24).

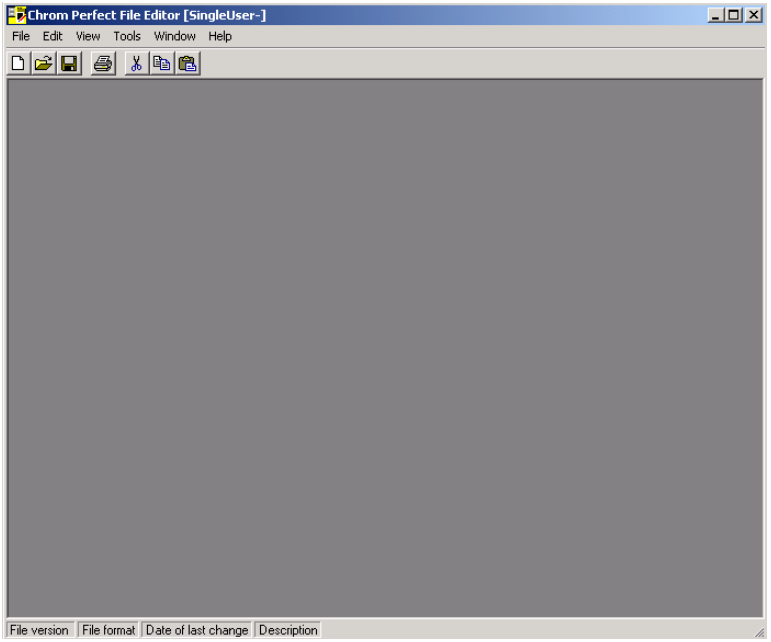


Figure A-24: *File Editor*

2. In the window, click **File** > **Open** to open a file (Figure A-25).

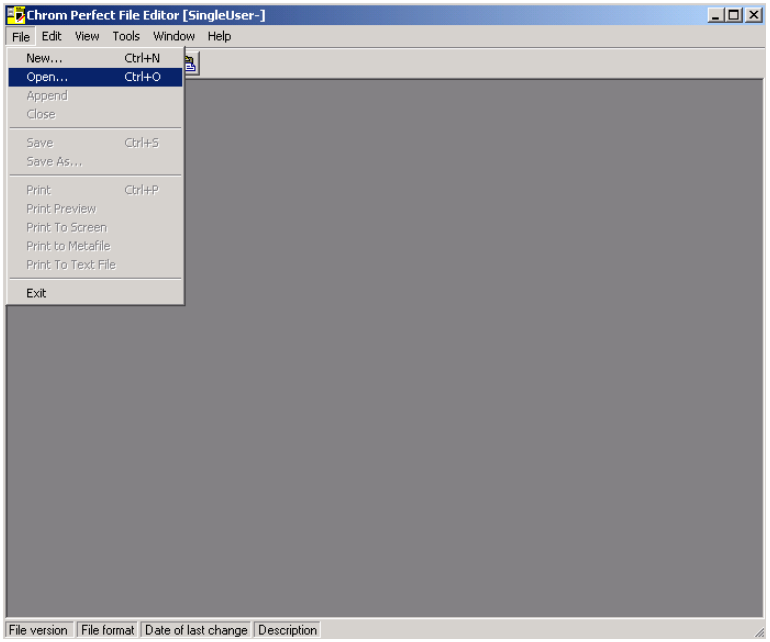


Figure A-25: *File > Open Menu*

Open Existing File window will open (Figure A-26).

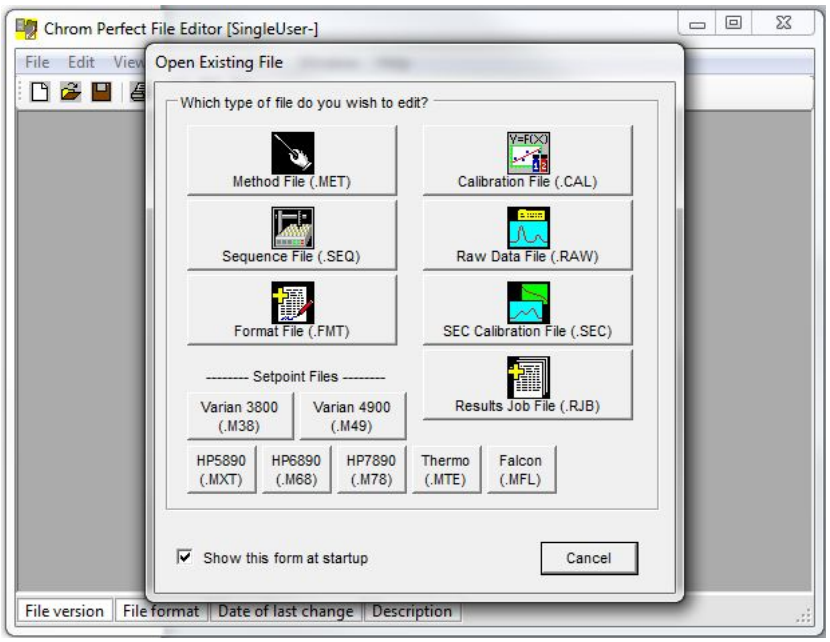


Figure A-26: *Open Existing File*

3. In the window select **Format File** button.

   A file name inquiry window is open (Figure A-27).

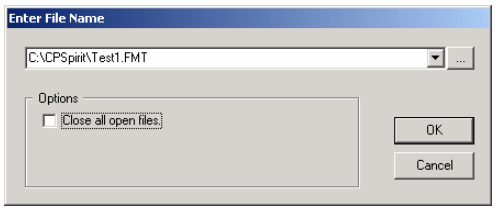


Figure A-27: *Enter File Name*

4. Use browse key (**…**) to open Test1.FMT (Test1 was prepared by ProteinSimple) as generic customer report format, it only needs small change before used for most of application associated with iCE system.

   Figure A-28 is the report format of formatted report **Test1**.

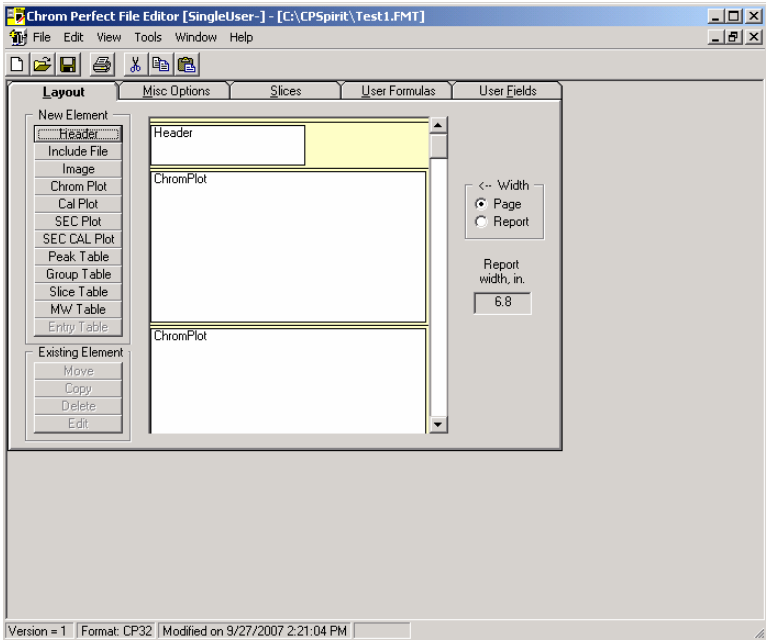


Figure A-28: *Formatted Report (Test1)*

5. In the report, click anywhere on the second ChromPlot.

   Now, as shown in Figure A-29, the second ChromPlot becomes green, and all buttons under Existing Element are active.

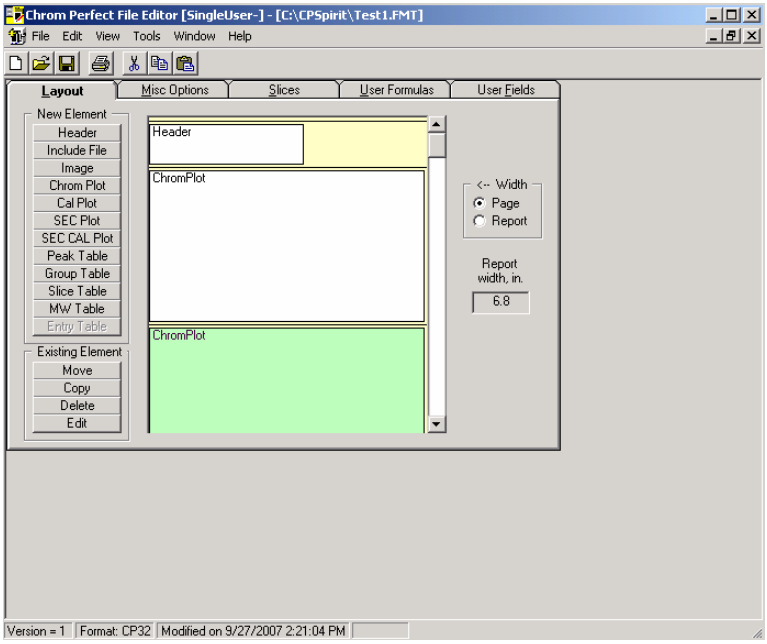


Figure A-29: *Second ChromPlot (Green)*

6. Select **Edit** button under Existing Element to open Chromatogram Plot Properties window (Figure A-30).

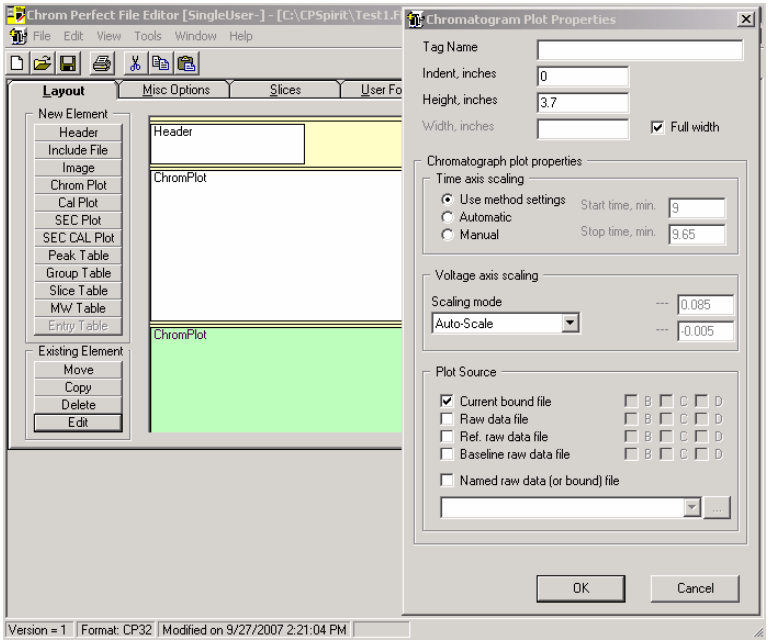


Figure A-30: *Chromatogram Plot Properties*

7. In the window check **Use method settings** under Chromatograph plot properties.
8. Click **OK**.
9. As shown in Figure A-31, click **Yes** button to save the change into Test1.FMT.

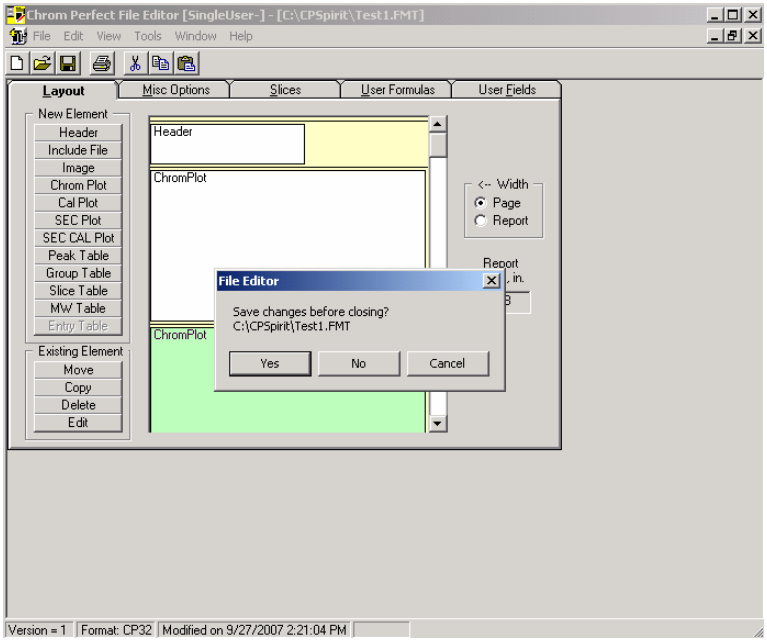


Figure A-31: Saving File Editor Changes

Edit Method File

1. Go back to main menu of Chrom Perfect to select **File Editor** button.
2. Once in Chrom Perfect File Editor window, select **Method File (.MET)** button (Figure A-32).

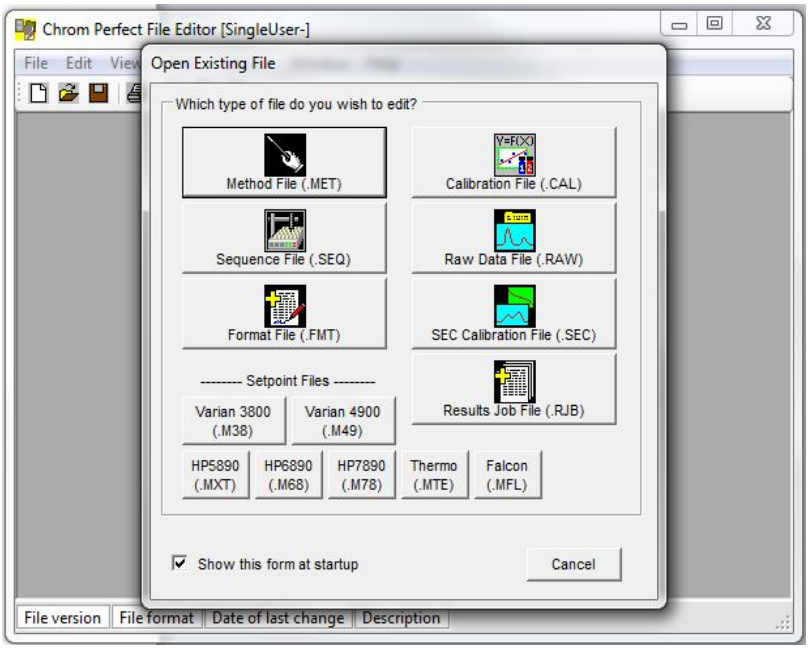


Figure A-32: *Open Method File (.MET)*

3. In the file name inquiry window use browse key (**…**) to open method file **Test1.MET** that saved before. The method file is open as shown in Figure A-33.

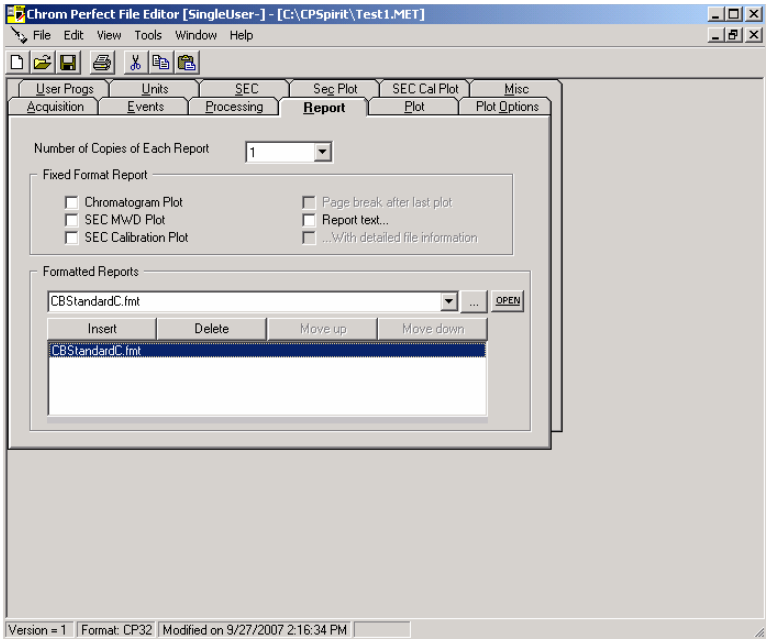


Figure A-33: Report Tab

4. In the window select **Report** tab.
5. Click the first line in the table under the title Formatted Reports.

   Now, browse (**…**) button and **Open** button are active.
6. Open the **Test1.FMT** (the formatted report file that was edited before in "Edit Formatted Report File" on page 272) using the browse button and **Open** button as shown in Figure A-34.

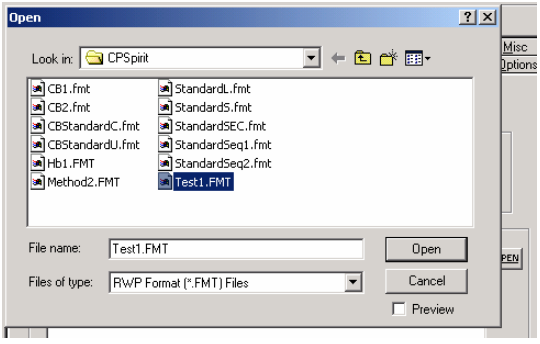


Figure A-34: Open Method File

Now **Test1.FMT** becomes default report format in the method **Test1.MET** as shown in Figure A-35.

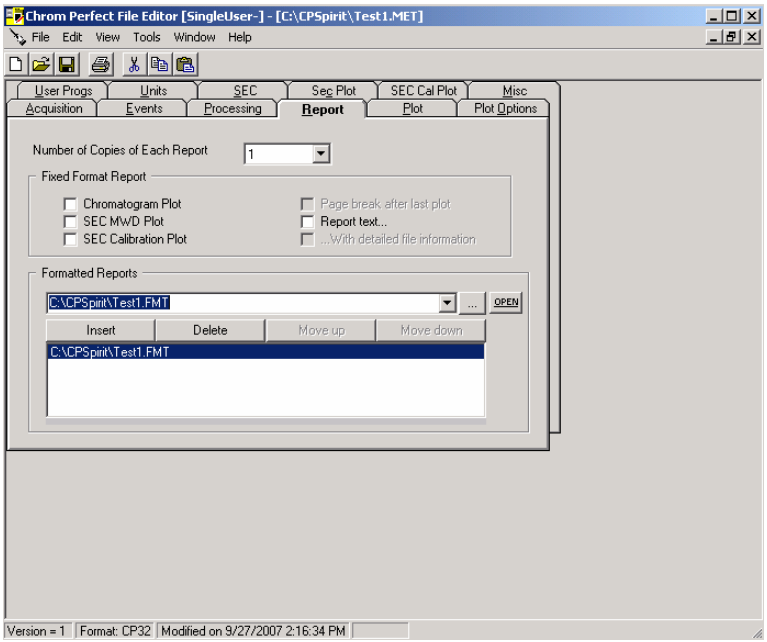


Figure A-35: Default Report Format

7. Click X box at the up-right corner of the window to close the window.
8. Save the changes into the method file **Test1.MET** as shown in Figure A-36.

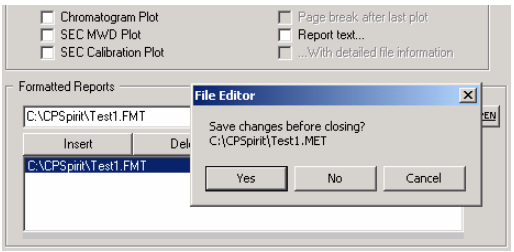


Figure A-36: Save Test1.MET Method File

9. Click **Yes**.

Process Data Files Using the Saved Customer Method

Open Multi Data Files

1. In the main menu, click **Analysis** button to open Chrom Perfect Analysis window.
2. Select **File** > **Open** to open Chrom Perfect Analysis window.
3. In the window select **File** > **Open** to open a file name inquiry window.
4. Use browse button (**...**) at the right side of the window to browse from a directory of data files as shown in Figure A-37.

   In this example, there are six data files of the same sample in the directory.

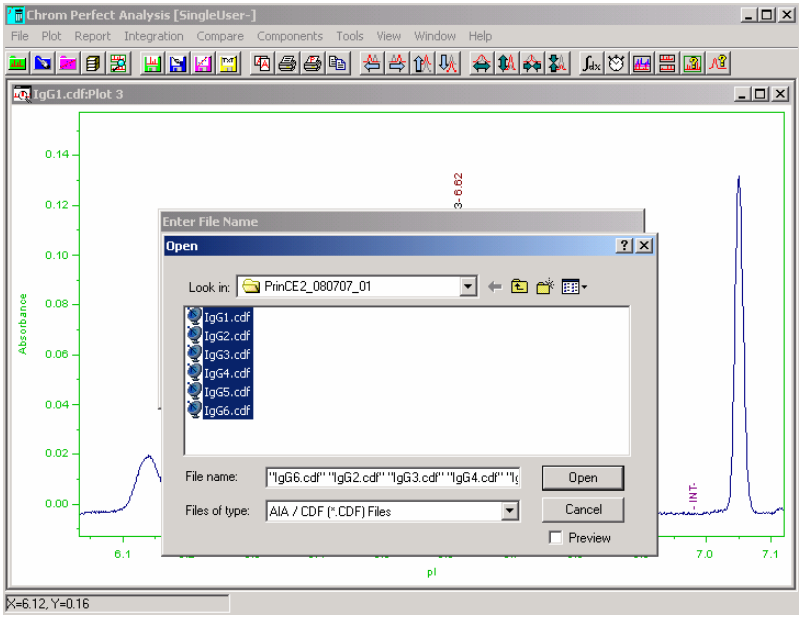


Figure A-37: *Opening Six Data Files*

5. Highlight all the six data files and then click **Open** button to open all these six data files.

   The six data files are display in "Tile" style as shown in Figure A-38.

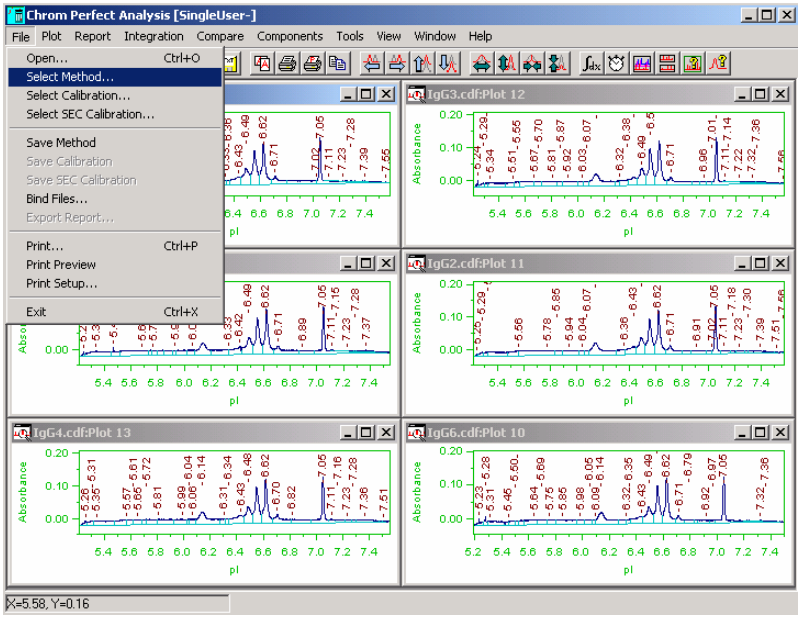


Figure A-38: Data Files Organized in a Tile Format

Apply the Save Method File (Test1.MET) to All Six Data Files

1. Click **File** > **Select Method** to select method file to open the file name inquiry window.
2. In the file name inquiry window (Figure A-39) ensure that the box of **Apply to all chromatograms** is checked.

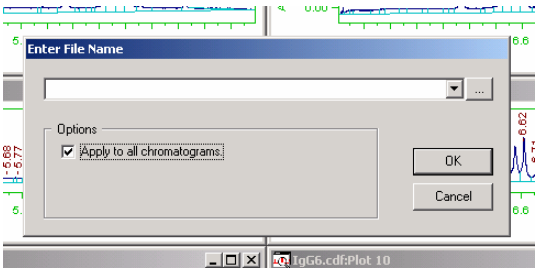


Figure A-39: Apply to All Chromatograms Check Box

3. Then, use the drop-down button to select the cached method filename or use browse button (**…**) at the right side of the window to open the method file (**Test1.MET**) as shown in Figure A-40.

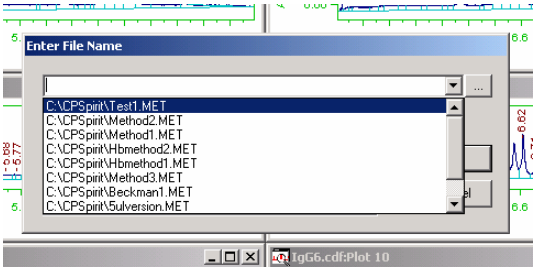


Figure A-40: *Selecting Saved Method Files to Open*

Once the method file is open, it automatically applies to all these six data files. As shown in Figure A-41, sample peaks are labeled as defined in the method.

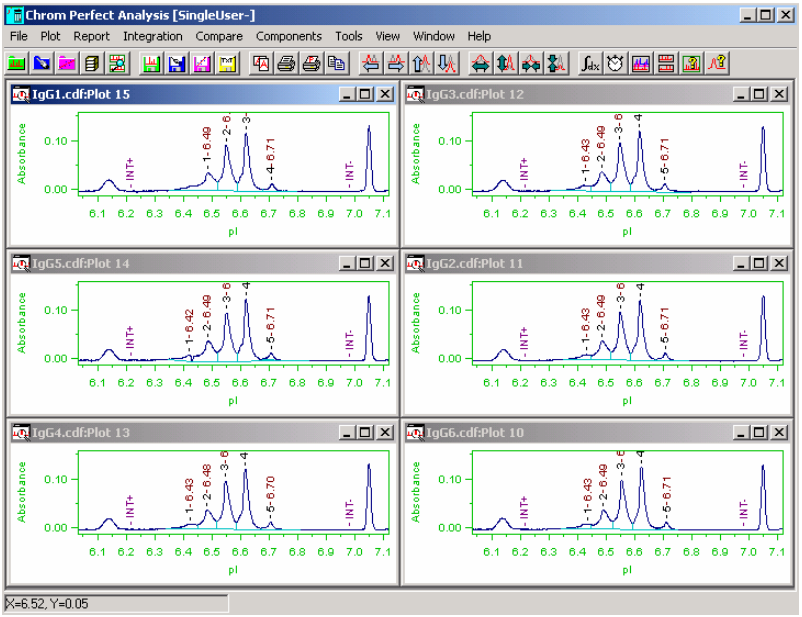


Figure A-41: *Labeled Sample Peaks*

Display or Print the Customer Report for a Data File

1. Highlight a data file by click anywhere on its window.

   In this example, data file **IgG2** is highlighted.

2. As shown in Figure A-42, click **Report** > **Method Format Files** to display customer report of this data file (IgG2) as shown in Figure A-42 and Figure A-43.

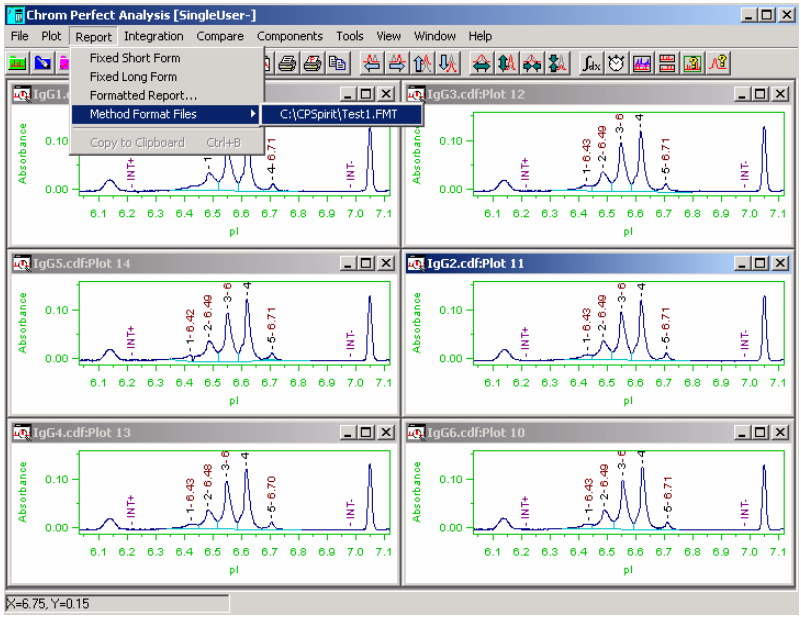


Figure A-42: Displaying Customer Report for Selected Data Files

3. After the customer report is displayed, click **File** > **Print Preview** or **File** > **Print** to display or print out the customer report for the highlighted data file (IgG2).

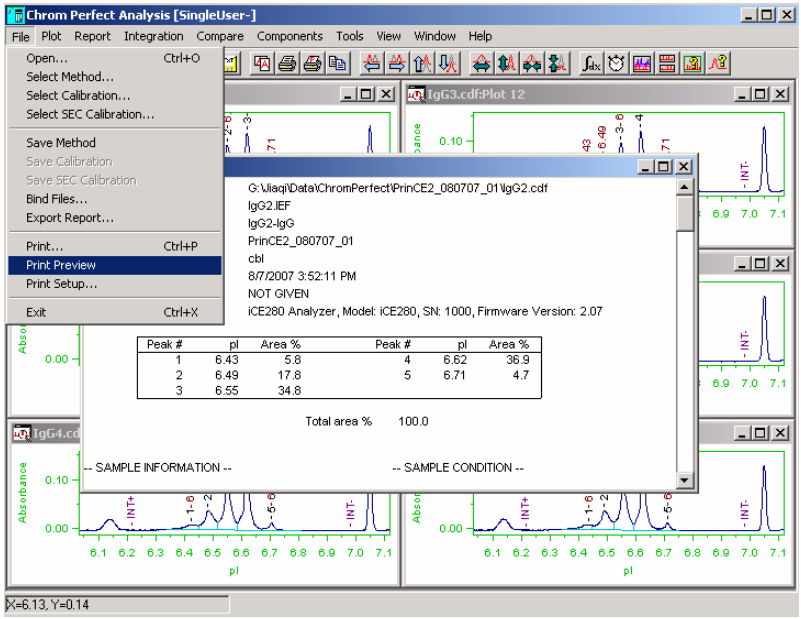


Figure A-43: *File > Print Preview*

Figure A-44 shows the example of Print Preview.

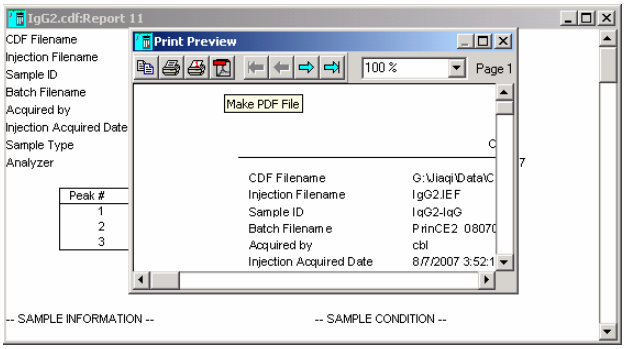


Figure A-44: *Print Preview Sample*

Below is the printed customer report for the data file **IgG6** (Figure A-45 and Figure A-46).

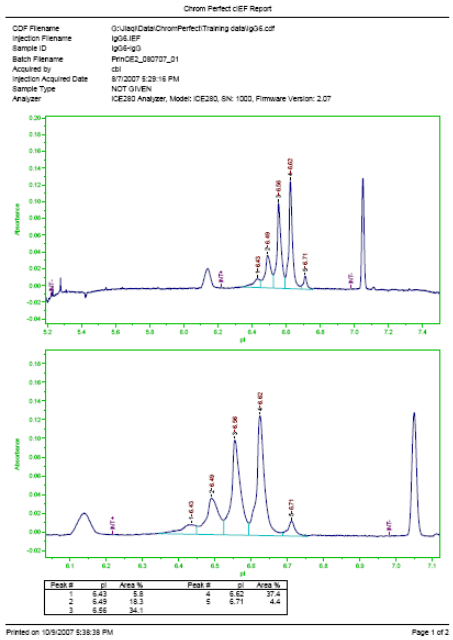
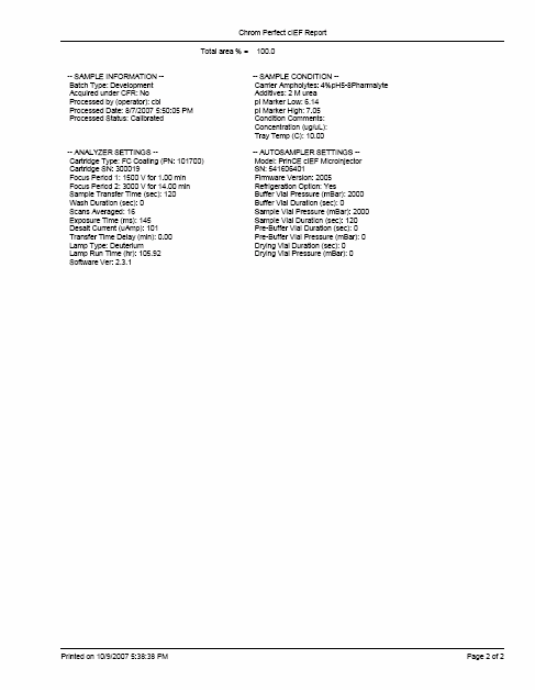


Figure A-45: *Printed Customer Report for IgG6 (page 1)*


Chrom Perfect cIEF Report

Total area % = 100.0

-- SAMPLE INFORMATION --
Batch Type: Development
Acquired under CFR: No
Processed by (operator): cst
Processed Date: 8/7/2007 5:50:08 PM
Processed Status: Calibrated

-- SAMPLE CONDITION --
Carrier Ampholytes: 4%pH3-9Pharmalyte
Additives: 2 M urea
pI Marker Low: 6.14
pI Marker High: 7.05
Condition Comments:
Concentration (ug/uL):
Tray Temp (C): 10.00

-- ANALYZER SETTINGS --
Cartridge Type: FC Coating (PN: 101700)
Cartridge SN: 302019
Focus Period 1: 1500 V for 1.00 min
Focus Period 2: 3000 V for 14.00 min
Sample Transfer Time (sec): 120
Wash Duration (sec): 0
Scans Averaged: 16
Exposure Time (ms): 145
Desalt Current (uAmp): 101
Transfer Time Delay (min): 0.00
Lamp Type: Deuterium
Lamp Run Time (hr): 105.52
Software Ver: 2.3.1

-- AUTOSAMPLER SETTINGS --
Model: PrinCE cIEF MicroInjector
SN: 541635421
Firmware Version: 2005
Refrigeration Option: Yes
Buffer Vial Pressure (mBar): 2000
Buffer Vial Duration (sec): 0
Sample Vial Pressure (mBar): 2000
Sample Vial Duration (sec): 120
Pre-Buffer Vial Duration (sec): 0
Pre-Buffer Vial Pressure (mBar): 0
Drying Vial Duration (sec): 0
Drying Vial Pressure (mBar): 0

Printed on 10/9/2007 5:38:38 PM Page 2 of 2

Figure A-46: *Printed Customer Report for IgG6 (page 2)*

Overlay Multiple Data Traces

NOTE: In this section, the above six pi calibrated data files are used the example.

1. Repeat the procedures in *"Process Data Files Using the Saved Customer Method,"* starting on page 283 and *"Apply the Save Method File (Test1.MET) to All Six Data Files"* to open the six example data traces and apply the Testl method on these six data files.

   The result is shown in Figure A-47.

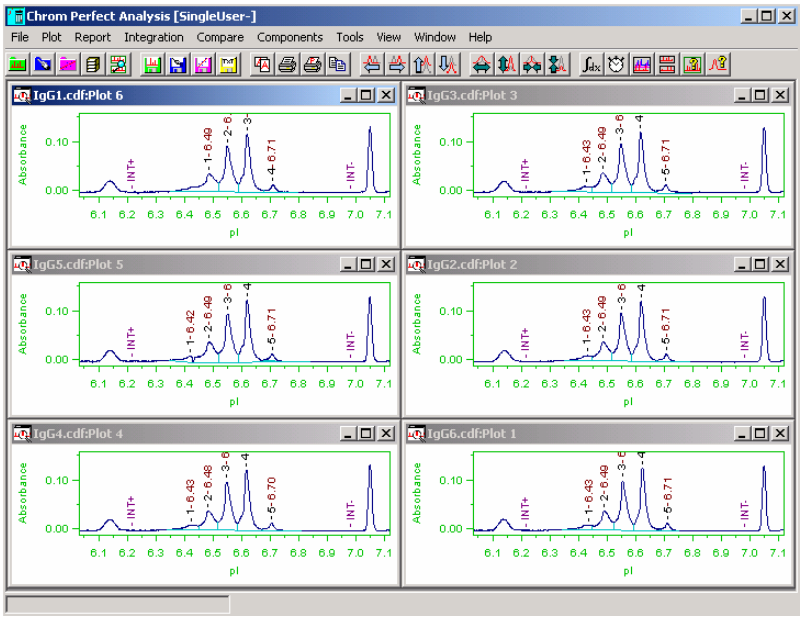


Figure A-47: *Overlay Data Files*

2. In Figure A-47, click **Compare** and select **Overlay** to change the display mode from **Tiled** to **Overlay**, as shown in Figure A-48.

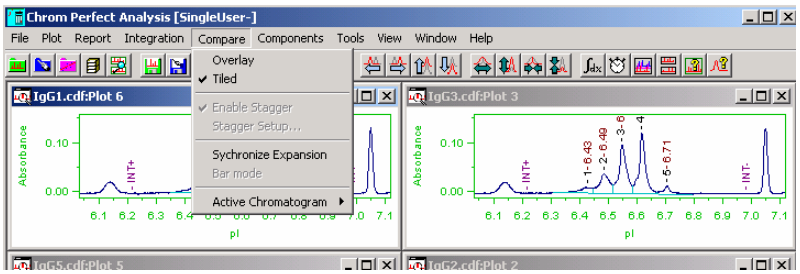


Figure A-48: *Tiled (Display) Mode*

The six data traces are overlaid as shown in Figure A-49.

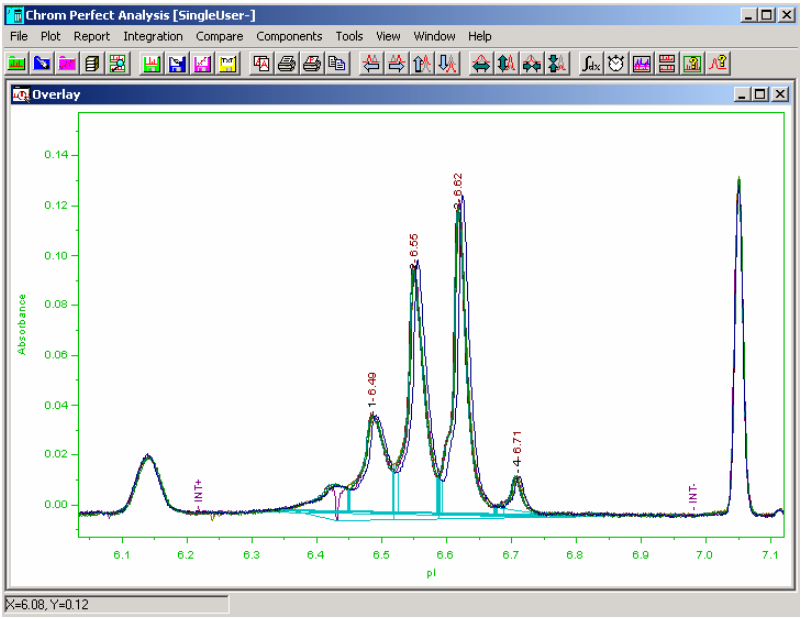


Figure A-49: Overlaid Traces

Any expansion or change in display scale while multiple traces are displayed in **Overlay** mode will only apply to the Active data trace (the highlighted trace when the display in **Tiled** mode).

3. To apply the expansion and change to all the traces, the data traces should be "Synchronized". This can be done by clicking **Compare** and check **Synchronize Expansion** as shown in Figure A-50.

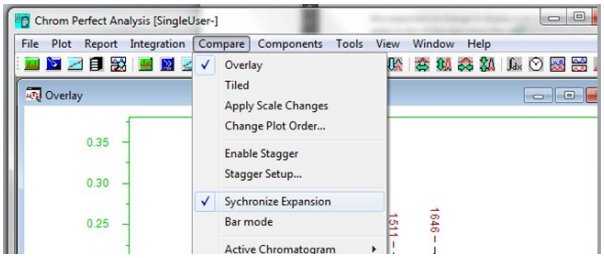


Figure A-50: Synchronize Expansion

Now, all the data traces can be expanded at the same time.

4. As shown in Figure A-51 and Figure A-52, use the mouse to draw a box on the sample peaks and right-click on the mouse to expand the scale of the display.

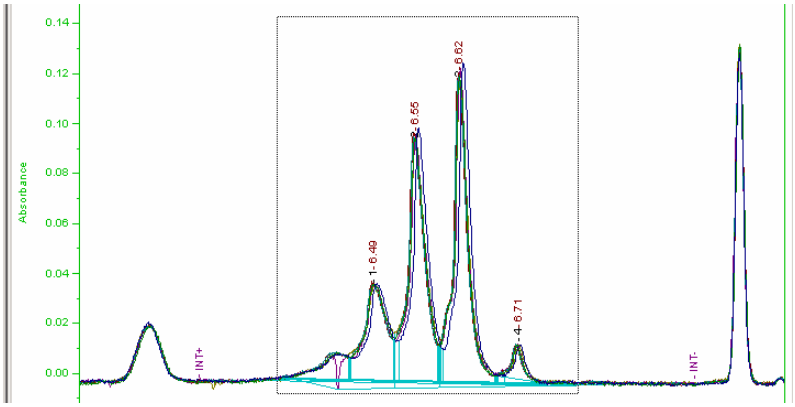


Figure A-51: *Expanding Scale—Example A*

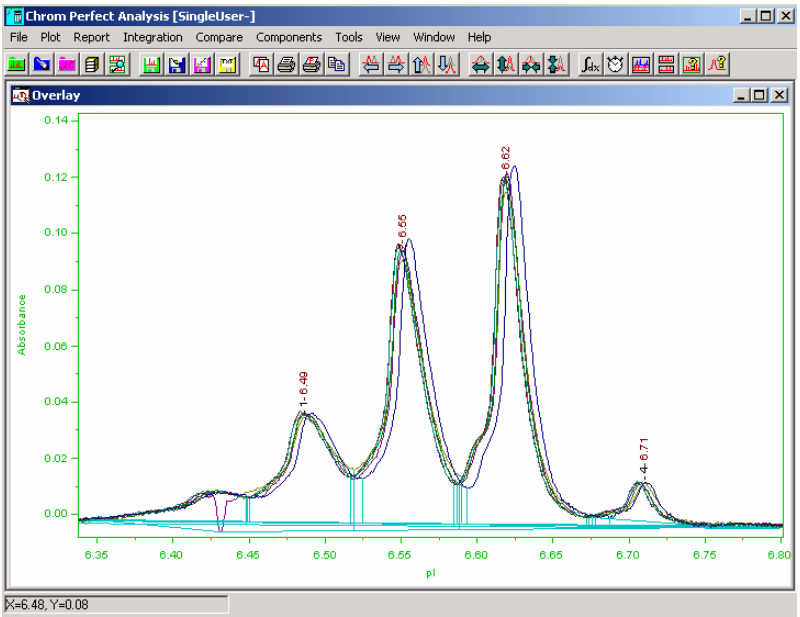


Figure A-52: *Expanding Scale—Example B*

As described earlier, if the **Synchronize Expansion** is not checked, the above action is only applied to the Active data trace.

The Active data trace can be seen and changed by **Click Compare** > **Active Chromatogram** as shown in Figure A-53. The checked file name is the Active data trace.

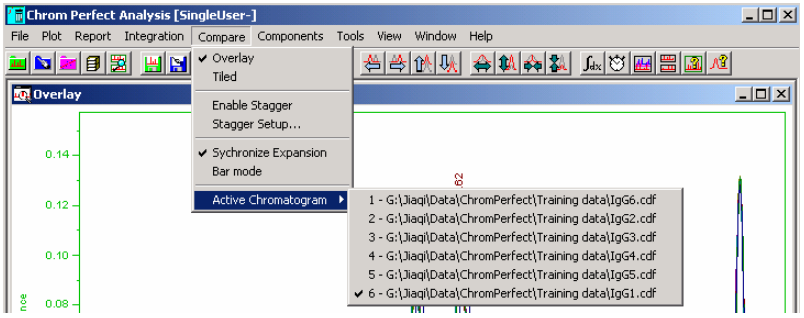


Figure A-53: Active Data Trace

5. Click any other date file name to change the Active data trace.
6. To stagger the multiple data traces, as shown in Figure A-54 and Figure A-55, click **Compare** > **Stagger Setup** to open the Stagger Chromatograms window.

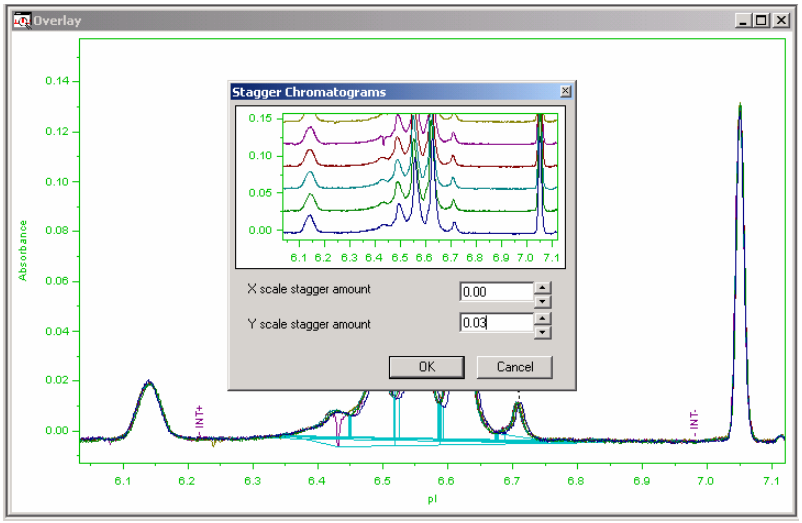


Figure A-54: Staggering Multiple Data Traces (Example A)

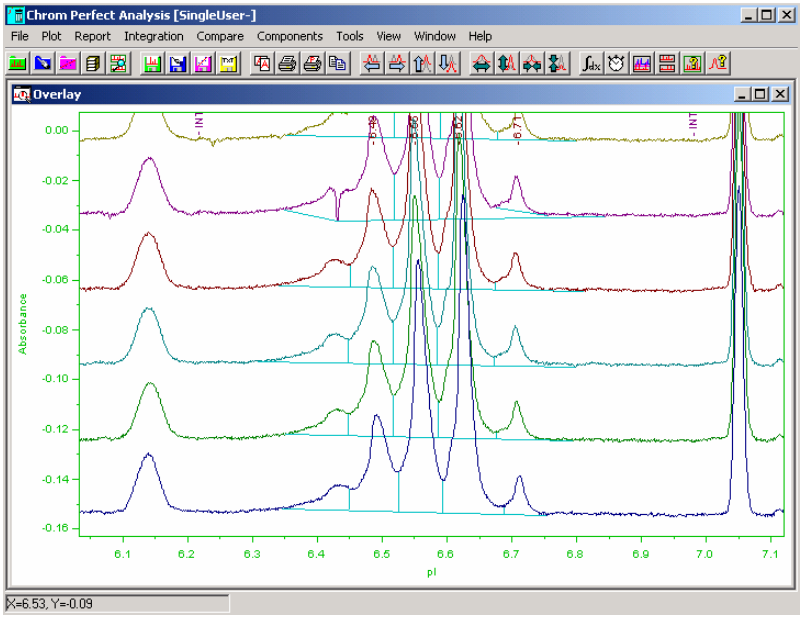


Figure A-55: *Staggering Multiple Data Traces (Example B)*

7. Set X and Y scale stagger amount, then, click **OK** to close the window.

   Now the stagger setup is applied to the display. In the example, the offset at Y scale is set at 0.03.

   In the Figure A-55 example, after the staggering, the data traces are out of maximum Y scale. The maximum Y scale needs to be changed.

8. To change the scale, as shown in Figure A-56, right-click on the mouse, then, click **Scale**… to open Plot Scale window.

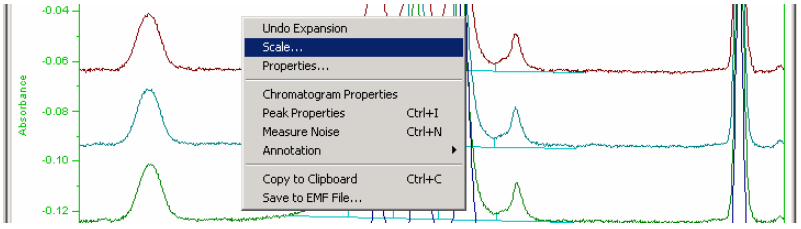


Figure A-56: *Scale Menu—Changing the Scale*

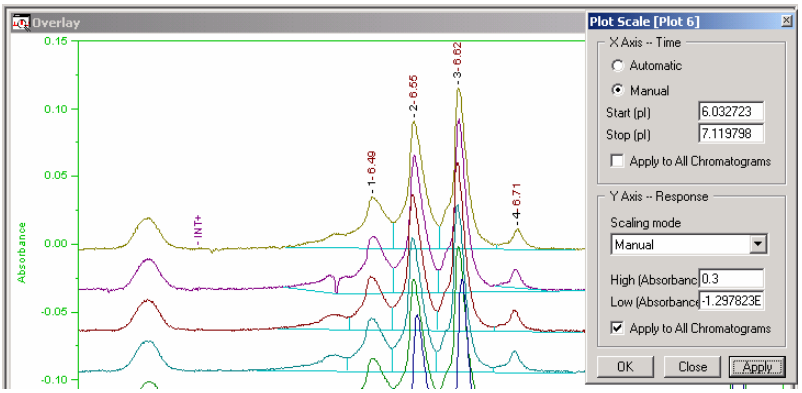


Figure A-57: Changing Plot Scale

9. As shown in Figure A-56:
   a. In the window under the title Y-Axis select **Manual**, change the **High** and **Low**.
   b. Check the box of **Apply to All Chromatograms**.
   c. Click button **Apply** without closing the window.
   d. Repeat the adjustment of the **High** and **Low**, and click **Apply** until achieve satisfactory result.
   e. Click **OK** or **Close** to close the window.

   The final result should be similar to Figure A-58.

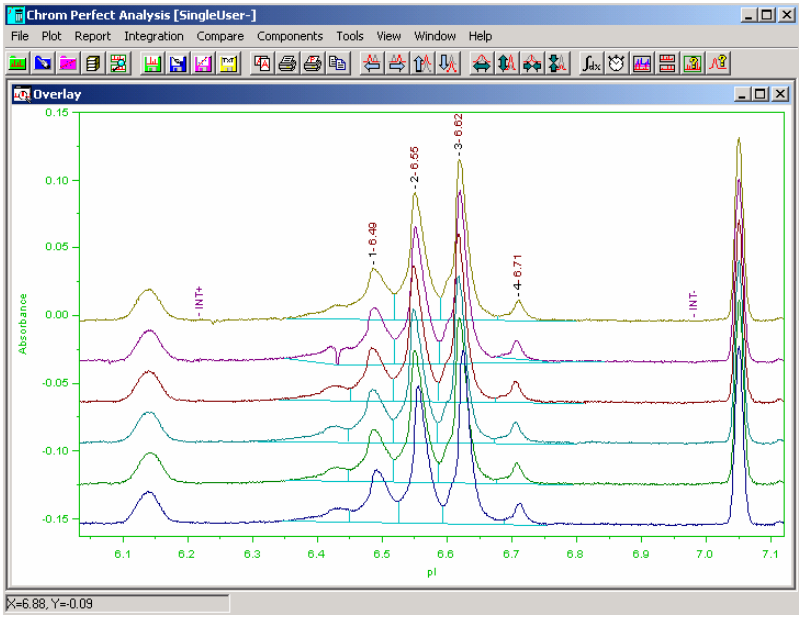


Figure A-58: *Plot Scale Change Results*

Some General Features of Chrom Perfect

What Constitutes a Chrom Perfect System?

All programs sharing a common program directory are part of the same Chrom Perfect system. By default, programs using different program directories are in separate Chrom Perfect systems, but if it is desirable, these may be joined into the same system.

Optional User Management

Chrom Perfect supports user names and passwords when enabled by a flag in the System Manager. When this feature is enabled, users will be forced to log-on, supplying a recognized user name and password. Based on this user name, Chrom Perfect either grants or withholds permission to perform file writes and other actions, according to the permission flags for that user. One user logon name is reserved for the Administrator. Only the Administrator may use the System Manager program and thereby change the security settings, logon names, or passwords.

User log-on names are system-wide in scope, so a user can log-on to any station in the Chrom Perfect system. A user may log-on to more than one station at a time.

When users are forced to log on, Chrom Perfect remembers each user's preferences, so that users do not interfere with one another's work. User preferences are station-local. The following user preferences are supported:

- Any number of search directories may be declared. If a file name is supplied without a path, Chrom Perfect will try to find the file in the search directories, and will append the path of the first search directory that contains the named file.
- Chrom Perfect programs generally remember their last settings and restore these settings the next time they are launched.
- For each type of file, Chrom Perfect remembers the names of the last ten files that the user opened or saved. When the user encounters a file prompt, these names are available in a pull-down list.

Users with responsibility for many projects may possess several logon names, and use one for each project. In this way the user preferences will become more convenient, as they will reflect the last visit to the particular project. Furthermore, it becomes a simple matter to print all log entries for a particular project.

When the user management feature is disabled, the logon dialog is skipped, all users have total power (including Administrator power), and all users share a common user name, "SingleUser". The aforementioned preferences are also common to all users.

User preferences are maintained in a configuration (.INI) file that resides in the working directory, which is often, but not always, the same as the Chrom Perfect program directory. The name of this file is "CP-userNameNN.INI", where *userName* is the user's logon name and *NN* is two numeric characters. In single-user mode, the file name is "CP- SingleUser68.INI".

NOTE: These security features limit user actions only within Chrom Perfect and are not intended as a substitute for the usual Windows NT and network-based security features. In particular, they cannot prevent users from altering or deleting files outside of Chrom Perfect.

Error Messages

When Chrom Perfect encounters a situation where it cannot continue normally, it will generate an error. Errors may be logged and may appear on the screen. Errors that are directly generated by user actions (for example, failure to find a misspelled file) cause an error message to appear on the screen, but in general do not generate error log entries.

Serious errors, such as program errors, normally cause an error message to appear on the screen and generate error log entries. However, in certain cases, when the user may not be present, the screen message is suppressed.

NOTE: If an error message appears on the screen, it must be acknowledged before the program will continue.

File Name Conventions

Chrom Perfect supports long file names. However, certain precautions are in order. First, if they are truly long, the file name may not fit in the display area, making it difficult to see the information. Second, space characters, although legal in long file names, are not recommended. A directory or file name containing a space character may cause trouble when the file is processed with a Method that specifies a user program.

File name specifications are called "unqualified" when the file name does not include a drive and path, or "fully qualified" when the drive and path are present. Most applications assume that an unqualified file name refers to a file in the current working directory. By contrast, when Chrom Perfect encounters an unqualified file name, it first looks in the search directories, in order, and prepares the path of the first search directory that contains a matching file. Only if no search directory contains the specified file will look in the working directory.

When entering file names, the user must decide whether or not to use a path. There are advantages and disadvantages either way. For example, consider the case of a Method file that contains the name of a Calibration file.

If the Calibration file name includes the path, then the Method file is guaranteed to refer to that Calibration file, but the Method file will be "broken" if the Calibration file is moved to another directory, or if these files are moved to another station with a different directory structure. This is more of a problem now than it was under earlier versions of Chrom Perfect because the search directory paths are personal to each user. There-

fore, when users decide to include paths, they must also ensure that their several stations all use the same drive and directory structure, or difficulties may arise when using one station to analyze files that were collected on another station.

If the Calibration file name does not include the path, then the Calibration file must reside in a search directory. If there are Calibration files of the same name in more than one search directory, then the Method file will refer to the first Calibration file found, and the Method file will not be "broken" if the Calibration file is moved to another search directory. Therefore, these files may be shared among users and even moved to another station with a different search directory structure without difficulty.

The following file extensions are reserved by Chrom Perfect:

Extension	File Type
.RAW	Raw files
.BND	Bound files
.MET	Method files
.CAL	Calibration files
.SEQ	Sequence files
.FMT	Format files
.RJB	Results job files
.MXT	HP5890 setpoint files
.M68	HP6890 setpoint files
.ASC	ASCII area files
.LOG	Data-log and error-log files
.ASQ	ASCII to sequence map files

The following file types are used, but not reserved, by Chrom Perfect:

Extension	File Type
.BMP	Bitmap files
.WMF	Windows metafiles
.EMF	Enhanced metafiles
.CDF	AIA Chromatography data format files
.ZED	Formatted reports in an ASCII file

The names of Raw files, and of those files that are derived from Raw files, consist of three parts: the base name, the cycle number (a nonnegative integer in the range 0 - 30000), and the extension. These three sections are separated by period ("dot") characters, for example; "MyFile.1.raw", "MyFile.15.zed", and "VeryLongFileName.0015.bnd".

There is potential ambiguity in the process of creating these file names. For example, the files "MyFile.1.raw" and "MyFile.0001.raw" have the same base name, cycle number, and extension, yet they are not identical. It is possible for both of these files to exist in the same directory. Given a cycle number, how does Chrom Perfect decide which is the right file name? Here are the rules:

1. When browsing for files, the user sees all files and makes the choice.
2. When creating file names, the number of digits is controlled by a system-wide setting.
3. Certain programs support a button which adds leading zeros as necessary until a match is found.

Configurable Page Headers

Chrom Perfect can print a number of different reports and hard copies of most of its file types. The following page properties may be separately configured for each of the supported file types:

- Page margins (top, bottom, left, right)
- Page border lines (top, bottom, left, right)
- Page gutter (just inside the border lines)
- Page header and footer
- Inter-line spacing
- Header and footer font name, size, and other properties
- Watermark

Most of these items are self-explanatory, but some require further explanation.

The page header and footer may contain any constant text, as well as any of several substitution codes that expand at print time to the following items:

- Current date
- Current time
- Current page number
- Total number of pages
- Serial number (system-wide, incremented every time a report is generated)

Any part of the header or footer text may be left-, center- or right-justified.

The watermark is a single line of text, printed vertically in small font within the left margin of the page. A typical watermark is reproduced below:

Chrom Perfect® 4.4.23-10002-MyHostName-00001419

The watermark contains the Chrom Perfect version number (“4.4.23”), the software serial number (“10002”), the host name of the station that generated the report ("MyHostName"), and the print serial number described above (“00001419”). These items provide an audit trail identifying the version of Chrom Perfect that generated the report and the location where it was created. If the report has more than one page, then the identical watermark will appear on each page. Since the print serial number increments every time a report is generated, the watermark provides a way to verify that the several pages in fact belong to the same report.

The “About” Form

All Chrom Perfect programs have a menu item that will launch the “About CP” form. For the Main Menu program, this item is in the popup menu that appears when the user right-clicks on the Menu form. For all other programs, this item is under the Help menu.

The “About” form looks like this:

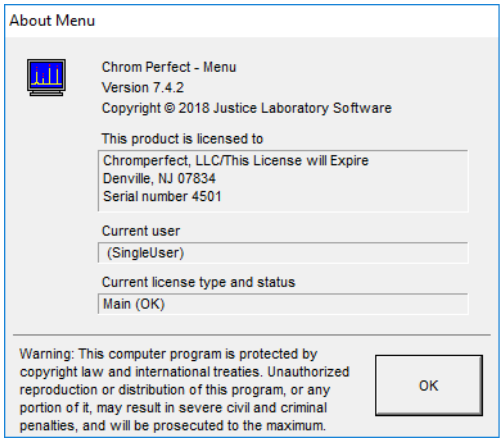


Figure A-59: *About Form*

- The name of the Chrom Perfect program appears in the caption of the form and also in the first line. The software version (Major : Minor : Revision) appears next.
- The customer's name and city and the software serial number are listed next. This information uniquely identifies this copy of Chrom Perfect and its intended user.
- The current user's log-on name and full name appear next.
- The final box lists the type of operating license required by this program. The type is "Main" for most Chrom Perfect programs, or "Programmer" for certain add-on programs and any user-created program that calls Chrom Perfect modules. The if "(OK)" appears after the license type, and then the license was granted. If it does not appear, then the license was denied. A blank field indicates no license. This information becomes valuable when there is some doubt as to the operation of the license.
- Pressing the **System Info** button invokes the standard Microsoft System Information form.

Data, Error, And Alarm Logging

Chrom Perfect supports two log files, the data-log and the error-log.

The data-log records normal actions, such as file writes, printing, and downloading instruments. Most actions that are protected by permission flags are logged.

The error-log records abnormal actions, such as program errors. Errors that are directly generated by user actions (for example, failure to find a misspelled file) cause an error message to appear on the screen, but in general do not generate error log entries.

The alarm-log records user-defined alarm actions, as well as configuration changes within the Alarm utility.

The logging settings consist of the following:

- a master enable flag, that determines whether data logging is active (error logging is always active)
- the log file path, that determines which directory contains the data- and error-log files
- the huge-log notification flag, that determines whether users should be notified whenever the data log becomes huge
- the number of entries that constitutes "huge"
- a number of flags that control which actions result in data-log entries

Logging may be central or station-local, as determined by a flag in the System Manager. If logging is central, then the log settings are editable within the System Manager, all stations in the CP System will use the same settings, and only the Administrator may change these settings. If logging is station-local, then the log settings are editable within the Station Manager, each station in the CP System will set its own settings, and any user with permission may change these settings.

Regardless of whether the log file path is determined at the system or at the station level, the log file directory may reside either on the local station or on a central server. The choice is entirely determined by the path. For example, if the log file path is "C:\logDir", where "C:" represents the local hard drive and "logDir" is a directory on that drive, then each station will maintain its own log files, which will contain only events generated on that station. On the other hand, if the log file path is "ServerName\\logDir", where "ServerName" represents the name of a central server and "logDir" is a shared directory name on that server, then all stations will use the same log files, and those log files will contain all events generated

within the CP System. The difference between local and central log files becomes apparent when the files are to be viewed. Local log files must be viewed locally. Central log files may be viewed from any station in the CP System. When there are several stations, central log files tend to become huge more quickly than do local log files.

If the log file path is blank, then the log files will reside in the working directory, which is usually, but not always, the same as the Chrom Perfect program directory.

The data log can be configured to record all events, or just selected events. If some events are excluded, then the data-log file will not have to be archived as often.

The events include both manual actions (those performed directly by the user) and automatic actions (those performed by Traffic).

Optionally, users may be allowed, or forced, to enter a brief reason for saving the file. This text is stored in the saved file and is also added to the data-log file. This feature is not active for files that are not logged and for files that are saved as part of automatic processing.

The data-log, error-log, and alarm-log files may be viewed and archived from within the History program.