MauriceFlex

cIEF Fractionation Method Development Guide

Introduction

The MauriceFlex cIEF Fractionation Method Development Kit is a one-stop shop for all your method development needs! It includes all sample preparation reagents and a wide range of ampholytes, pI markers, and additives, as well as Fluorescence Calibration Standard and fractionation reagents. We've even included a System Suitability Kit to make sure your MauriceFlex is ready to go. This cIEF Fractionation Method Development Guide will help you every step of the way, and with MauriceFlex's generic methods for multiple molecules, method development has never been easier.

MauriceFlex cIEF Fractionation Method Development Kit contents (PN PS-MDK01-F)

pI Markers

Store at -10 to -30 °C

PN	REAGENT	QTY/AMT*
046-028	Maurice cIEF pI marker – 3.38	210 µL
046-029	Maurice cIEF pI marker – 4.05	210 µL
046-030	Maurice cIEF pI marker – 5.85	210 µL
046-031	Maurice cIEF pI marker – 6.14	210 µL
046-032	Maurice cIEF pI marker – 7.05	210 µL
046-033	Maurice cIEF pI marker – 8.40	210 µL
046-047	Maurice cIEF pI marker – 9.50	210 uL
046-034	Maurice cIEF pI marker – 9.99	210 µL
046-035	Maurice cIEF pI marker – 10.17	210 µL

*After reconstitution

Reagents

Store at 2 to 8 °C

PN	REAGENT	DESCRIPTION	QTY/AMT
102506	Anolyte Solution	0.08 M H_3PO_4 in 0.1% Methyl Cellulose	2 x 10 mL
102506	Catholyte Solution	0.1 M NaOH in 0.1% Methyl Cellulose	2 x 10 mL
102505	0.5% MC	0.5% Methyl Cellulose	2 x 10 mL
101876	1.0% MC	1.0% Methyl Cellulose	1 x 10 mL
046-025	Maurice cIEF Fluorescence Calibration Standard	Fluorescence standard for cIEF Calibration	1 x 5.5 mL
Not sold separately	Urea	Lyophilized urea	5 vials
046-574	SimpleSol Protein Solubilizer	SimpleSol stock solution for protein solubilization for cIEF	1 x 24 mL
042-691	500 mM Arginine	500 mM arginine. For use as a cathodic blocker	1 x 500 µL
046-044	Maurice cIEF System Suitability Kit	The kit contains 8 tubes of lyophilized System Suitability Peptide Panel and 1 vial of System Suitability Test Mix	1 kit
046-580	2 M Ammonium Acetate	2 M ammonium acetate. For use as fractionation mobilizer	1 x 350 µL

Ampholytes

Store at 2 to 8 °C

pH RANGE	DESCRIPTION	QTY/AMT
3–10	Pharmalyte pH 3–10 (Pharmalyte PN 17-0456-01)	200 µL
5–8	Pharmalyte pH 5–8 (Pharmalyte PN 17-0453-01)	100 µL
2.5–5	Pharmalyte pH 2.5–5 (Pharmalyte PN 17-0451-01)	100 µL
8–10.5	Pharmalyte pH 8–10.5 (Pharmalyte PN 17-0455-01)	200 µL
2–9	Servalyt pH 2–9 (seed grade, Servalyt PN 42935)	50 µL

Consumables

Store at 18 to 25 °C

PN	DESCRIPTION	QTY/AMT
101-0059*	cIEF Fractionation Cartridge	1
110-0019	MauriceFlex crimp top glass reagent vials, 2 mL	2 x 100
110-0018	MauriceFlex glass vials with insert, 0.3 mL	15
102-0012**	Maurice 96-well plates	15

*To re-order cIEF Fractionation Cartridges, use PN PS-MC02-F
**To re-order Maurice 96-well plates (quantity 10), use PN 046-021

Ordering info

This kit can be reordered by:

- **Phone:** 1-888-607-9692, option 1
- **Fax:** 1-408-520-4831
- **Email:** orders@proteinsimple.com

Other Things You'll Need

- Maurice cIEF consumables
  - Maurice cIEF Cartridge PN PS-MC02-C
  - Maurice glass reagent vials, 2 mL PN 046-017
  - Maurice clear screw caps for sample and reagent vials, PN 046-138
  - Maurice cIEF blue pressure caps, PN 046-573
- Maurice sample vials with integrated inserts, 0.2 mL, PN 046-083 (optional)
- Deionized (DI) or LC-MS grade water
- Iminodiacetic acid (Millipore PN 22000)
- 15 mL centrifuge tube
- Pipettes and tips
- Microcentrifuge and tubes
- Vortex
- Ice and ice bucket
- Centrifuge with plate adapter (or vial adapter for 12 mm, 2 mL vials if using sample vials PN 046-083)
- Spectrophotometer with 96-well plate adapter or plate reader with 280/350 nm fluorescence (optional)

Buffer exchange - see "Appendix D: Desalting and concentrating samples"

- Amicon Ultracel 50K membrane centrifugal filter (Millipore PN UFC9050)

  NOTE: Select a filter with a molecular weight cutoff appropriate for your protein

- 1 M Tris-HCl buffer pH 7.0 (Life Technologies PN AM9851)

Denaturing samples - see "Appendix E: Denaturing sample preparation."

- Urea, electrophoresis grade (Sigma-Aldrich PN U6504)

  NOTE: A secondary source of urea is only needed if your sample requires 4 M urea to address protein precipitation.

Guidelines to Great Results

- Lyophilized urea must be stored dry, and is provided in a foil pouch with desiccant. Please be sure to reseal the pouch after opening.
- Urea should be prepared fresh. Once the urea is reconstituted, it's good for one day only. Don't reuse.
- Lyophilized peptides should be aliquoted and stored at -20 °C after reconstitution. Reconstituted peptides have a 1-month shelf-life at 2 to 8 °C and a 6-month shelf-life at -20 °C
- pI markers should be diluted at least 50-fold into the sample solution for MauriceFlex Fractionation and MauriceFlex cIEF batches. pI markers should be diluted 100-fold into the sample solution for Maurice cIEF batches.
- Keep your reconstituted pI markers and prepared samples on ice until you're ready to run your batch on MauriceFlex.
- Use LC-MS grade water if fractions will be used for mass spectrometry analysis.
- Use fresh Anolyte, Catholyte, Fluorescence Calibration Standard, 0.5% MC and prepared Mobilizer Solution for each batch.
- Don't reuse reagents or vials.
- Whenever you handle the cartridge or remove it from its packaging, make sure the cartridge inlet doesn't come in contact with any surfaces. Avoid touching the capillary while holding the cartridge.

- Always perform the cartridge post-run cleanup before storing, and always store the cartridge in its original packaging at room temperature.
- Make sure you read the entire cIEF Fractionation Method Development Guide before getting started.

Method development overview

A successfully defined and optimized cIEF fractionation method gives you:

- A peak profile with similar number of peaks as observed for Maurice cIEF
- All peaks for samples and pI markers are visible in the final focusing image

Method development starts with a previously developed Maurice cIEF method, and you'll typically only need to optimize a few parameters from there. For many molecules, adding an additional 20 mM arginine to the final sample with the separation mix used for Maurice cIEF methods provides sufficient fractionation performance and don't need further development.

For more challenging molecules that do not fractionate well and/or have limited solubility, method optimization is accomplished using the strategy in FIGURE 1. The first step is to start with a generic method and evaluate the separation current and focused peak profile. Once you have the desired peak profile, fractionation can be optimized for the desired peak purity and concentration.

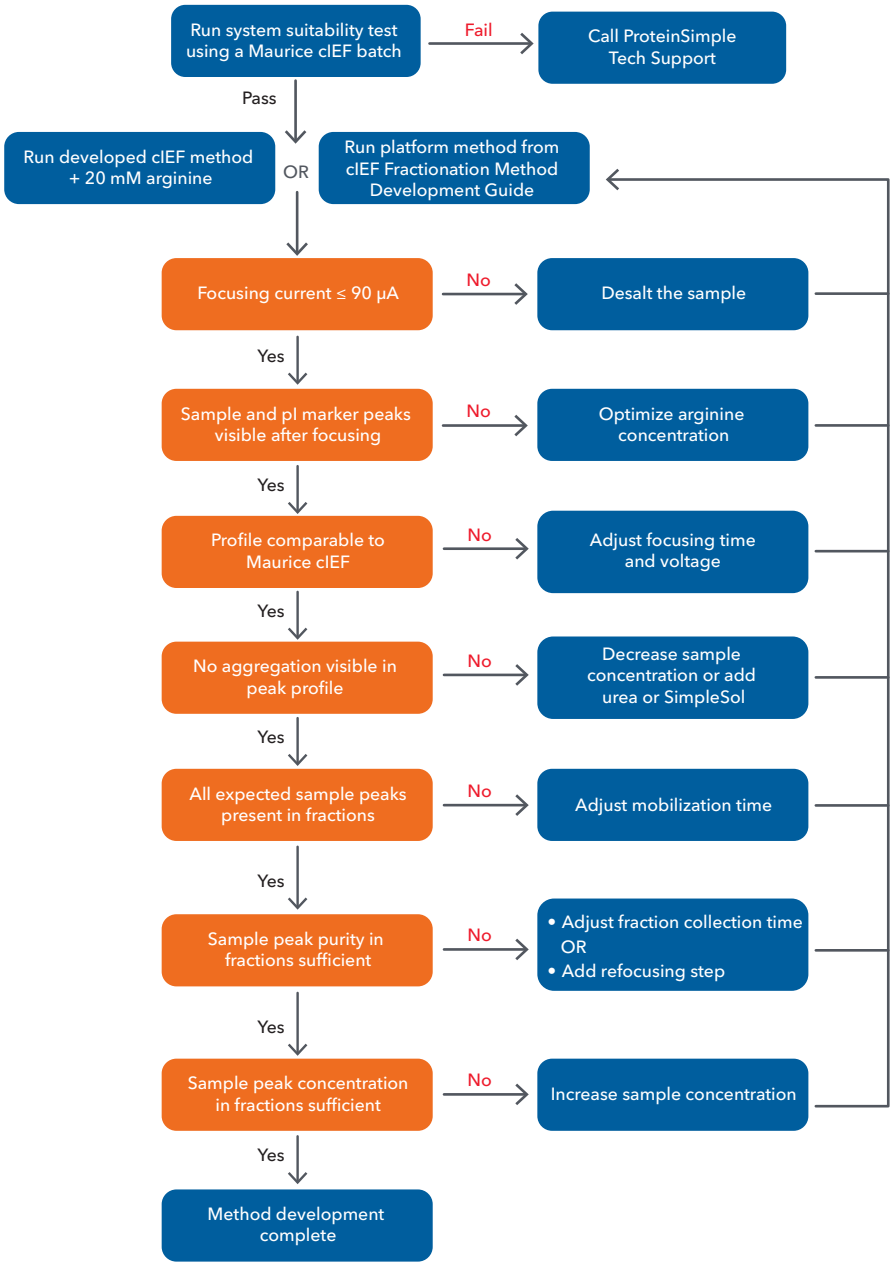


FIGURE 1. cIEF fractionation method development workflow.

Step 1.
MauriceFlex system performance check

The MauriceFlex cIEF Fractionation Method Development Kit includes a System Suitability Test Sample that lets you check cIEF performance before you begin method development.

PREPARE THE SYSTEM SUITABILITY PEPTIDE PANEL

1. Using scissors, carefully cut the top of the foil package leaving the sealing strip intact.
2. Take out the strip of tubes and cut one clear tube of lyophilized System Suitability Peptide Panel from the strip. Return the remaining tubes to the original package, reseal tightly and store at 2 to 8 °C.
3. Pierce the foil on the tube with a pipette tip.
4. Add 40 µL of DI water to the tube. Gently resuspend by pipetting the solution up and down to mix.
5. Add 160 µL of the System Suitability Test Mix to the freshly reconstituted peptide panel. Gently mix by pipetting up and down. Transfer this solution to a 1.5 mL microcentrifuge tube.
6. Vortex the tube 3 times, 5 seconds each.

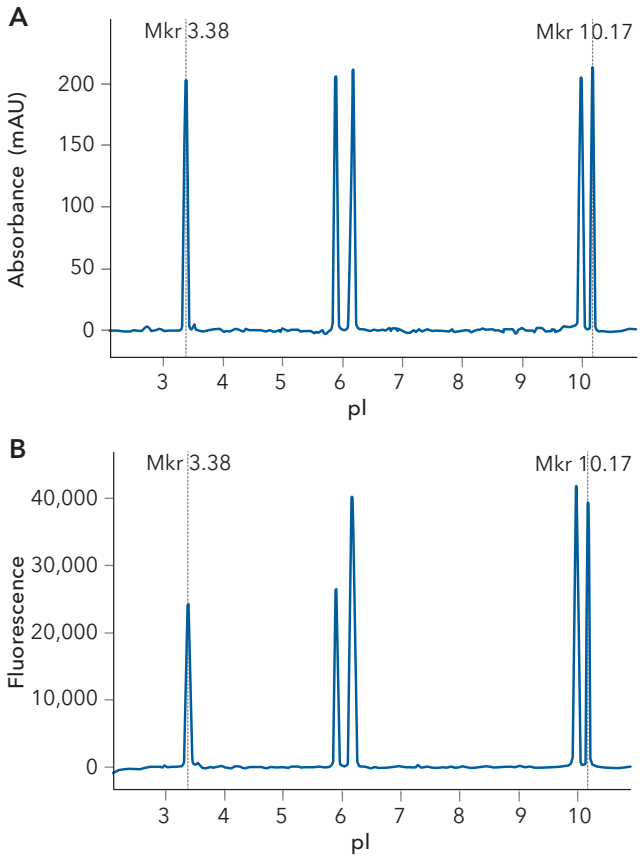


FIGURE 2. System Suitability test UV absorbance result (A) and fluorescence result (B) using a Maurice cIEF batch.

7. Centrifuge the tube at 10,000 xg for 3 minutes to sediment any particulates.
8. Carefully aspirate the top 160 µL of the solution and pipette it into a sample vial with integrated insert or well of a 96-well plate. You'll want to insert the pipette tip all the way to the bottom of the insert or well when you dispense the solution to avoid introducing bubbles.

NOTE: Make sure to check for and remove any bubbles at the bottom of the sample vial or well.

9. If you're using vials, close the sample vial with a clear screw cap.
10. Spin your sample plate or sample vials for 5 minutes at 1000 xg using the appropriate centrifuge adapter.

MAURICE cIEF SET UP AND START

1. Prepare your batch reagents and place them in MauriceFlex. See "Appendix A: Maurice cIEF reagent preparation" for prep details.
2. Lock the batch reagents in place by sliding the locking mechanism from the left to the right.
3. Place the metal 96-well plate insert or the metal vials insert in MauriceFlex and then place your sample plate or vials in the insert.
4. Prepare your cartridge using the procedure in "Appendix B: Maurice cIEF cartridge preparation", then install the cartridge in MauriceFlex.
5. Launch Compass for iCE.
6. Click the **Batch** screen.
7. In the File menu, click **New Batch** and select **Maurice cIEF**.
8. Add samples by highlighting the sample location(s) in the Layout pane and clicking **Add**.
9. Use the default System Suitability method that uses these parameters:

Method Parameter	Setting
Focus Period 1	Time: 1.0 minutes Voltage: 1500 V
Focus Period 2	Time: 4.5 minutes Voltage: 3000 V
Detection	Absorbance: 0.005 seconds Fluorescence: 3, 5, 10, 20 seconds
Sample Load Duration	55 seconds
Standards	pI 3.38: 300 pixels pI 10.17: 1800 pixels

10. Do two sequential replicate injections. Highlight the injection in the Injections pane and click **Replicate** to add a second injection.

11. Save your batch.

12. Click **Start**.

13. After the batch has completed, perform a post-run cleanup of the cartridge as described in "Appendix C: Maurice cIEF Cartridge post-run cleanup"

EXPECTED RESULTS

1. Review your results. If your system is functioning correctly you will see:

- Five peaks for the pI Markers 3.38, 5.85, 6.14, 9.99 and 10.17 as shown in **FIGURE 2A** and **2B**.
- The estimated pI for the 5.85 and 6.14 markers should be between the ranges specified by the COA.
- Peak heights of >100 mAU (absorbance mode with 0.005 second exposure time) and <60,000 RFU (fluorescence mode with 10 second exposure time).
- Resolution of ≥2.1 for the 6.14 marker and ≥1.7 for the 10.17 marker (fluorescence mode with 10 second exposure time).

NOTE: Relative peak heights for the system suitability peptides will change between absorbance and fluorescence measurements as they have different primary amino acid sequences which affects fluorescence emissions.

2. If the System Suitability test results are outside the specified range, we recommend replacing the cIEF Cartridge and repeating the test. In cases where the new cartridge provides the same result, carefully prep a new System Suitability Test Sample and rerun. If the System Suitability test continues to not meet specifications, please contact ProteinSimple Technical Support at support@proteinsimple.com.

Step 2. cIEF Fractionation Method Development

FOCUSING OPTIMIZATION

MauricFlex cIEF fractionation method development starts by verifying the profile of your molecule on a cIEF Fractionation Cartridge.

- Option 1: If you have a Maurice cIEF method already developed for your molecule, we recommend you first perform a run using your cIEF separation mix with additional arginine and increased pI marker added to check if the peak profile of your molecule is similar on a cIEF Fractionation Cartridge.
- Option 2: If you do not have a previously developed Maurice cIEF method, you can develop one following the guidance in the Maurice cIEF Method Development Guide and then perform a follow-up run on the cIEF Fractionation Cartridge following option 1 and this guide.
- Option 3: Use one of the MauriceFlex platform methods listed in this guide to check the peak profile on the cIEF Fractionation Cartridge. Additional optimization may be required.

For all approaches, focusing optimization is first accomplished by running a MauriceFlex cIEF batch using the cIEF Fractionation Cartridge to evaluate the separation current and peak profile. This batch type will only perform cIEF separation and does not include fractionation, allowing for quick verification of the focusing method on MauriceFlex. Due to the longer separation length of the capillary for a cIEF Fractionation Cartridge compared to the Maurice cIEF Cartridge (PN PS-MC02-C), additional arginine must be added to the sample as a cathodic blocker to focus the peaks within the cartridge's optical window. Instructions for preparing samples for MauriceFlex cIEF and MauriceFlex Fractionation batches will follow in the next sections.

Sample components, especially salts, can compromise separation in cIEF methods. Salt concentrations above 10 mM in the sample solution compress the pH gradient and generate a higher separation current that can damage your cIEF Fractionation Cartridge. When you have samples with high protein concentration (>20 mg/mL), diluting the protein down to the concentration in the final sample solution (1 mg/mL) will eliminate enough ionic strength for successful analysis. For samples with high salt and lower protein concentrations, you can use one of the following approaches to optimize your methods for focusing and fractionation on the cIEF Fractionation Cartridge:

- A buffer exchange step to lower or remove salt

  or

- Dilute the sample to reduce ionic strength and pool fractions from more than one fractionation run to collect desired fraction quantity

For more information on how sample components affect cIEF methods, please see the Maurice cIEF Method Development Guide.

Prepare Sample Solution

For your initial run, the sample's final protein concentration should be ~1 mg/mL with a salt concentration ≤10 mM. For example, a 15 mg/mL sample in 150 mM NaCl will need to be diluted 3-fold prior to mixing with the separation mix so you'll have 1 mg/mL protein and 10 mM NaCl in the final prepared sample.

If your sample is low in protein concentration or is high in salt, you may not be able to reach the recommended salt concentration. In cases where further dilution does not generate the desired result, we recommend desalting your sample. Please see "Appendix D: Desalting and concentrating samples" for procedures on how to desalt and concentrate samples.

If you already have a Maurice cIEF method for your molecule, add an additional 20 mM arginine in the final prepared sample when using the cIEF Fractionation Cartridge to ensure all peaks and pI markers are visible after focusing. See FIGURE 3 for a comparison of peak profiles using a developed Maurice cIEF method on Maurice cIEF, and MauriceFlex cIEF before and after addition of 20 mM arginine. If you do not have a previously developed Maurice cIEF method, you can prepare your sample using one of the MauriceFlex platform methods in TABLE 1-3.

1. Dilute each sample to 5 mg/mL in water. If your protein is <5 mg/mL we suggest concentrating the sample. Please see "Appendix D: Desalting and concentrating samples" for the procedure.

2. Prepare 160 µL of MauriceFlex cIEF Master Mix per sample:

    a. If using your own cIEF method, adjust your Master Mix to include 1.5% of each pI marker and an additional 20 mM arginine in the final sample solution.

    b. If using a platform method, prepare a Master Mix based on the quantities in TABLES 1–3, where the number of samples to prepare = X. Choose a Master Mix based on the pI of your molecule.

3. Add 100 µL of MauriceFlex cIEF Master Mix solution to an individual microcentrifuge tube for each sample.

4. Add 25 µL of 5.0 mg/mL protein sample to the 100 µL MauriceFlex cIEF Master Mix in the tube.

5. Thoroughly vortex each sample to mix completely.

6. Centrifuge at 13,000 xg for 5 minutes to remove air bubbles and sediment any particulates.

7. Carefully aspirate the top 100 µL of the solution and pipette it into a well of a 96-well plate. Insert the pipette tip all the way to the bottom of the well when you dispense the solution to avoid introducing bubbles.

    NOTE: Any row in the plate can be used for samples when running a MauriceFlex cIEF batch.

    NOTE: 100 µL of sample will support up to 2 injections. If you want to test multiple methods on the same prepared sample, prepare 150 µL of sample to support up to 4 injections.

8. Spin your sample plate for 5 minutes at 1000 xg using a centrifuge plate adapter.

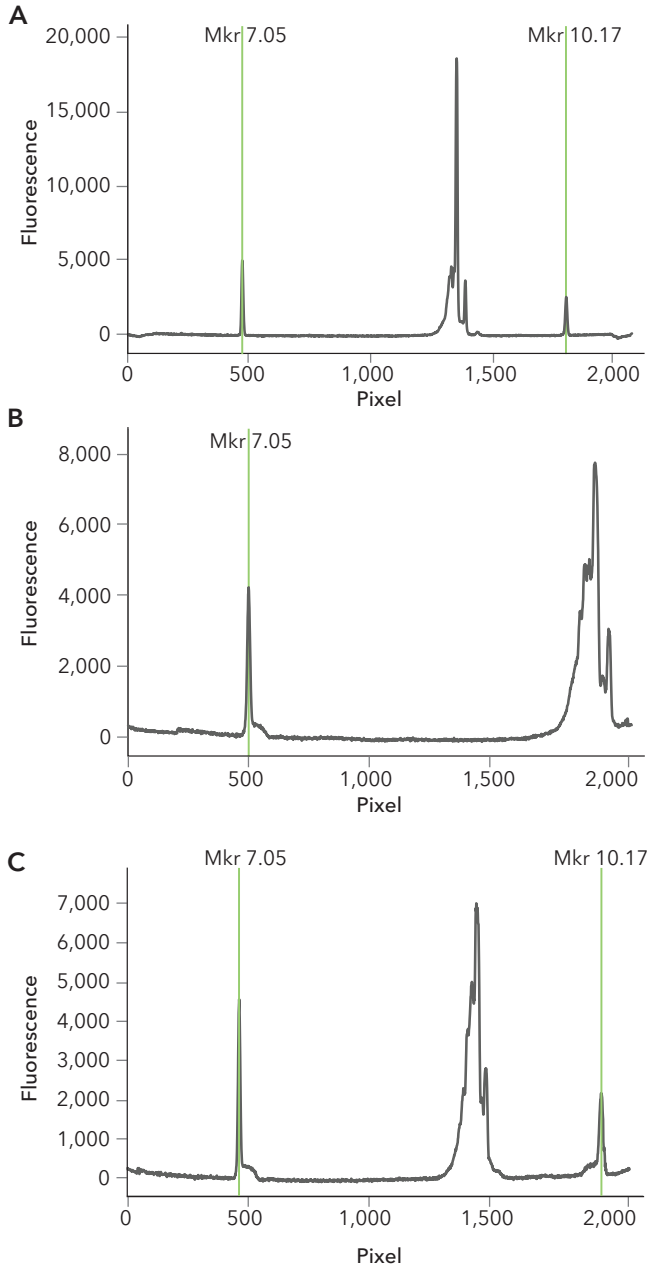


FIGURE 3. Effect of adding an additional 20 mM arginine to the prepared sample for MauriceFlex cIEF methods. NIST mAb was run using a Maurice cIEF sample solution at 0.2 mg/mL on a cIEF Cartridge (A), at 0.5 mg/mL on a cIEF Fractionation Cartridge (B) and at 0.5 mg/mL using the Maurice cIEF sample solution with an additional 20 mM arginine on a cIEF Fractionation Cartridge (C). The addition of 20 mM arginine to the sample solution results in the basic pI marker (10.17) being visible in the cIEF Fractionation Cartridge optical window. The Maurice cIEF sample solution contained 0.35% methyl cellulose, 1% Pharmalyte 3–10, 3% Pharmalyte 8–10.5, and 5 mM arginine as a cathodic blocker. The sample was separated on Maurice cIEF by applying 1500 V for 1 minute, followed by 3000 V for 8 minutes. Samples were separated on MauriceFlex cIEF by applying 500 V for 10 minutes, then 1000 V for 10 minutes, followed by 1500 V for 25 minutes.

MauriceFlex cIEF Master Mix	Final Sample Concentration	Single Sample	Master Mix ((Single Sample * X) + 20%)		
# of samples		1	2	3	4
DI water	N/A	66 µL	158 µL	238 µL	317 µL
1% Methyl Cellulose	0.35%	70 µL	168 µL	252 µL	336 µL
Pharmalyte 3–10	1%	2 µL	4.8 µL	7.2 µL	9.6 µL
Pharmalyte 8–10.5	3%	6 µL	14.4 µL	22 µL	29 µL
500 mM Arginine	25 mM	10 µL	24 µL	36 µL	48 µL
pI Marker 7.05	1.5%	3 µL	7.2 µL	10.8 µL	14.4 µL
pI Marker 10.17	1.5%	3 µL	7.2 µL	10.8 µL	14.4 µL
Total volume		160 µL	384 µL	576 µL	768 µL

TABLE 1. MauriceFlex cIEF Master Mix solution for molecules with most charge variants in pI range 8–10.

MauriceFlex cIEF Master Mix	Final Sample Concentration	Single Sample	Master Mix ((Single Sample * X) + 20%)		
# of samples		1	2	3	4
DI water	N/A	68 µL	163 µL	245 µL	326 µL
1% Methyl Cellulose	0.35%	70 µL	168 µL	252 µL	336 µL
Pharmalyte 3–10	0.8%	1.6 µL	3.8 µL	5.8 µL	7.7 µL
Pharmalyte 5–8	1.6%	3.2 µL	7.7 µL	11.5 µL	15.4 µL
Pharmalyte 8–10.5	1.6%	3.2 µL	7.7 µL	11.5 µL	15.4 µL
500 mM Arginine	20 mM	8 µL	19.2 µL	29 µL	38 µL
pI Marker 6.14	1.5%	3 µL	7.2 µL	10.8 µL	14.4 µL
pI Marker 9.50	1.5%	3 µL	7.2 µL	10.8 µL	14.4 µL
Total volume		160 µL	384 µL	576 µL	768 µL

TABLE 2. MauriceFlex cIEF Master Mix solution for molecules with most charge variants in pI range 7–9.

MauriceFlex cIEF Master Mix	Final Sample Concentration	Single Sample	Master Mix ((Single Sample * X) + 20%)		
# of samples		1	2	3	4
DI water	N/A	60 µL	144 µL	217 µL	288 µL
1% Methyl Cellulose	0.35%	70 µL	168 µL	252 µL	336 µL
Pharmalyte 3–10	1%	2 µL	4.8 µL	7.2 µL	9.6 µL
Pharmalyte 5–8	3%	6 µL	14.4 µL	22 µL	29 µL
200 mM Iminodiacetic Acid	14 mM	14 µL	34 µL	50 µL	67 µL
500 mM Arginine	5 mM	2 µL	4.8 µL	7.2 µL	9.6 µL
pI Marker 4.05	1.5%	3 µL	7.2 µL	10.8 µL	14.4 µL
pI Marker 7.05	1.5%	3 µL	7.2 µL	10.8 µL	14.4 µL
Total volume		160 µL	384 µL	576 µL	768 µL

TABLE 3. MauriceFlex cIEF Master Mix solution for molecules with most charge variants in pI range 5–8.

MauriceFlex cIEF Set Up and Start

1. Place the fractionation adapter in the instrument.

   NOTE: Remove all the vials in rows P and N of the reagent platform before installing the fractionation adapter.

2. Prepare your batch reagents and place them in MauriceFlex. See "Appendix F: MauriceFlex cIEF reagent preparation" for prep details.

3. Place your sample plate on the sample platform.

4. Prepare your cartridge using the procedure in "Appendix G: cIEF Fractionation Cartridge preparation," then install the cartridge in MauriceFlex.

5. Launch Compass for iCE.

6. Click the **Batch** screen.

7. In the File menu, click **New Batch** and select **MauriceFlex cIEF**.

8. By default, the sample location is in well A1. To change location, select A1 and click **Remove** in the Layout pane. Then add a sample by highlighting the sample well location and click **Add**.

9. Use the default method in Compass with the parameters shown in the table below.

   NOTE: All detection images are fluorescence only.

Method Parameter	Setting
Focus Period 1	Time: 10 minutes Voltage: 500 V
Focus Period 2	Time: 10 minutes Voltage: 1000 V
Focus Period 3	Time: 25 minutes Voltage: 1500 V
Focus Period Imaging	Detection Interval: 5 minutes Exposure: 0.2 seconds
Final Focus Detection	Exposure: 0.2 seconds
Sample Load	20 seconds

10. Save your batch.

11. Click **Start**.

12. After the batch has completed, perform a post-run cleanup of the cartridge as described in the "Appendix I: cIEF Fractionation Cartridge post-run cleanup"

Review and Optimize Your Focusing Results

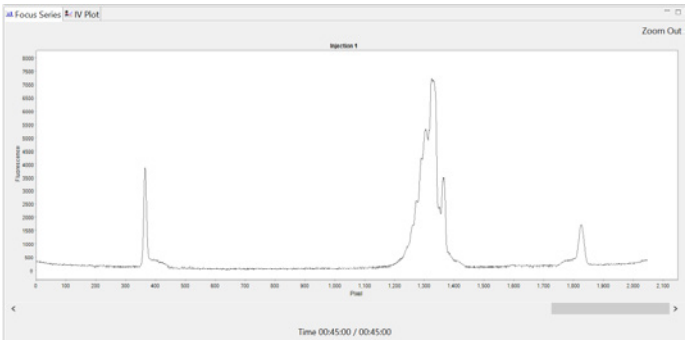
A focusing method is optimized when all peaks (molecule and pI markers) are visible within the cartridge's optical window and has an initial current below 90 µA. An example of the peak profile, and current and voltage plots observed when running the NIST mAb using default separation settings for a MauriceFlex cIEF batch are shown below for reference (FIGURE 4).

Use the following guidelines if additional optimization is required.

1. **Optimize arginine concentration to ensure all peaks are visible.** We recommend adding an additional 20 mM arginine to methods developed on Maurice cIEF that are used for MauriceFlex cIEF fractionation. Increase the arginine concentration if the basic pI marker is not visible or the pI marker position is >1900. You can also adjust the arginine concentration as needed if the pH gradient formed within the capillary is suboptimal. Compression of the pH gradient by arginine is non-linear and may impact peak profile resolution and degree of peak shifting differently depending on the pI of the molecule (FIGURE 5).

2. **Optimize your sample concentration for fluorescence.** When optimizing the sample concentration to verify the peak profile, it's important to avoid artifacts caused by signal saturation. Signal saturation can be identified by peak shapes that flatten near their maxima and may distort the peak profile or reduce resolution between peaks. Peak intensity should be below 60,000 fluorescence units to avoid signal saturation. While MauriceFlex batches are not meant for assessing peak quantitation or resolution, the number of peaks should be comparable to those seen on a Maurice cIEF batch used for the same molecule.

A



B

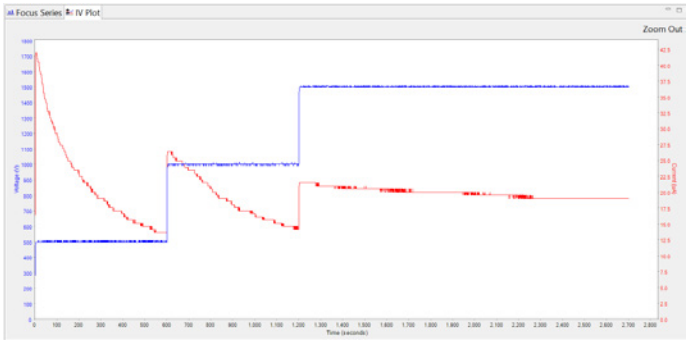
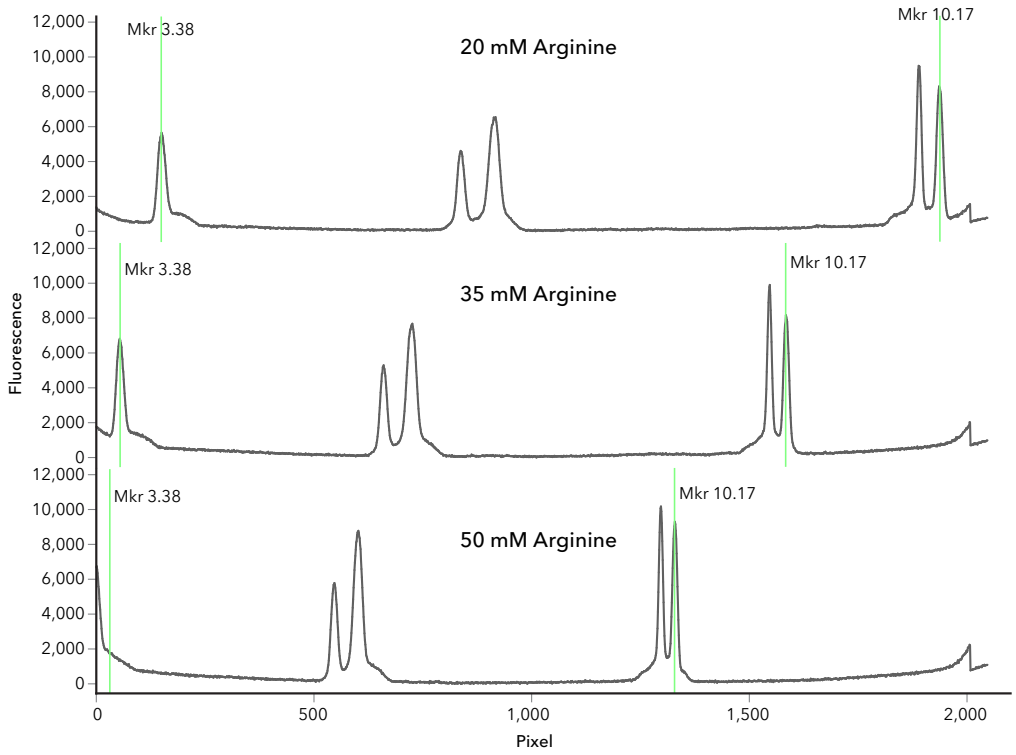


FIGURE 4. MauriceFlex cIEF separation profile (A) and IV Plots (B) of 0.5 mg/mL NIST mAb in Compass for iCE. The sample contained 0.35% methyl cellulose, 1% Pharmalyte 3–10, 3% Pharmalyte 8–10.5, and 25 mM arginine as a cathodic blocker. The sample was separated using a cIEF Fractionation Cartridge on MauriceFlex by applying 500 V for 10 minutes, then 1000 V for 10 minutes, followed by 1500 V for 25 minutes.

A



B

Additional Arginine Added to Sample Solution	3.38 pI Marker Pixel Position	10.17 pI Marker Pixel Position
20 mM	150	1936
35 mM	54	1584
50 mM	34	1329

FIGURE 5. Characterizing the effect of arginine on the pH gradient. An additional 20, 35, or 50 mM arginine was added to the System Suitability test sample (A). Pixel positions for pI markers 3.38 and 10.17 in samples shown in A (B). An unmodified sample contains 0.35% methyl cellulose, 4% Pharmalyte 3-10, 10 mM iminodiacetic acid as an anodic spacer, and 10 mM arginine as a cathodic blocker (results not shown). Samples were separated using a cIEF Fractionation Cartridge on MauriceFlex and applying 500 V for 10 minutes, then 1000 V for 10 minutes, followed by 1500 V for 10 minutes.

3. **Optimize your focusing time and voltage.** The default focusing method does not result in a fully focused profile at the end of separation for some molecules. In these cases, decrease the Focus Period 3 time to 10 minutes and add additional focusing steps as needed (Focus Periods 4 and 5) (FIGURE 6). We recommend starting with 5 minute increments at 2000 V for Focus Period 4, up to a maximum of 10 minutes. Add Focus Period 5 at 2500 V for 5 minutes if needed. Adding additional Focus Periods with increased voltage is recommended over increasing the focusing time for Focus Period 3 as this approach minimizes diffusion and effects from electro-osmotic flow (EOF) on focusing when using a cIEF Fractionation Cartridge. Adjust focusing parameters as needed to get a stable peak profile with minimal EOF.

4. **Optimize your sample to minimize precipitation/aggregation.** Some molecules may show evidence of precipitation/aggregation during the focusing step of a MauriceFlex batch due to the higher sample concentration typically used compared to Maurice cIEF batches (for more information on aggregation/precipitation, please see the Maurice cIEF Method Development Guide). If you have sufficient peak intensity but observe an unexpected peak profile (e.g., spikes), you can either reduce the sample concentration or add protein solubilizer to the sample (FIGURE 7). Adding 2 M urea (final concentration in the prepared sample) to your sample solution will eliminate sample aggregation most of the time. If you prefer to limit the amount of additives used in your sample, you can screen samples using lower concentrations in a single MauriceFlex cIEF batch.

Urea as a Protein Solubilizer

If using urea, first reconstitute the urea and then add to the Master Mix:

1. Add 320 µL of DI water to one vial of lyophilized urea.
2. Vortex to dissolve. This will result in a total volume of 560 µL of 10 M urea.

   NOTE: Prepare urea fresh each time. Once urea is reconstituted, it's only good for one day. Don't reuse.

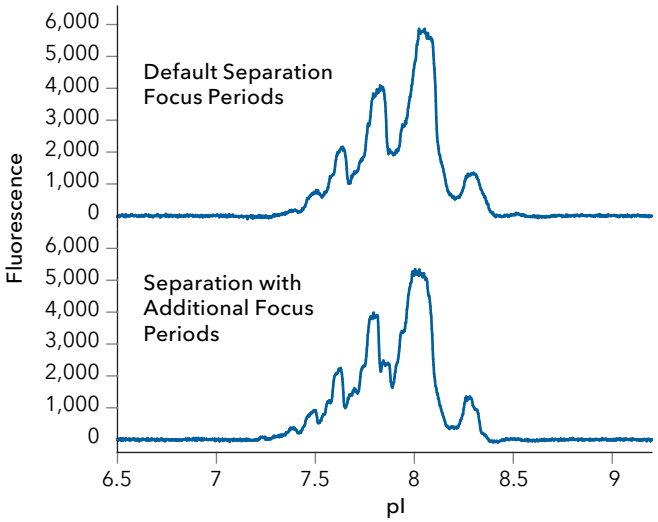


FIGURE 6. Adding additional Focus Periods can improve peak resolution. A mAb at 1 mg/mL was separated using the MauriceFlex cIEF default batch Separation parameters (10 minutes at 500 V for Focus Period 1, 10 minutes at 1000 V for Focus Period 2, and 25 minutes at 1500 V for Focus Period 3) and using optimized Separation parameters with additional focus periods (10 minutes at 500 V for Focus Period 1, 10 minutes at 1000 V for Focus Period 2, 10 minutes at 1500 V for Focus Period 3, 10 minutes at 2000 V for Focus Period 4, and 5 minutes at 2500 V for Focus Period 5). The additional Focus Periods resulted in a peak profile similar to that observed on Maurice cIEF (data not shown). The sample contained 0.33% methyl cellulose, 0.36% Phamalyte 3–10, 1.02% Pharmalyte 5–8, 1.02% Pharmalyte 8-10.5, 3.5 M urea, and 10 mM arginine as a cathodic blocker.

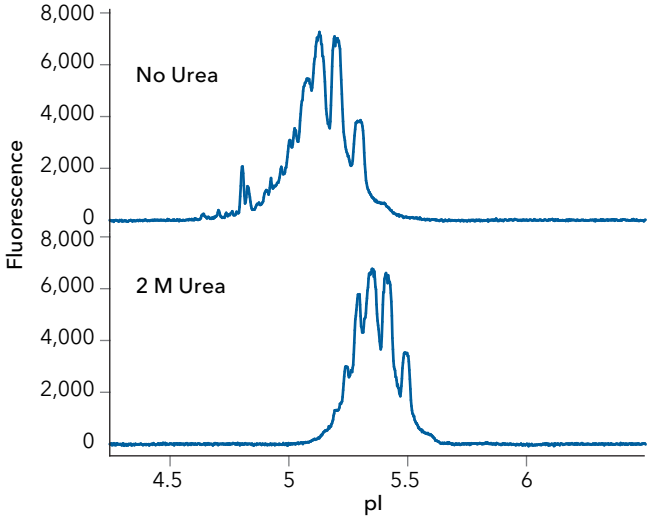


FIGURE 7. Eliminating precipitation/aggregation by adding urea to the sample solution. At 0.5 mg/mL, a separation without urea shows spikes in the peak profile for IgG Standard (PN 046-039). Adding 2 M urea results in well resolved peaks. The slight shift in measured pI is expected for samples with urea. The sample contained 0.35% methyl cellulose, 1% Pharmalyte 3–10, 3% Pharmalyte 5–8, 14 mM iminodiacetic acid as an anodic spacer, and 15 mM arginine as a cathodic blocker. The samples were separated using a cIEF Fractionation Cartridge on MauriceFlex and applying 500 V for 10 minutes, then 1000 V for 10 minutes, followed by 1500 V for 25 minutes.

3. Dilute your sample to the optimal protein concentration determined in your initial screen.

4. Prepare 160 µL of MauriceFlex cIEF Master Mix per sample:

   a. If you are using your own cIEF method, adjust your Master Mix to include 2 M urea, 1.5% of each pI marker, and an additional 20 mM arginine in the final sample solution.

   b. If you are using one of the platform methods in this guide, prepare a Master Mix based on the quantities in TABLES 4–6, where the number of samples to prepare = X. Choose a Master Mix based on the pI of your molecule.

5. Add 100 µL of MauriceFlex cIEF Master Mix solution to an individual microcentrifuge tube for each sample.

6. Add 25 µL of your protein sample to the 100 µL MauriceFlex cIEF Master Mix in the tube.

7. Vortex each sample to mix completely.

8. Centrifuge at 13,000 xg for 5 minutes to remove air bubbles and sediment any particulates.

9. Carefully aspirate the top 100 µL of the solution and pipette it in to a well of a 96-well plate. Insert the pipette tip all the way to the bottom of the well when you dispense the solution to avoid introducing bubbles.

   NOTE: Any row in the plate can be used for samples when running a MauriceFlex cIEF batch.

10. Spin your sample plate for 5 minutes at 1000 xg using a centrifuge plate adapter.

FRACTIONATION OPTIMIZATION

There are two steps in a MauriceFlex Fractionation batch that occur after focusing has completed – a Mobilization step to move the sample peaks closer to the end of the capillary and a Fractions step to collect fractions of mobilized charge variants in a 96-well plate. The same Mobilizer Solution (5 mM ammonium acetate) is used to initiate peak mobilization and to collect fractions in, however no fractions are collected during the Mobilization step. The default time for Mobilization is 25 minutes and the default collection time for Fractions is 45 seconds per well for 36 wells, which takes approximately 31 minutes.

MauriceFlex Fractionation Set Up and Start

1. Place the fractionation adapter in the instrument.

   NOTE: Remove all the vials in row P and N of the reagent platform before installing the fractionation adapter.

2. Prepare your batch reagents and place them in MauriceFlex. See "Appendix H: MauriceFlex Fractionation reagent preparation" for prep details.

MauriceFlex cIEF Master Mix	Final Sample Concentration	Single Sample	Master Mix ((Single Sample * X) + 20%)		
# of Samples		1	2	3	4
DI water		26 µL	62 µL	94 µL	125 µL
1% Methyl Cellulose	0.35%	70 µL	168 µL	252 µL	336 µL
10 M urea	2 M	40 µL	96 µL	144 µL	192 µL
Pharmalyte 3–10	1%	2 µL	4.8 µL	7.2 µL	9.6 µL
Pharmalyte 8–10.5	3%	6 µL	14.4 µL	22 µL	29 µL
500 mM Arginine	25 mM	10 µL	24 µL	36 µL	48 µL
pI Marker 7.05	1.5%	3 µL	7.2 µL	10.8 µL	14.4 µL
pI Marker 10.17	1.5%	3 µL	7.2 µL	10.8 µL	14.4 µL
Total volume		160 µL	384 µL	576 µL	768 µL

TABLE 4. MauriceFlex cIEF Master Mix solution with urea for molecules with most charge variants in pI range 8–10.

MauriceFlex cIEF Master Mix	Final Sample Concentration	Single Sample	Master Mix ((Single Sample * X) + 20%)		
# of Samples		1	2	3	4
DI water		28 µL	67 µL	101 µL	134 µL
1% Methyl Cellulose	0.35%	70 µL	168 µL	252 µL	336 µL
10 M urea	2 M	40 µL	96 µL	144 µL	192 µL
Pharmalyte 3-10	0.8%	1.6 µL	3.8 µL	5.8 µL	7.7 µL
Pharmalyte 5-8	1.6%	3.2 µL	7.7 µL	11.5 µL	15.4 µL
Pharmalyte 8-10.5	1.6%	3.2 µL	7.7 µL	11.5 µL	15.4 µL
500 mM Arginine	20 mM	8 µL	19.2 µL	29 µL	38 µL
pI Marker 6.14	1.5%	3 µL	7.2 µL	10.8 µL	14.4 µL
pI Marker 9.50	1.5%	3 µL	7.2 µL	10.8 µL	14.4 µL
Total volume		160 µL	384 µL	576 µL	768 µL

TABLE 5. MauriceFlex cIEF Master Mix solution with urea for molecules with most charge variants in pI range 7–9.

MauriceFlex cIEF Master Mix	Final Sample Concentration	Single Sample	Master Mix ((Single Sample * X) + 20%)		
# of samples		1	2	3	4
DI water	N/A	20 µL	48 µL	72 µL	96 µL
1% Methyl Cellulose	0.35%	70 µL	168 µL	252 µL	336 µL
10 M urea	2 M	40 µL	96 µL	144 µL	192 µL
Pharmalyte 3–10	1%	2 µL	4.8 µL	7.2 µL	9.6 µL
Pharmalyte 5–8	3%	6 µL	14.4 µL	22 µL	29 µL
200 mM Iminodiacetic Acid	14 mM	14 µL	34 µL	50 µL	67 µL
500 mM Arginine	5 mM	2 µL	4.8 µL	7.2 µL	9.6 µL
pI Marker 4.05	1.5%	3 µL	7.2 µL	10.8 µL	14.4 µL
pI Marker 7.05	1.5%	3 µL	7.2 µL	10.8 µL	14.4 µL
Total volume		160 µL	384 µL	576 µL	768 µL

TABLE 6. MauriceFlex cIEF Master Mix solution with urea for molecules with most charge variants in pI range 5–8.

3. Prepare your sample using the method determined from “Focusing Optimization” and pipet the sample in to a well in row A of a 96-well plate. For your first fractionation run, add 30 µL of Mobilizer Solution (5 mM ammonium acetate) to all wells in rows B–D of the same 96-well plate.

   NOTE: When running a MauriceFlex Fractionation batch, samples can only be added to row A of a 96-well plate.

4. Place your sample plate with Mobilizer Solution on the fractionation adapter.

5. Prepare your cartridge using the procedure in "Appendix G: cIEF Fractionation Cartridge preparation", then install the cartridge in MauriceFlex.

6. Launch Compass for iCE.

7. Click the **Batch** screen.

8. In the File menu, click **New Batch** and select **MauriceFlex Fractionation**.

9. By default, the sample location is in well A1. To change location, select A1 and click **Remove** in the Layout pane. Then add a sample by highlighting the sample well location in row A and click **Add**.

10. Use the default method with the parameters shown in the table below.

    NOTE: All detection images are fluorescence only.

Method Parameter	Setting
Focus Period 1	Time: 10 minutes Voltage: 500 V
Focus Period 2	Time: 10 minutes Voltage: 1000 V
Focus Period 3	Time: 25 minutes Voltage: 1500 V
Focus Period Imaging	Detection Interval: 5 minutes Exposure: 0.2 seconds
Final Focus Detection	Exposure: 0.2 seconds
Sample Load	20 seconds
Mobilization Period	Time: 25 minutes Voltage: 1000 V Detection Interval: 1 minute Exposure: 0.2 seconds
Fractionation Period	Time: 45 seconds per well Voltage: 1000 V Detection Interval: 1 minute Exposure: 0.2 seconds
Fraction Well Location	Rows: B–D Wells: 1–12 (Total of 36 wells)

11. Save your batch.

12. Click **Start**.

13. After the batch has completed, perform a post-run cleanup of the cartridge as described in "Appendix I: cIEF Fractionation Cartridge post-run cleanup"

    NOTE: Depending on the stability of your molecule, you can seal and store the fractionation plate at 4 °C or -20 °C, or you can remove individual fractions and store as appropriate.

Review Your cIEF Fractionation Results

An optimized cIEF fractionation method should result in mobilization and collection of the sample peaks in fractions in the 96-well plate. An example of peak mobilization and current and voltage plots when running the NIST mAb using the default settings for a MauriceFlex Fractionation batch are shown below for reference (FIGURE 8).

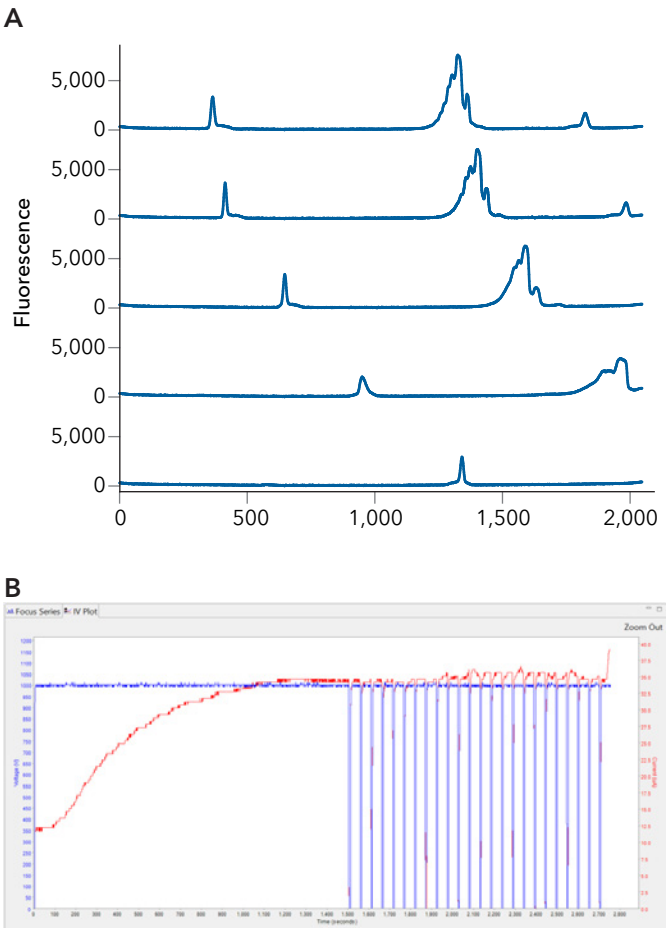


FIGURE 8. Mobilization electropherograms (A) and IV Plots (B) of 0.5 mg/mL NIST mAb during fractionation in Compass for iCE. The sample contained 0.35% methyl cellulose, 1% Pharmalyte 3–10, 3% Pharmalyte 8–10.5, and 25 mM arginine as a cathodic blocker. The sample was separated using a cIEF Fractionation Cartridge on MauriceFlex and applying 500 V for 10 minutes, then 1000 V for 10 minutes, followed by 1500 V for 25 minutes, and then mobilized by applying 1000 V for 25 minutes. After the Mobilization step, fractions were collected every 45 seconds while applying 1000 V.

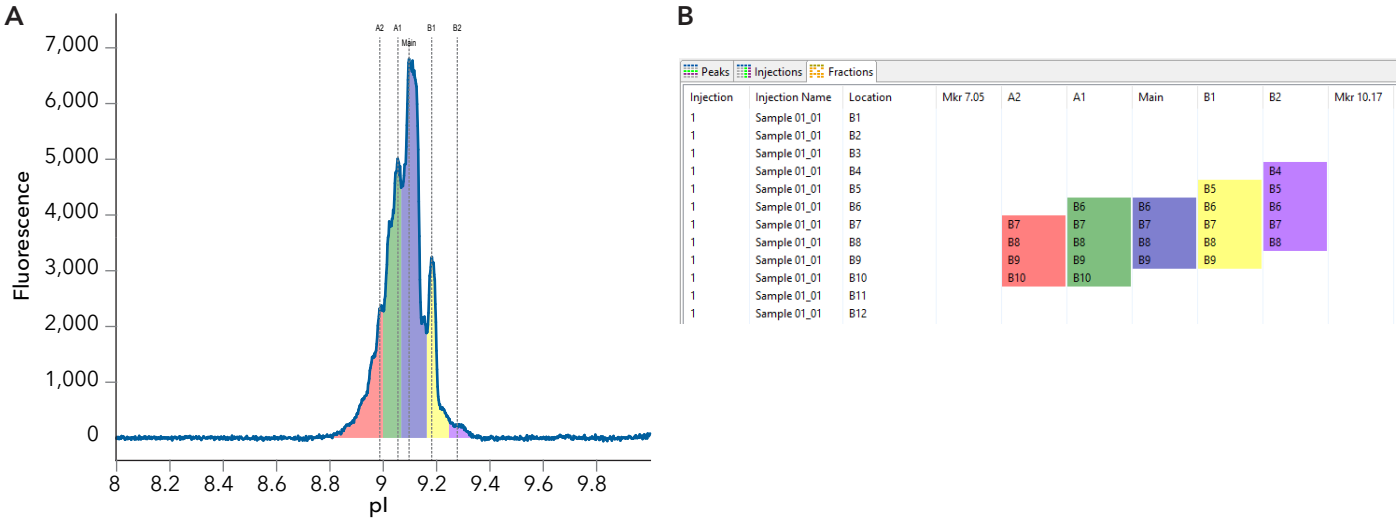


FIGURE 9. Peak profile for NIST mAb (A) and corresponding predicted fraction well locations for each charge variant (B) in a MauriceFlex Fractionation batch. The sample was prepared, separated, and fractionated using the same conditions as in FIGURE 8.

To verify the presence and identity of charge variants in specific fractions, we recommend performing a Maurice cIEF batch with the fractions and a sample with the unfractionated molecule as a reference. Peaks detected in the fractions can be aligned based on pI with peaks in the unfractionated reference sample to verify identify. Peak purity can be assessed in the fractions by measuring percent peak area for each peak detected in a fraction. We recommend using fluorescence detection in a Maurice cIEF batch for calculating peak purity and estimating concentration of charge variants within a fraction.

Choosing Fractions to Check Purity and Identity

You can choose which fractions to include by using the predicted well location of the peaks under the Fractions window in Analysis (see FIGURE 9). The predicted fraction well locations in Compass for iCE are a best estimate of which peaks are present in fractions collected in the 96-well plate. The prediction algorithm was designed using a mAb as a model, so you may need to check wells that are outside of the predicted range for the peaks depending on your molecule.

To ensure the best prediction possible, you may need to adjust the Peak Find - Threshold and Width settings under Analysis - Peak Fit. Due to wider peaks when using the cIEF Fractionation Cartridge, we recommend using two Peak Fit Analysis Groups. When using the default MauriceFlex Fractionation analysis settings, the “Peak Fit” Analysis Group is applied to the electropherogram for the focused sample and “Peak Fit Fractions” is applied to Fractionation data, which are the mobilization electropherograms. See default settings in FIGURE 10. Peak Find Threshold and Width settings for "Peak Fit Fractions" should be adjusted as

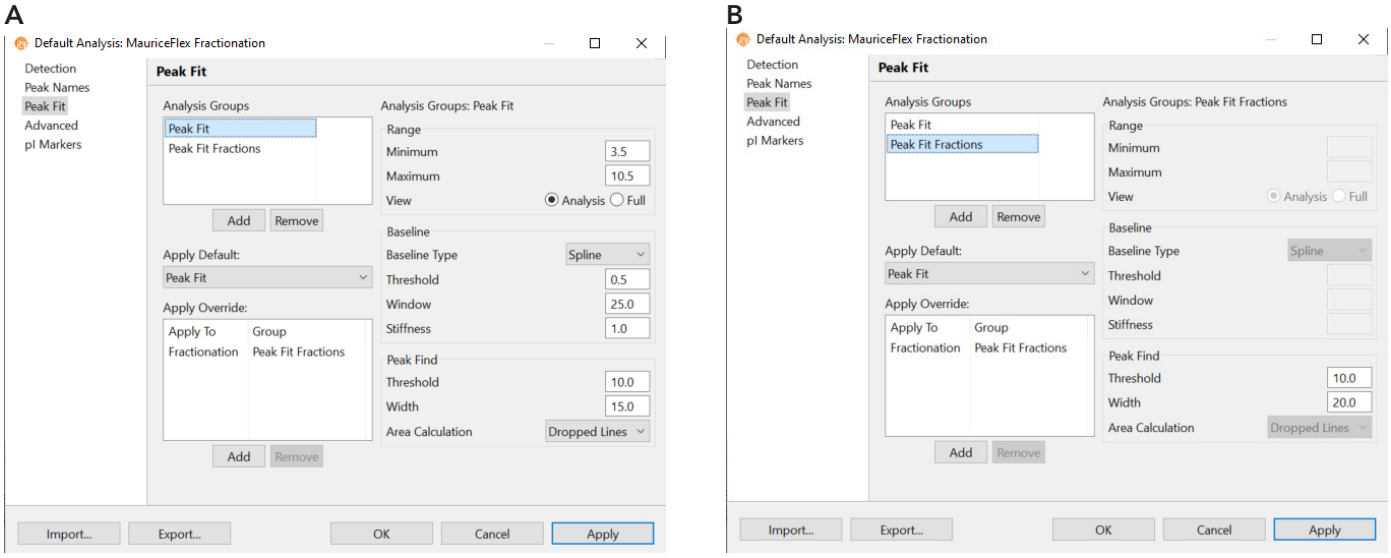


FIGURE 10. Default Peak Fit settings for a MauriceFlex Fractionation batch. The Width for the “Peak Fit” Analysis Group is set to 15 (A) and is automatically applied to the electropherogram for the focused sample. The Width for “Peak Fit Fractions” Analysis Group has Peak Width set to 20 and is applied to Fractionation data, which are the mobilization electropherograms (B). Only the Threshold and Width settings can be changed for Peak Fit groups applied to Fractionation data.

needed to ensure that the most acidic pI marker is accurately identified in all mobilization electropherograms as this is important for predicting the well location of collected peaks.

Once you are satisfied with the Peak Fit settings, we recommend running all fractions that are predicted to have peaks as well as five fractions before the first fraction containing the most basic peak of interest is predicted and five fractions after the last fraction containing the most acidic peak of interest is predicted.

You may also use a spectrophotometer with a plate adapter that measures fluorescence to estimate the well location of the most abundant charge variants. You will need a spectrophotometer with "read from top" capabilities. Read the fractionation plate containing the fractions with an excitation setting of 280 nm and emission setting of 350 nm to identify wells that may contain charge variants. You may need to adjust your plate reader settings to account for the height of the MauriceFlex 96-well plate, which has a height of 16.1 mm. However, while this is a quick method to identify fraction(s) with the most abundant charge variant, it is not as sensitive as checking fractions using a Maurice cIEF batch and will not identify fractions containing low abundance charge variants. We recommend choosing the seven wells before and seven wells after the well with the highest measured signal from the plate reader to run in a Maurice cIEF batch to confirm identity and purity of the sample in the fractions.

Step 3: Running Fractions on Maurice cIEF

Prepare Fraction Samples

If you have previously developed a Maurice cIEF method for your molecule, use the same separation mix (Master Mix) and separation parameters for the fractions and reference sample, but dilute the unfractionated reference sample in Mobilizer Solution (5 mM ammonium acetate) prior to mixing with the Master Mix. We recommend diluting the unfractionated reference sample in Mobilizer Solution to a concentration that is 5-fold lower than the sample concentration used for the MauriceFlex Fractionation batch. Then mix both the diluted unfractionated reference sample and the fraction samples with the Master Mix using the same sample to Master Mix ratio. If you are using one of the platform methods from this guide, prepare the unfractionated reference sample and fractions with one of the Master Mixes in TABLES 7–9 and use the Maurice cIEF assay parameters as described in the following sections. Example sample preparations are listed below for the unfractionated and fraction samples.

1. Dilute the unfractionated reference sample in Mobilizer Solution (5 mM ammonium acetate) to a concentration 5-fold lower than the concentration used for fractionation in 100 µL total volume. For example, if you used 1 mg/mL for the fractionation batch, dilute the unfractionated reference sample by mixing 20 µL of 1 mg/mL sample in 80 µL of Mobilizer Solution.
2. Prepare your Master Mix:
    a. If you are using your own cIEF method, prepare enough Master Mix for all samples plus 20% extra volume.
    b. If you are using a platform method from this guide, prepare a Master Mix based on the quantities in TABLE 7–9, where the number of samples to prepare = X. Use the same Pharmalyte formulation as in the Master Mix used for the fractionation batch.
3. Add 32 µL of Maurice cIEF Master Mix solution to an individual microcentrifuge tube for each sample.
4. Add 8 µL of each sample (diluted unfractionated sample and individual fractions) to the 32 µL Maurice cIEF Master Mix in the tube.
5. Thoroughly vortex each sample to mix completely.
6. Centrifuge at 13,000 xg for 5 minutes to remove air bubbles and sediment any particulates.
7. Carefully aspirate the entire 40 µL of each sample and pipette it into a well of a 96-well plate. Insert the pipette tip all the way to the bottom of the well when you dispense the solution to avoid introducing bubbles.
8. Spin your sample plate for 5 minutes at 1000 xg using a centrifuge plate adapter.

Maurice cIEF Set Up and Start

1. Prepare your batch reagents and place them in MauriceFlex. See "Appendix A: Maurice cIEF reagent preparation" for prep details.
2. Lock the batch reagents in place by sliding the locking mechanism from the left to the right.
3. Place the metal 96-well plate insert in MauriceFlex and then place your sample plate in the insert.
4. Prepare your cartridge using the procedure in "Appendix B: Maurice cIEF cartridge preparation", then install the cartridge in MauriceFlex.
5. Launch Compass for iCE.
6. Click the **Batch** screen.
7. In the File menu, click **New Batch** and select **Maurice cIEF**.
8. Add samples by highlighting the sample location(s) in the Layout pane and clicking **Add**.

Maurice cIEF Master Mix	Final Sample Concentration	Single Sample	Master Mix ((Single Sample * X) + 20%)		
# of samples		1	5	10	15
DI water	N/A	15.2 µL	91 µL	182 µL	274 µL
1% Methyl Cellulose	0.35%	14 µL	84 µL	168 µL	252 µL
Pharmalyte 3–10	1%	0.4 µL	2.4 µL	4.8 µL	7.2 µL
Pharmalyte 8–10.5	3%	1.2 µL	7.2 µL	14.4 µL	22 µL
500 mM Arginine	5 mM	0.4 µL	2.4 µL	4.8 µL	7.2 µL
pI Marker 7.05	1%	0.4 µL	2.4 µL	4.8 µL	7.2 µL
pI Marker 10.17	1%	0.4 µL	2.4 µL	4.8 µL	7.2 µL
Total volume		32 µL	192 µL	384 µL	576 µL

TABLE 7. Maurice cIEF Master Mix solution for molecules with most charge variants in pI range 8–10.

Maurice cIEF Master Mix	Final Sample Concentration	Single Sample	Master Mix ((Single Sample * X) + 20%)		
# of samples		1	5	10	15
DI water	N/A	15.6 µL	94 µL	187 µL	281 µL
1% Methyl Cellulose	0.35%	14 µL	84 µL	168 µL	252 µL
Pharmalyte 3–10	0.8%	0.3 µL	1.9 µL	3.8 µL	5.8 µL
Pharmalyte 5–8	1.6%	0.6 µL	3.8 µL	7.7 µL	11.5 µL
Pharmalyte 8-10.5	1.6%	0.6 µL	3.8 µL	7.7 µL	11.5 µL
pI Marker 6.14	1%	0.4 µL	2.4 µL	4.8 µL	7.2 µL
pI Marker 9.50	1%	0.4 µL	2.4 µL	4.8 µL	7.2 µL
Total volume		32 µL	192 µL	384 µL	576 µL

TABLE 8. Maurice cIEF Master Mix solution for molecules with most charge variants in pI range 7–9.

Maurice cIEF Master Mix	Final Sample Concentration	Single Sample	Master Mix ((Single Sample * X) + 20%)		
# of samples		1	5	10	15
DI water	N/A	12.8 µL	77 µL	154 µL	230 µL
1% Methyl Cellulose	0.35%	14 µL	84 µL	168 µL	252 µL
Pharmalyte 3–10	1%	0.4 µL	2.4 µL	4.8 µL	7.2 µL
Pharmalyte 5–8	3%	1.2 µL	7.2 µL	14.4 µL	22 µL
200 mM Iminodiacetic Acid	14 mM	2.8 µL	16.8 µL	34 µL	50 µL
pI Marker 4.05	1%	0.4 µL	2.4 µL	4.8 µL	7.2 µL
pI Marker 7.05	1%	0.4 µL	2.4 µL	4.8 µL	7.2 µL
Total volume		32 µL	192 µL	384 µL	576 µL

TABLE 9. Maurice cIEF Master Mix solution for molecules with most charge variants in pI range 5–8.

9. Define the method:

   a. If you already have a Maurice cIEF method developed for your molecule, use the same separation parameters and change the Detection to include the Fluorescence exposure times listed in the table below.

   b. If you do not already have a Maurice cIEF method developed for your molecule, create a new method and set up parameters as shown in the table below.

Method Parameter	Setting
Focus Period 1	Time: 1 minute Voltage: 1500 V
Focus Period 2	Time: 8 minutes Voltage: 3000 V
Detection	Absorbance: 0.005 seconds Fluorescence: 20, 40, 80 seconds
Sample Load	55 seconds

10. Set up your batch for one injection per sample.

11. Save your batch.

12. Click **Start**.

13. After the batch has completed, perform a post-run cleanup of the cartridge as described in "Appendix C: Maurice cIEF Cartridge post-run cleanup"

Step 4: Optimize Your Fractionation Parameters

If no fractions contain the charge variants of interest or desired peak purity is not achieved, you can optimize the fractionation parameters as described in this section.

Mobilization Step

If no fractions contain the charge variants of interest, then you may need to change the Mobilization parameters to ensure that the charge variants have moved close to the end of the capillary but not out of it prior to the Fractions step. To optimize the Mobilization time, first select the Fractions view in the Analysis Screen and review the image for the 25 minute timepoint (T25) under the Experiment pane. You can also view by peak mobilization in the Focus Series pane of the Run Summary Screen. In the Injections pane, select Fractionation in the Method column and then drag the slider bar under the plot to the left or right (see the Maurice User Guide for more information). See FIGURE 11 for Mobilization time method development workflow.

If sample peaks are still visible at 25 minutes, then you should increase the time in the Mobilization step in the batch. We recommend increasing the Mobilization time by the total time of fraction collection from your fractionation run. For example, if using the default settings for a MauriceFlex Fractionation batch, then increase the Mobilization time from 25 minutes to 56 minutes (25 minutes plus 31 minutes for the Fractions step). Perform a second fractionation run and check the fractions for charge variants using the methods described in "Choosing Fractions to Check Purity and Identity".

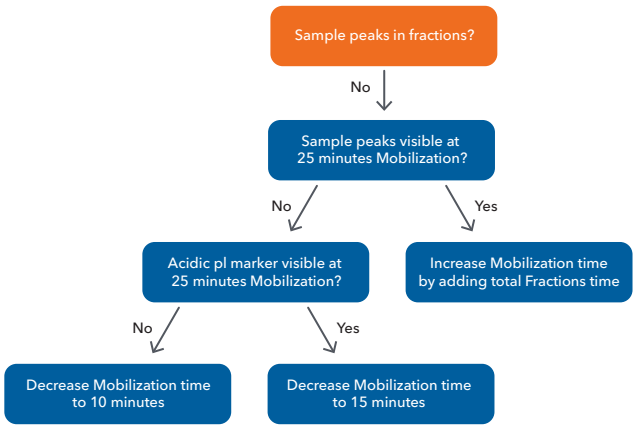


FIGURE 11. Workflow for optimizing Mobilization time.

The Mobilization time may need to be decreased if the sample has moved out of the capillary before the Fractions step has started. In general, if the acidic pI marker is not visible at the end of the Mobilization step (25 minutes), then decrease the Mobilization time to 10 minutes and perform a second fractionation run, followed by checking the fractions for your desired charge variants.

When running samples containing urea, samples typically mobilize slower during the Mobilization and Fractions steps (FIGURE 12). This is a result of lower current after applying voltage to samples containing urea. We recommend increasing the Mobilization time to 35 minutes to ensure that the desired charge variants are close to the capillary end when starting the Fractions step.

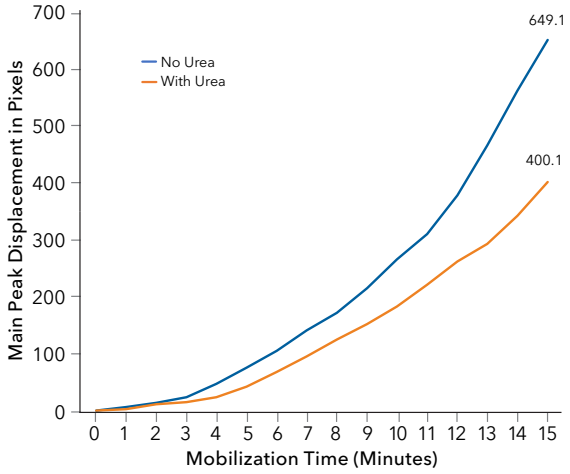


FIGURE 12. Samples mobilize slower when urea is included in the sample solution. The pixel displacement of the main peak of NIST mAb was measured for each detection interval during the Mobilization step of a MauriceFlex Fractionation batch. The sample without urea had larger pixel displacement (649.1 pixels) after 15 minutes of mobilization time versus only 400.1 pixel displacement for a sample with urea. Displacement was used instead of absolute pixel position to normalize for the initial pixel position of the main peak in each run. The sample was prepared, separated, and fractionated using the same conditions as in FIGURE 8.

In some cases, increasing the voltage during the Mobilization may be beneficial. Samples that have low current (<15 µA) once the current has stabilized during Mobilization may lose resolution during this step, resulting in lower purity of individual charge variants in the fractions. The current for the Mobilization and Fractions steps is visible when the IV Plot is selected in the Run Summary view (see the Maurice User Guide for more information). Increasing the voltage can help maintain resolution during fractionation and result in better charge variant purity. When increasing the voltage, make sure the stabilized current is below the values listed in TABLE 10 for each corresponding voltage to minimize Joule heating. Samples with high urea have require up to 2000 V during mobilization and fractionation. The first time you increase the Mobilization voltage, we recommend shortening the Mobilization time to 15 minutes to prevent mobilization of your charge variants out of the capillary. Note that the voltage settings for the Mobilization and Fractions step are linked, and that changing the Mobilization voltage will also change the Fractions voltage setting.

Mobilization Voltage (V)	Current (µA)
1000	50
1500	33
2000	25

TABLE 10. Voltage settings and corresponding maximum recommended current during Mobilization and Fractions steps.

Fractions and Refocus Steps

If the fractions do not contain the desired purity of charge variants, then you may need to change the parameters in the Fractions step. The default MauriceFlex Fractionation batch collects fractions at 45 seconds per well across 36 wells. If more than one charge variant is present in the well and the purity is below 80% for a single charge variant, then you can decrease the Fractions time to improve purity (FIGURE 13). If you started with the default MauriceFlex Fractionation batch settings, we recommend decreasing the time to 25 seconds and increasing the number of fraction wells to 60. Increasing the number of wells is to accommodate the shorter collection time per well. To minimize the number of fractions collected, you can also increase the Mobilization time (see "Collecting Charge Variants in Earlier Fractions"). If 25 seconds results in individual charge variants split across too many fractions, then we recommend using 35 seconds for the Fractions time. Note that the shortest time permitted for the Fractions step is 20 seconds.

For some molecules, reducing the Fractions time does not improve charge variant purity in the fractions. In these cases, we recommend adding a Refocus step to the fractionation batch, which allows for refocusing of the sample after the Mobilization step and before the Fractions step. An example of using the Refocus step to improve charge variant purity is shown in FIGURE 14. The Refocus parameters can be determined based on the results of your previous fractionation run and the voltage setting used for the Separation step.

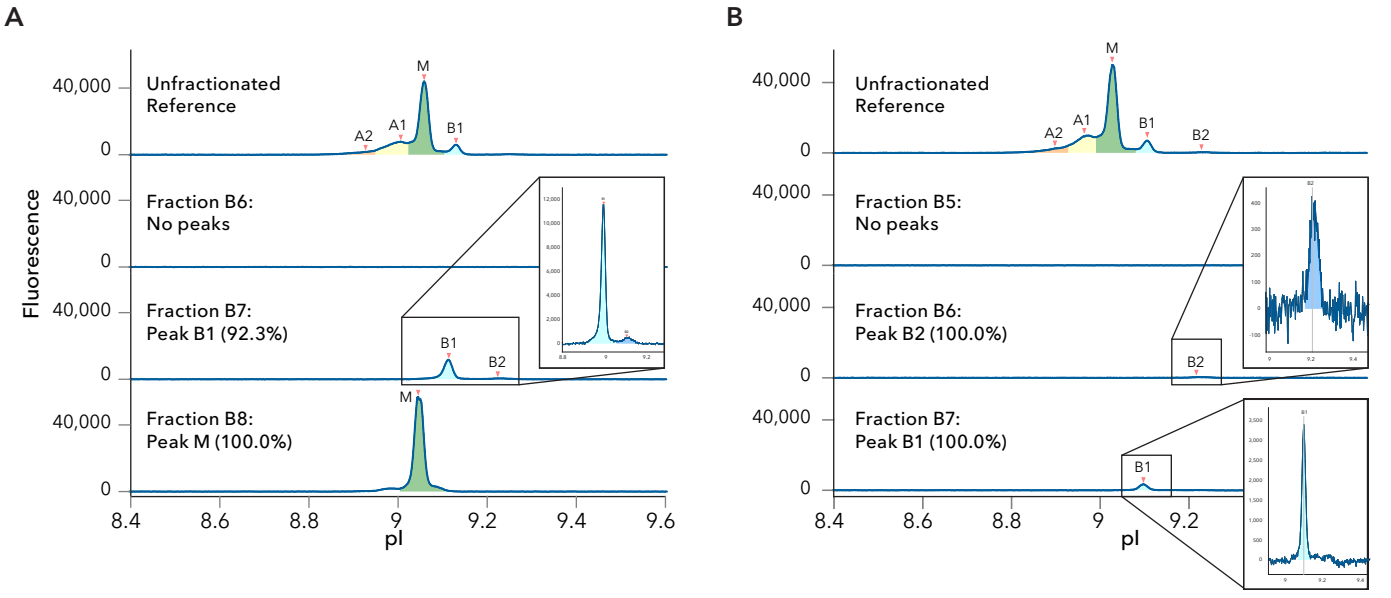
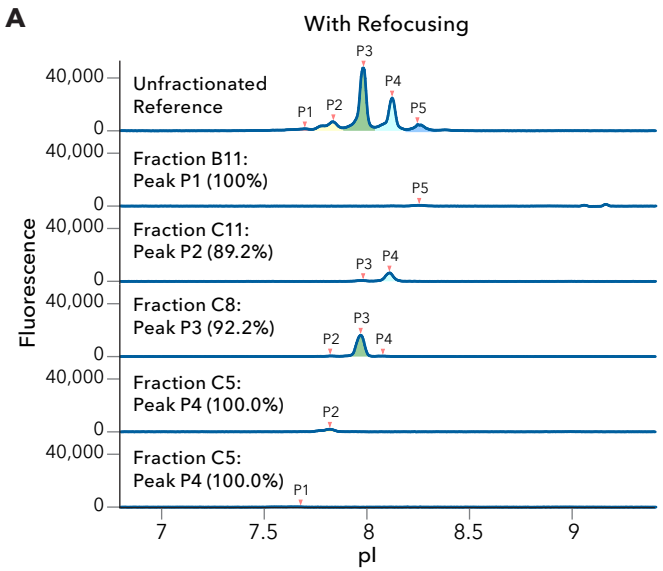
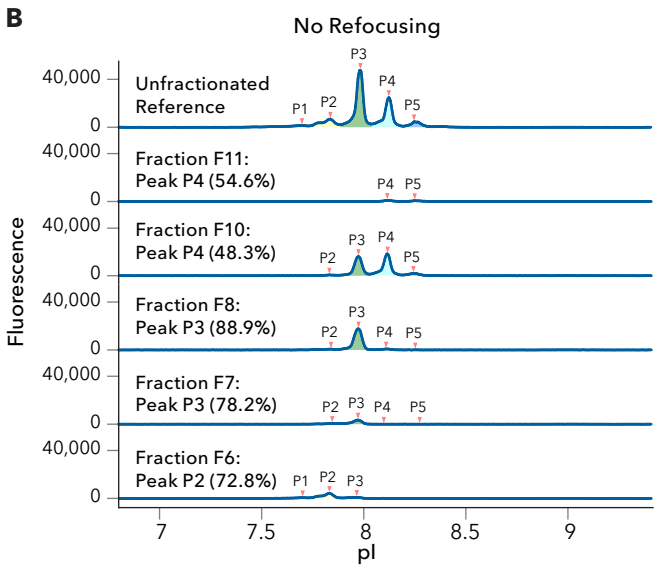


FIGURE 13. Reducing Fractions collection time from 45 seconds (A) to 25 seconds (B) can improve charge variant purity based on Maurice cIEF results. The B2 and B1 peaks for the NIST mAb were collected with increased purity using 25 second fraction collection time as verified when running the fractions on Maurice cIEF using 40 seconds fluorescence exposure. Charge variant percent purity is shown for representative fractions. The NIST mAb was fractionated at 1mg/mL and contained 0.35% methyl cellulose, 1% Pharmalyte 3–10, 3% Pharmalyte 8–10.5, and 25 mM arginine as a cathodic blocker. MauriceFlex Fractionation separation was performed by applying 500 V for 10 minutes, then 1000 V for 10 minutes, followed by 1500 V for 25 minutes, and then mobilized by applying 1000 V for 25 minutes. After the Mobilization step, fractions were collected every 45 seconds (fractions used for A) or 25 seconds (fractions used for B) while applying 1000 V. Reference sample (0.2 mg/mL) and fraction samples were separated on Maurice cIEF by applying 1500 V for 1 minute, followed by 3000 V for 8 minutes. The samples contained 0.35% methyl cellulose, 1% Pharmalyte 3–10, 3% Pharmalyte 8–10.5, and 5 mM arginine as a cathodic blocker.





C

With Refocusing					
Fraction Well	P1	P2	P3	P4	P5
B10					100
B11					100
B12					100
C12			9.38	90.62	
C11			10.83	89.17	
C10			28.49	71.51	
C9		4.48	88.41	7.11	
C8		4.32	92.23	3.46	
C7			100		
C6		74.03	25.97		
C5		100			
C4	35.75	64.25			
C2	100				

D

No Refocusing					
Fraction Well	P1	P2	P3	P4	P5
F11				54.65	45.35
F10		2.16	42.54	48.26	7.04
F9		3.44	63.03	17.49	1.43
F8		6.2	88.88	4.92	
F7		21.85	78.15		
F6	11.68	72.78	15.54		
F5	30.22	62.16	7.62		

FIGURE 14. Refocusing during cIEF fractionation can improve peak purity in fractions. Running fractions for a mAb on Maurice cIEF at 20 seconds fluorescence exposure showed better charge variant peak purity when using refocusing (A, C) versus without refocusing (B, D). Percent purity is shown for the most abundant charge variant in representative fractions (**A & B**). Fractions with greater than 80% purity for each charge variant are highlighted in tables for each fractionation run (C & D). The mAb was run on a MauriceFlex Fractionation batch at 1 mg/mL and contained 0.28% methyl cellulose, 0.62% Pharmalyte 3–10, 1.55% Pharmalyte 5–8, 1.55% Pharmalyte 8–10.5, 3.8 M urea, and 14.6 mM arginine as a cathodic blocker. The fractionation samples were separated by applying 500 V for 10 minutes, then 1000 V for 10 minutes, followed by 1500 V for 25 minutes, and then mobilized by applying 1500 V for 30 minutes. Fractions were collected every 25 seconds while applying 1000 V. The batch with refocusing (A & C) included a Refocus step using 2000 V for 5 minutes. Reference sample (0.2 mg/mL) and fraction samples were separated on Maurice cIEF by applying 1500 V for 1 minute, followed by 3000 V for 8 minutes. The samples contained 0.30% methyl cellulose, 3.7% Pharmalyte 3–10, 0.9 M urea, and 9.3 mM arginine as a cathodic blocker.

The Refocus time should be calculated as follows:

1. Identify the first well in the fractionation sequence that contains your sample. This can be confirmed by running your fractions on a Maurice cIEF batch.
2. Calculate at what time that well was used for collection during the Fractions step by multiplying the Fractions time by the fraction number (e.g., 45 seconds multiplied by fraction 10 = 450 seconds).
3. Convert the time in step 2 to minutes and then subtract 5 minutes.
4. Add the time calculated in step 3 to 25 minutes.
5. Input the time calculated in step 4 for Time in the Mobilization step in a new MauriceFlex Fractionation batch. Round up to the nearest minute.
6. Input the same voltage value that is used in the last focus period in the Separation step in the Refocus Profile window (FIGURE 15). Set the Refocus Time at 5 minutes.

   NOTE: Peak prediction will not be visible in Compass for iCE if refocusing is used in the batch.

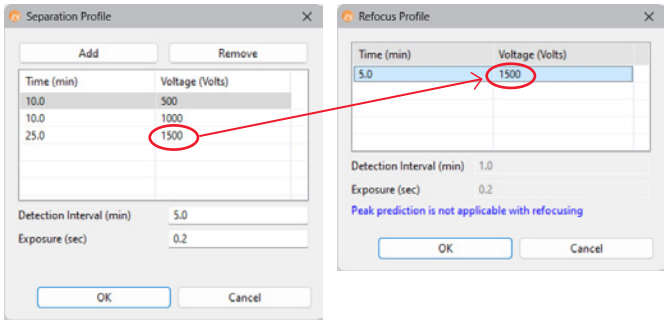


FIGURE 15. Inputting values for the Refocus Profile based on the final Focus Period in the Separation Profile.

Collecting Charge Variants in Earlier Fractions

Once your optimal focusing, mobilization and fractionation collection times have been determined, you can increase the Mobilization Time to collect the desired charge variants in earlier fractions. We only recommend making this change after you have completed multiple fractionation batches to ensure fractions are reproducibly collected in similar wells across batches. The new Mobilization Time should be calculated as follows:

1. Identify the first well in the fractionation sequence that contains your sample.
2. Calculate at what time that well was used for collection during the Fractions step by multiplying the Fractions time by the fraction number (e.g., 45 seconds multiplied by fraction 10 = 450 seconds).
3. Convert the time in step 2 to minutes and then subtract 5 minutes.
4. Input the time calculated in step 3 for Time in the Mobilization step in a new MauriceFlex Fractionation batch. Round up to the nearest minute.

Fraction Concentration

Use the method described below to calculate the approximate concentration of the charge variants in your fractions. This equation assumes a sample dilution factor of 0.2 for the fraction, which is derived by dividing the total volume of the capillary (~6 µL) by the volume of the Mobilizer Solution in the fraction well (30 µL).

1. Perform a Maurice cIEF batch with the unfractionated reference sample and fractions as described in "Review Your cIEF Fractionation Results".
2. Using the data generated step 1, calculate the total peak area for all charge variants in each fraction (e.g., blue box for fraction E8 below).
3. From the data in step 1, sum the peak area for all fractions that contain the charge variants (orange box below).
4. Calculate the approximate concentration for a fraction using the formula:

$$\frac{\textit{Total peak area for each fraction (Step 2)}}{\textit{Total peak area for all fractions (Step 3)}} \times \textit{Fractionation sample concentration x 0.2}$$

An example of calculating fraction concentration using results from the fractionation of 1 mg/mL NIST mAb is shown below.

	Fraction Well										
NIST Charge Variants	E8	E9	E10	E11	E12	F12	F11	F10	F9	F8	Total peak area for all fractions
A2							59208.5	16436.9	23389.6	6325	
A1				66947.1	288778.5	105846					
M		76912.8	807819.9	382725.3							**1981284.8**
B1	66156.8	73404.5									
B2	7333.9										
Total peak area for each fraction	**73490.7**	150317.3	807819.9	449672.4	288778.5	105846	59208.5	16436.9	23389.6	6325	
Concentration (ug/mL)	7.4	15.2	81.5	45.4	29.2	10.7	6.0	1.7	2.4	0.6	

The concentration of fraction well E8 is calculated as

$$\frac{73490.7}{1981284.8} \times 1000\ \mu g/mL \times 0.2 = 7.4\ \mu g/mL$$

This method to calculate concentration should be considered a theoretical concentration. Actual concentrations may be lower due to sample loss to the plate well or instability of the protein at very low concentrations. To help retain fraction integrity, we recommend storing the fractions in low-binding tubes or sealed fractionation plate at either 4 ˚C or -20 ˚C depending on the stability of your molecule.

Appendix A: Maurice cIEF reagent preparation

Prepare your batch reagents as shown in TABLE 11 and place the reagent vials in MauriceFlex as shown in FIGURE 16.

Reagent	Volume	Cap	Position
0.5% Methyl Cellulose	2.0 mL	**Blue** pressure cap	P1
Fluorescence Calibration Standard	500 µL	**Blue** pressure cap	P2
DI water	2.0 mL	**Blue** pressure cap	P3
Empty vial (air)	N/A	**Blue** pressure cap	P6
DI water	2.0 mL	**Clear** screw cap	N1

TABLE 11. Batch reagent preparation.

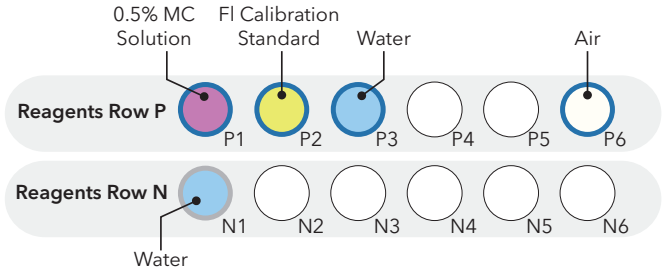


FIGURE 16. Reagent vial placement.

NOTE: Reagent vials on a MauriceFlex must be locked in place by sliding the locking mechanism from the left to right before starting a batch.

Appendix B: Maurice cIEF cartridge preparation

1. Take the cartridge out of its packaging. Save the packaging, you'll need it later.
2. Place the cartridge on a flat surface with its electrolyte tanks facing up.
3. Remove the stoppers from both electrolyte tanks.
4. Add 2 mL of Catholyte solution to the OH^- electrolyte tank (white port).
5. Add 2 mL of Anolyte solution to the H^+ electrolyte tank (red port).

   NOTE: Make sure you don't overfill the electrolyte tanks.
6. Seal each tank with the rubber stoppers. Use the grey stopper for the OH^- and the red one for the H^+ tank. If excess liquid comes out of the tank, make sure to wipe it with a lint-free laboratory wipe.

Appendix C: Maurice cIEF Cartridge post-run cleanup

1. Open MauriceFlex's door.
2. Remove your samples. Leave the Water (P3) and Air (P6) vials in place if your cartridge still has injections left since they will be needed for the cleanup step.
3. Remove the cartridge.
4. Place the cartridge on a flat surface with its electrolyte tanks facing up.
5. Remove the stoppers from both the electrolyte tanks.
6. Using a pipette or low vacuum, aspirate the solution from the tank.
7. Fill the tank with 2 mL of DI water, then aspirate it out. Repeat two more times.

   NOTE: Make sure not to get any liquid on the capillary.
8. Aspirate all the remaining liquid and make sure that the tanks are dry.
9. Place both stoppers back on the electrolyte tanks. They should be firmly closed.
10. Verify the reagent vials are placed as shown in FIGURE 17. Check that there is at least 1.5 mL of water in vial P3.

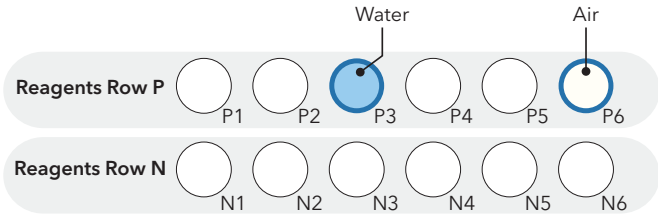


FIGURE 17. cIEF Cartridge Post-run cleanup vial placement.

11. Insert the cartridge in MauriceFlex.
12. In the Compass main menu, select Instrument and click **Cartridge Post-Run Cleanup**. It'll only take 5 minutes.
13. Once the cleanup procedure is done, remove the cartridge and reagent vials.
14. Leave the stoppers off to allow the tanks to air dry.
15. Put the cartridge and stoppers in the protective packaging and store at room temperature.

Appendix D: Desalting and concentrating samples

1. Add 500 µL of your sample into an Amicon Ultracel 50K Membrane Centrifugal Filter (Millipore PN UFC9050)
2. Centrifuge for 5 minutes at 12,000 xg.
3. Replace the filtered volume with 20 mM Tris buffer pH 7.0 (Life Technologies PN AM9851).
4. Do two additional cycles of centrifugation and buffer replacement.
5. For simple desalting, replace the filtered volume to 500 µL. If you need to concentrate the sample, store the remaining volume of buffer-exchanged sample at -20 °C or below if you won't use it immediately.

Appendix E: Denaturing sample preparation.

In extreme cases, some proteins need a higher concentration than 2 M urea to stay in solution during cIEF analysis. When this happens, you'll need to denature the samples using 4 M urea in the cIEF Master Mix solution. To make sure the final urea concentration in the sample is 4 M, follow the example preparation.

The total sample volume should be 200 µL. You'll need the following amount of components per 160 µL of master mix prepared with 48 mg urea powder:

- 70 µL of 1% Methyl Cellulose
- 8 µL of carrier ampholytes (total)
- 1.5 µL of each pI marker
- 8 uL of 500 mM Arginine
- Add DI water to reach 160 µL total volume

Appendix F: MauriceFlex cIEF reagent preparation

Prepare your batch reagents as shown in TABLE 12 and place the reagent vials in MauriceFlex as shown in FIGURE 18.

Reagent	Volume	Vial type	Position
0.5% Methyl Cellulose	2 mL	Crimp top vial	R1
Fluorescence Calibration Standard	350 µL	Glass vial with insert, 0.3 mL	R2
Water	2 mL	Crimp top vial	R3
Water	2 mL	Crimp top vial	R4
Water	2 mL	Crimp top vial	R5
Empty (air)	N/A	Crimp top vial	R6
Catholyte	2 mL	Crimp top vial	K2
Water	2 mL	Crimp top vial	K5

TABLE 12. MauriceFlex cIEF batch reagent preparation.

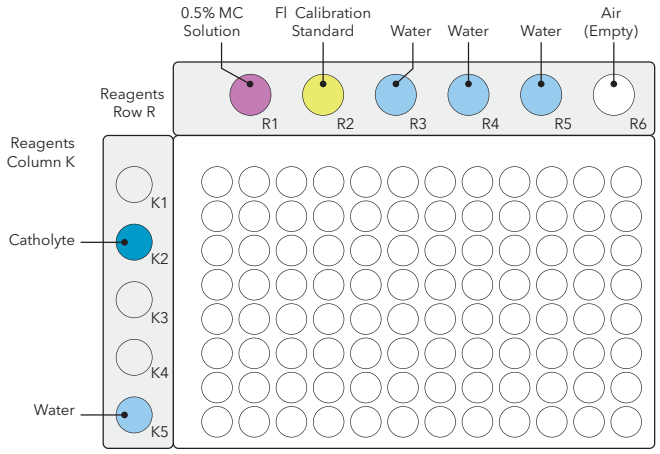


FIGURE 18. MauriceFlex cIEF batch reagent vial placement.

Appendix G: cIEF Fractionation Cartridge preparation

1. Take the cIEF Fractionation Cartridge out of its packaging. Save the packaging, you'll need it later.

   NOTE: Make sure the tip of the cartridge inlet doesn't come in contact with any surfaces. A damaged inlet may compromise the cartridge and cause a failed injection.

   NOTE: Avoid touching the capillary while holding the cartridge.

2. Place the cartridge on a flat surface with its electrolyte tank facing up.

3. Remove the red stopper from the tank.

4. Add 2 mL of Anolyte solution to the tank.

   NOTE: Make sure you don't overfill the tank.

5. Seal the tank with the rubber stopper. If excess liquid comes out of the tank, make sure to wipe it with a lint-free laboratory wipe.

Appendix H: MauriceFlex Fractionation reagent preparation

Prepare your batch reagents as shown in TABLE 13 and place the reagent vials in MauriceFlex as shown in FIGURE 19.

Reagent	Volume	Vial type	Position
0.5% Methyl Cellulose	2 mL	Crimp top vial	R1
Fluorescence Calibration Standard	350 µL	Glass vial with insert, 0.3mL	R2
Water	2 mL	Crimp top vial	R3
Water	2 mL	Crimp top vial	R4
Water	2 mL	Crimp top vial	R5
Empty (air)	N/A	Crimp top vial	R6
Catholyte	2 mL	Crimp top vial	K2
Mobilizer Solution (5 mM ammonium acetate)	2 mL	Crimp top vial	K3
Mobilizer Solution (5mM ammonium acetate)	2 mL	Crimp top vial	K4
Water	2 mL	Crimp top vial	K5

TABLE 13. MauriceFlex Fractionation batch reagent preparation.

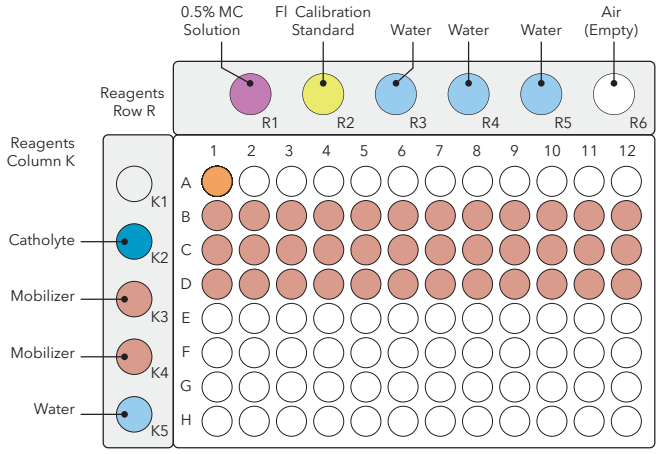


FIGURE 19. MauriceFlex Fractionation batch reagent vial placement.

Appendix I: cIEF Fractionation Cartridge post-run cleanup

1. Open MauriceFlex's door.
2. Remove your samples. Leave the Water (R3) and Air (R6) vials in place if your cartridge still has injections left since they will be needed for the cleanup step.
3. Remove the cartridge.
4. Place the cartridge on a flat surface with its electrolyte tank facing up.
5. Remove the red stopper on the electrolyte tank.
6. Using a pipette or low vacuum, aspirate the solution from the tank.
7. Fill the tank with 3 mL of DI water, then aspirate it out. Repeat two more times.

   NOTE: Make sure not to get any liquid on the capillary.
8. Aspirate all the remaining liquid and make sure that the tank is dry.
9. Place the stopper on the electrolyte tank. It should be firmly closed.
10. Verify the reagent vials are placed as shown in FIGURE 20. Check that there is at least 2 mL of water in vial R3.

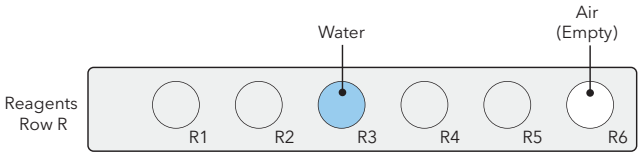


FIGURE 20. cIEF Fractionation Cartridge Post-run cleanup vial placement.

11. Insert the cartridge in MauriceFlex.
12. In the Compass main menu, select Instrument and click **Cartridge Post-Run Cleanup**. It'll only take 5 minutes.
13. Once the cleanup procedure is done, remove the cartridge and reagent vials.
14. Leave the stopper off to allow the tank to air dry.
15. Put the cartridge and stopper in the protective packaging and store at room temperature.

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