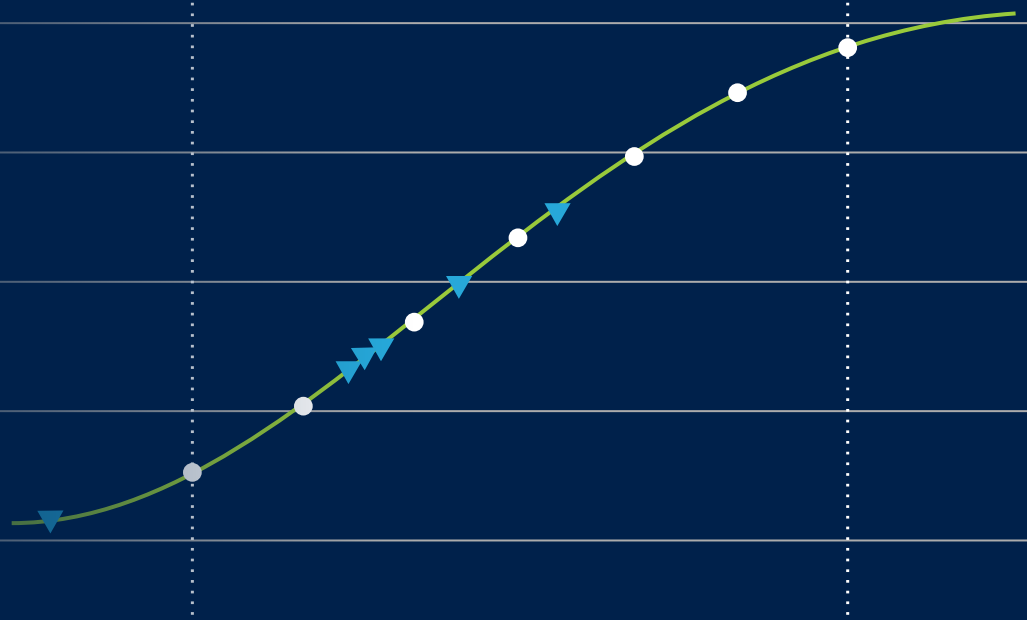


User Guide



Quantist Data Analysis Software

PL2-0016

Rev A | July 2022

biotechne®



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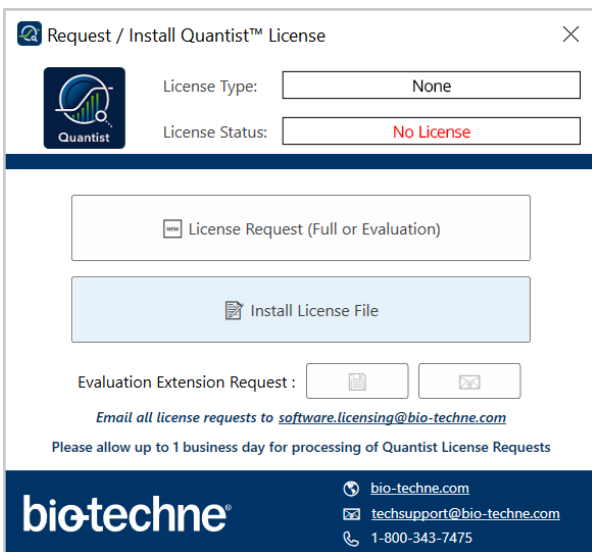
Minimum Software Requirements	
Processor	3.0 GHz i7 - 6th gen.
RAM	8 GB
Hard Drive	256 GB
Operating System	Windows 10 Pro 64 bit
Screen Resolution	1600x900 24 bit color

1.0 Install Quantist

1. Download Quantist software and install it on your computer. A Quantist licensing window will appear the first time you use the software.
2. Each software license is available for use on one computer only. Multiple users on the same computer will be able to access Quantist.
3. If you do have a license, skip to step 9.

If you do not have a license select License Request. Fill out the form and choose Evaluation License or a Full License.

Complete and save the form on the computer the software will be installed on.

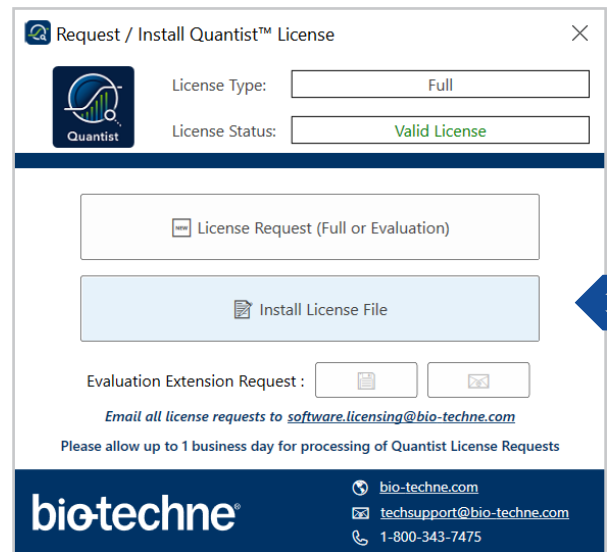


4. Quantist will generate a license request (.req) file.

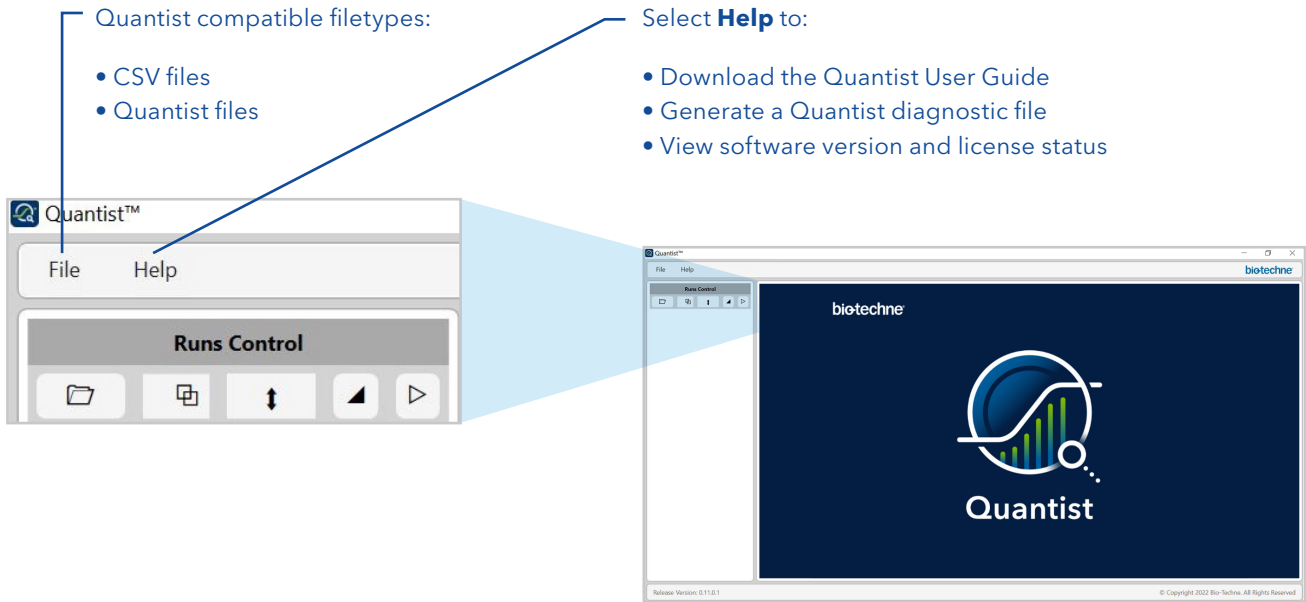


QuantistLicens
eRequest_20
220323_0639
41.req

5. Email the request to:
software.licensing@bio-techne.com
6. You will receive a license within 1 business day.
7. Save the license on the computer used to request it. Choose the file location that works for you. Make sure that you are able to navigate to the license via the Quantist software.

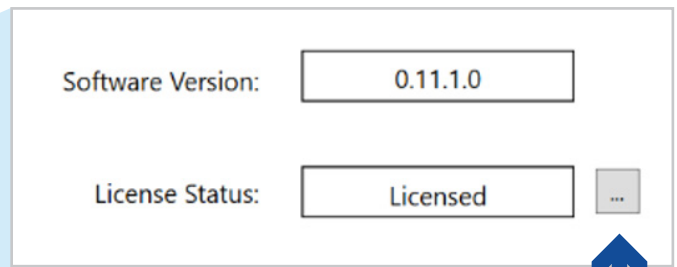


8. After you have received the license, open Quantist software. The licensing window will appear.
9. Select Install License File.
10. Navigate to the license that you saved on the computer and select it. Your copy of Quantist is now licensed. The License Type window will say "Full" or "Evaluation". The license status will say Valid License. You can close this window if it does not automatically.



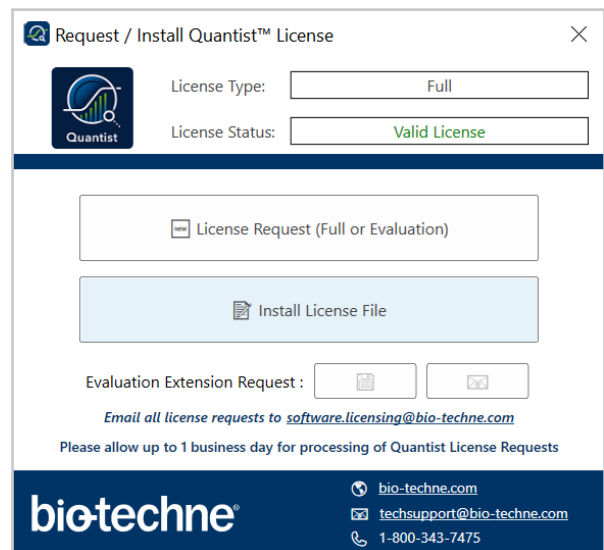
11. Open Quantist to start.
12. Select Help > About to view the software version and license status
13. Click "..."

Help > About Software



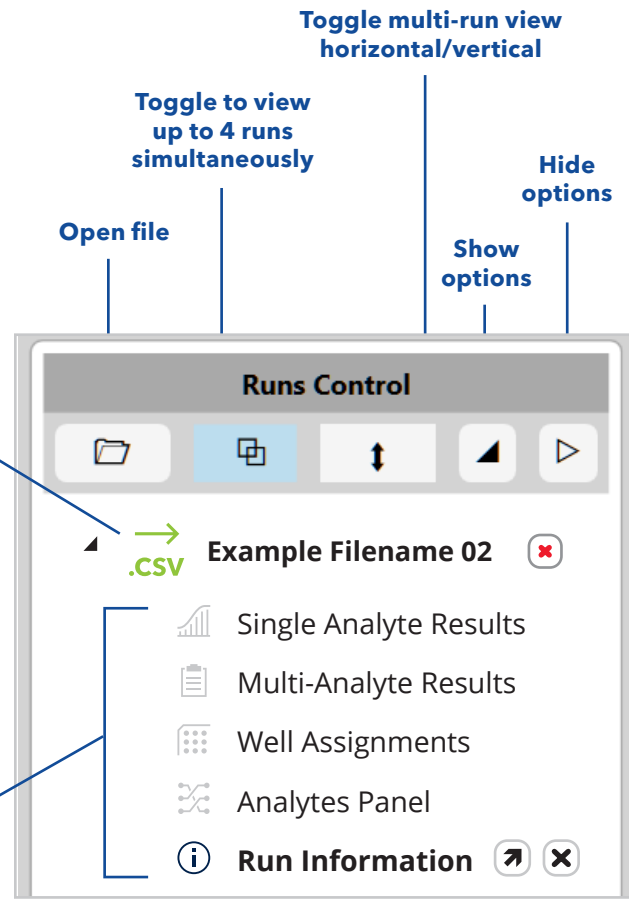
14. If moving from an Evaluation License to a Full License, repeat Steps 3-10 and reference your Sales Order number when filling out License Request form.

Software Licensing Window



How to Utilize the Runs Control Window

- Run File Icon**
- Quantist File
 - Green indicate an active file. Displays are showing details related to this file's options.
 - Luminex® xPONENT® files
 - Grey icons are inactive. Click a grey icon to make active.
 - Click X to completely close the .CSV or .Quantist file



File Options

- File Inactive (click to activate)
File Option Inactive
- File Active
File Option Inactive (click to activate)
- File Active
File Option Active

An active option has related window pane(s) displaying customizable details.

- Open option's display pane
- Close display pane

2.0 Data Analysis Using Quantist Software

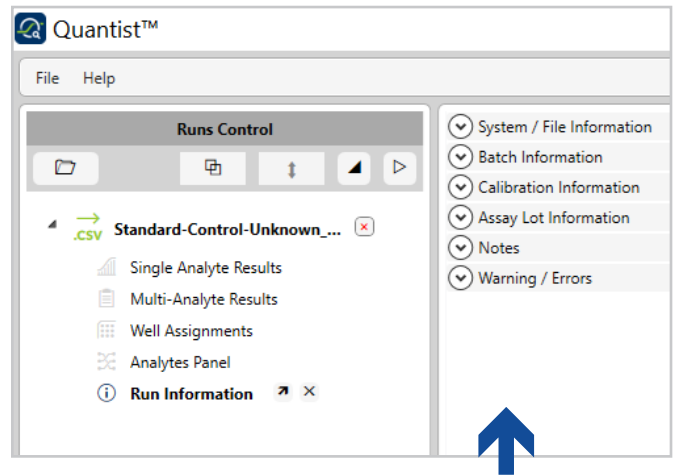
1. Launch Quantist software.
 2. Import CSV or Quantist files:
Drag and drop file into the Runs Control window or
File > Open Runs
 3. Green file icons are active
Grey file icons are inactive
- Quantist file icon: → Luminex® xPONENT® file icon: →
4. Shift + click to select multiple files from a folder.
 5. Quantist imports up to 50 CSV files.
Imported files will appear in the left column/window.

Run Information

6. Select **Run Information** for an overview of each file

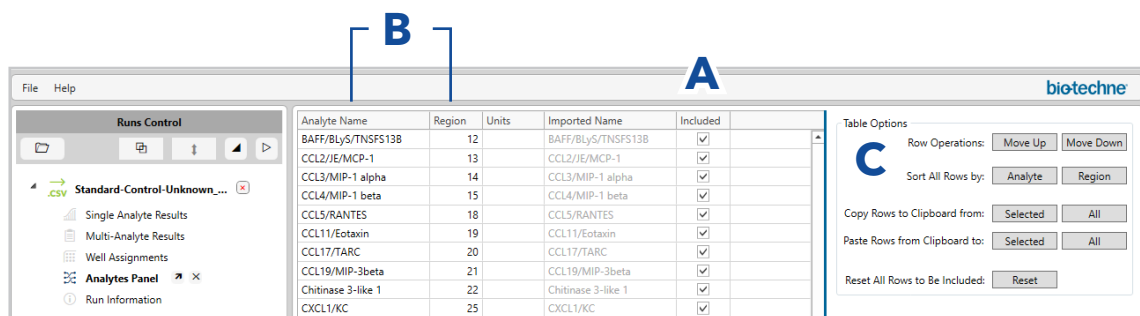
Click a section header to expand or collapse

Notes section is an editable field to add any run notes.



Analytes Panel

7. To begin, select **Analytes Panel** to confirm or update analyte assignment to bead region.
 - A. The Analyte name and Units columns are editable. Toggle the "included" box to include the target analyte/bead region or not.
 - B. Select the Analyte Name or Bead Region column headers to arrange columns in alphabetical or numerical order, respectively.
 - C. Use the right panel (**Table Options**) to:
 - Move target analyte up or down
 - Sort rows by analyte or bead region
 - Set units for selected rows
 - Copy rows to or from clipboard
 - Reset all rows to be included



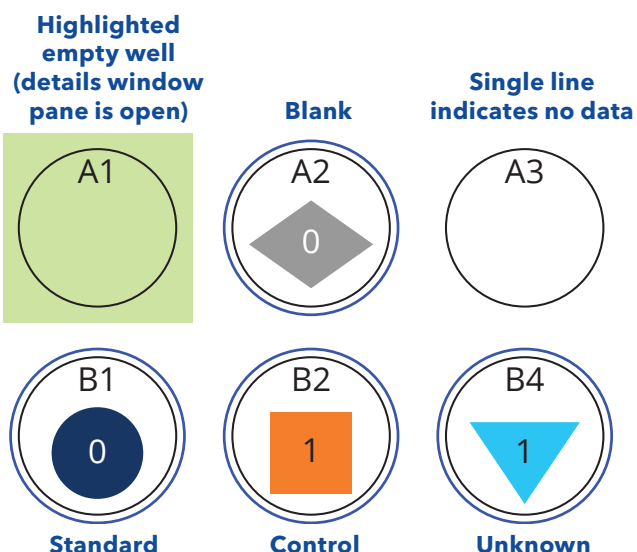
Modify Well Plate Details

Assign Wells

1. Highlight desired wells
contiguous area: shift + click
discontiguous areas: ctrl + click
2. With well(s) highlighted (light green)
Click the tool bar legend to assign type
3. Active wells are indicated with double line.
To edit details of a well see windows to right side.

Clear wells

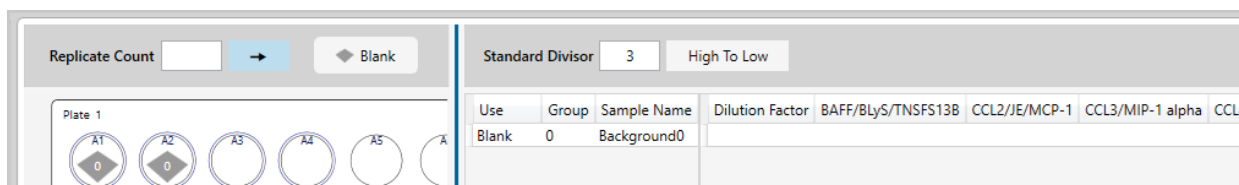
1. Select the well(s)
2. Click the trash can icon



Well Assignments



7. **Assign blank wells:**
Blank wells are indicated with a grey diamond.
Blanks will be detailed in the rightmost window.



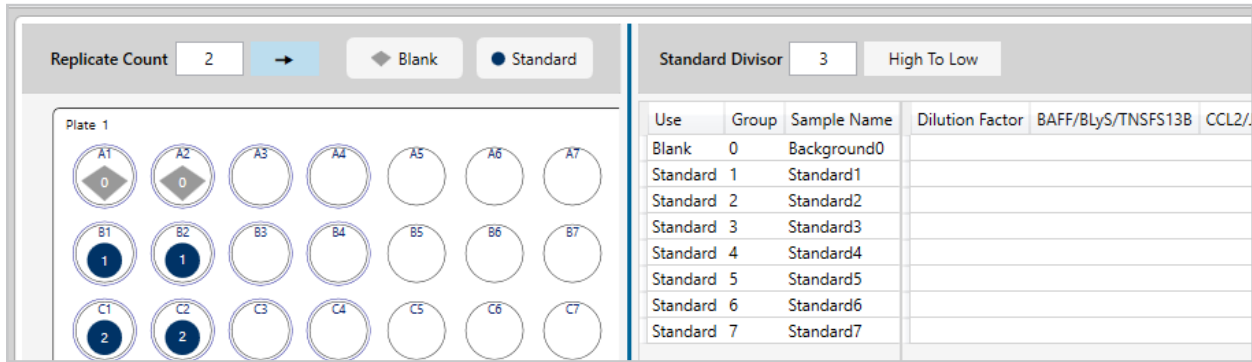
8. **Assign Standards:**
 - A. Set the replicate number in the Replicate Count field of the center window.
 - B. Toggle the arrow vertical or horizontal to choose the direction of replicates.
 - C. Highlight wells that contain standards
 - D. Click Standard.
Blue circles will appear in selected wells.
Numbers in blue circles indicate which standard is in each well.

- E. Use the right window to set the highest standard.
High To Low (Lowest Number is the High Standard). Low To High (The highest number is the High Standard)

The serial dilution will automatically populate based on the highest standard and the standard divisor. Serial Dilution is recalculated when a standard is deleted. Except for the case of the High Standard being deleted

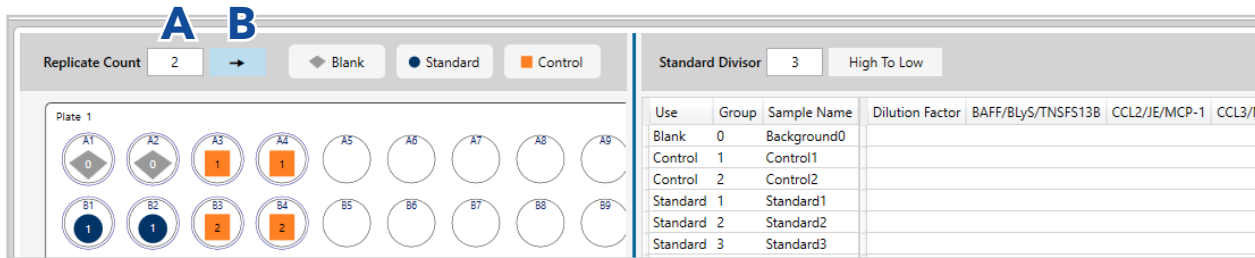
Use the Standard Divisor window to set the standard serial dilution. If no divisor is entered, you can manually enter the serial dilution series for each analyte.

- F. Otherwise, the highest standard must be entered for each analyte.



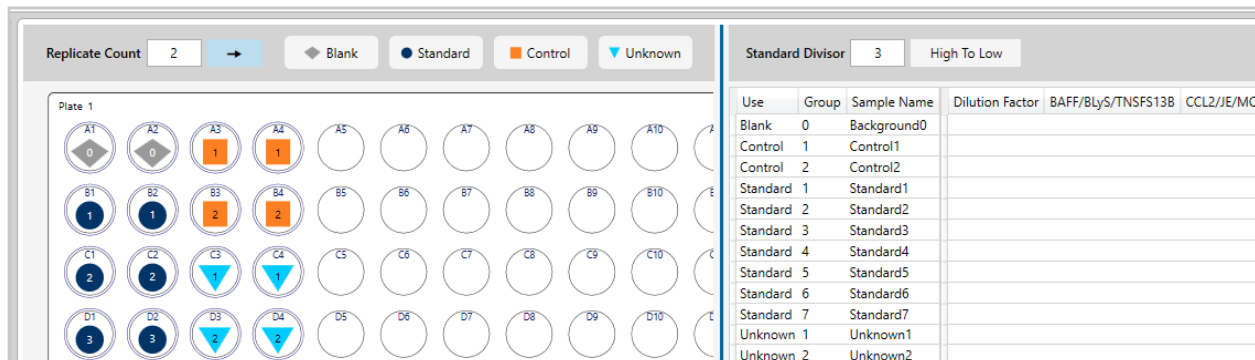
9. **Assign Controls:**

- A. Set replicate number at the top left of the center window.
- B. Toggle the direction of replicates using the arrow.
- C. Highlight the control wells.
- D. Select control.
Orange squares will appear in each of the selected wells. The controls will appear in the right window
- E. Add the concentration for each set of control replicates for each analyte.



10. **Assign Unknowns:**

- A. Set replicate number at the top left of the center window.
- G. Assign Unknown wells.
Blue triangles will appear in each selected well. Details of Unknown wells is open at right.
- H. Add dilution factor for unknowns in right column



H

DATA QUALITY CHECK

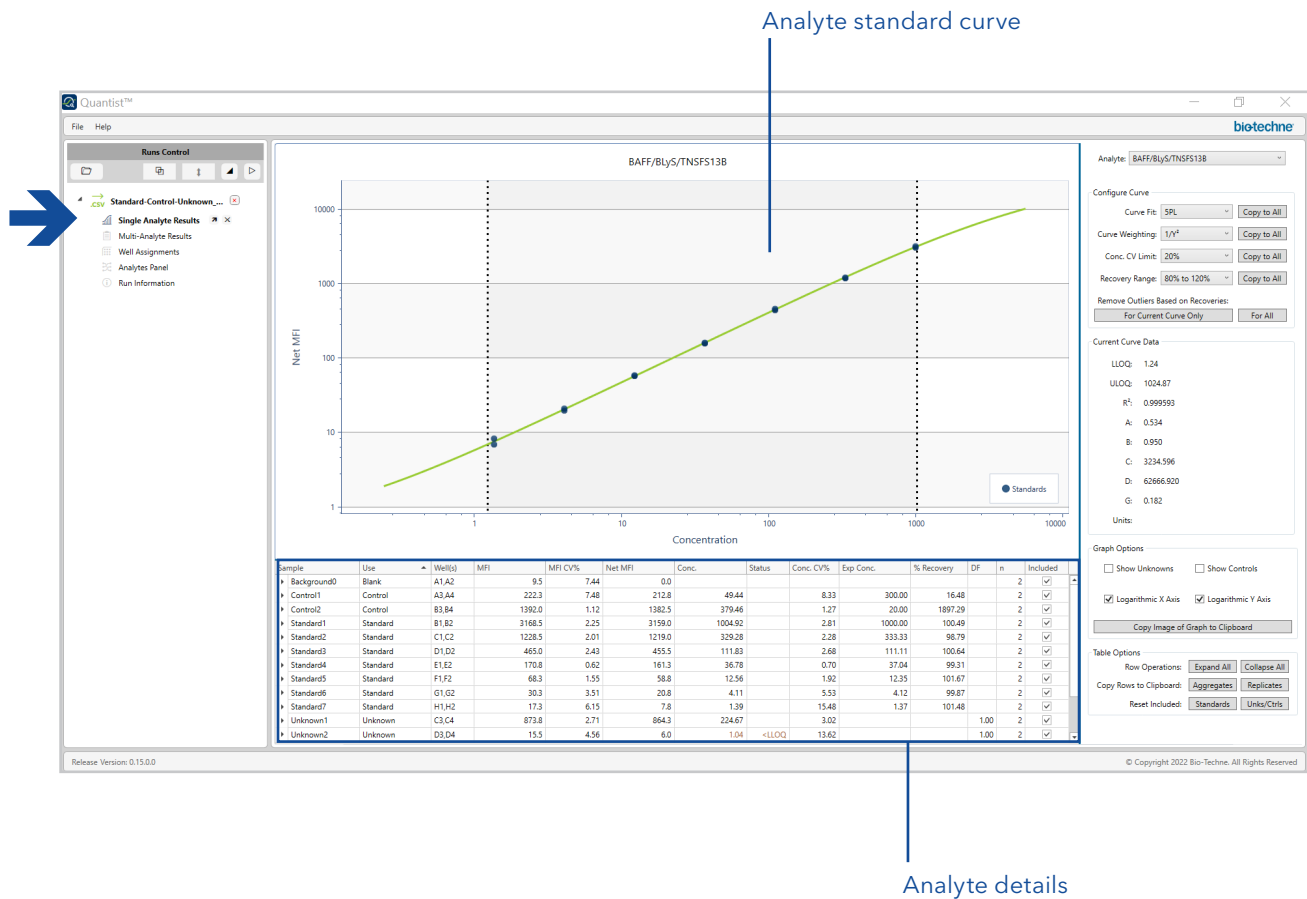
You can evaluate standard curve quality by assessing recovery and Coefficient of Variation (%CV).

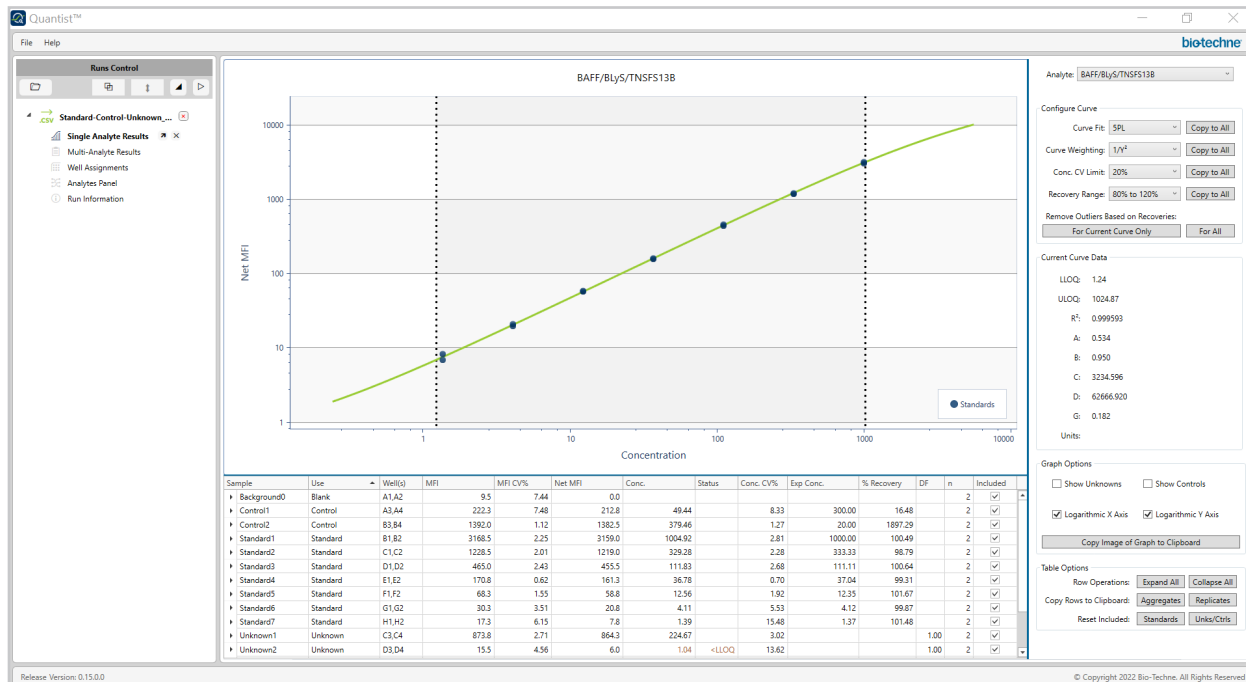
You can also configure standard curve properties such as curve fit, curve weighting, and recovery range.

Single Analyte Results

12. Within run controls menu click Single Analyte Results

Two windows are open at center:
 The standard curve for each analyte (top)
 Data for the analyte (bottom)





A
B
D
C

13. **Configure the Curve** for each standard curve using the right window.

- A. Toggle between analytes using the **Analyte** drop-down button.
- B. Toggle the **Curve Fit** button to choose between a 5PL or 4PL curve fit. All R&D Systems Luminex assays use a 5 parameter logistic (5PL) curve fit for asymmetrical curves. You have the option of selecting the 4PL curve fit for symmetrical curves.
- C. The **Current Curve Data** section of the right window shows all curve data, including Curve fit, weighting, recovery range, LLOQ, ULOQ, A, B, C, D, G, units

4PL

- a = estimated response at zero concentration
- b = slope factor
- c = mid-range concentration (C50)
- d = estimated response at an infinite concentration

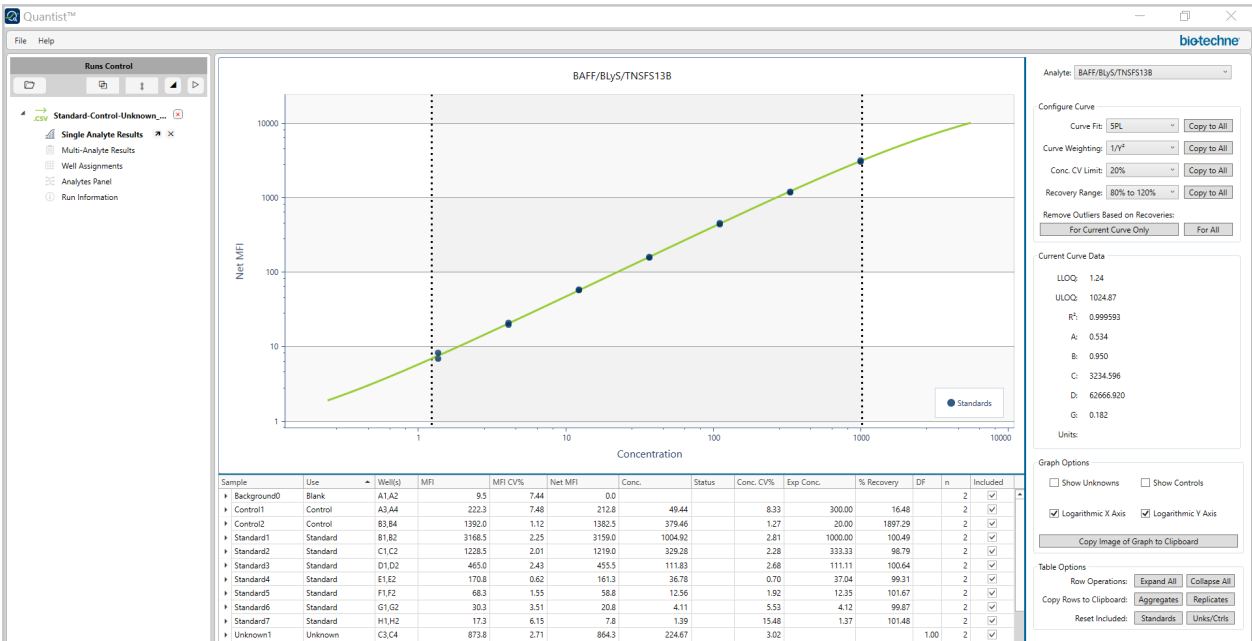
5PL

- a = estimated response at zero concentration
- b = slope factor
- c = mid-range concentration (C50)
- d = estimated response at infinite concentration
- g = asymmetry factor

- D. Toggle the **Curve Weighting** button to select curve weighting: none, 1/Y, or 1/Y².

Appropriate curve weighting is required to ensure that all data contribute equally to the final regression curve. Choose 1/Y² to compensate for high variability at the low end of the standard curve.

- E. The **Conc. CV Limit** button allows you to set the percent coefficient of variation limit. Options range from no limit to 5% CV
- F. Toggle the **Recovery Range** button to choose the appropriate recovery range



14. Configure the Curve (continued)

G. The **Graph Options** section of the right window allows you to:

- Visualize controls and unknowns on the standard curve.
- Standards are indicated by dark blue circles. Unknowns are indicated by light blue triangles. Controls are indicated by orange squares.
- Toggle on/off logarithmic scale of X and Y axis
- Copy the standard curve to a clipboard

H. The **Table Options** section of the right window has several functions:

- Expand/collapse all rows in the center bottom table. Triangles on the far left of rows in the bottom center. Windows can also be toggled to expand individual replicate rows
- Copy aggregates or replicates to a clipboard for pasting elsewhere
- Reset standards, controls, and unknowns.

15. Data Analysis Optimization - Remove Outliers

Assess Recovery

Scroll across the table in the bottom center window to view details in: the % Recovery column and the Included column.

For any standard that is outside of the selected % recovery range, confirm that the box is unchecked in the Included column.

The right window (in the Configure Curve section) there is an option to Remove Outliers Based on Recoveries for the current curve or all curves.

Assess %CV

Examine the % CV column and exclude samples that are outside of the acceptable %CV range by unchecking the included box.



Multi-Analyte Results

16. Select Multi-analyte Results to sort and compare sample data across target analytes.

A. The following data can be viewed across all target analytes on an assay by using the Selected Field drop-down button in the right window.

MFI:

Mean Fluorescent Intensity.
Excluded data is faint gray

MFI % CV:

MFI percent coefficient of variation.
Excluded data is absent.

Net MFI:

sample MFI-background well MFI.
Excluded data is faint gray.

Concentration:

Sample concentration relative to the standard curve. Excluded data is faint gray. Data outside of established curve parameter limits is brown.

Concentration Status:

Is the concentration > or < LLOQ or ULOQ?
Data outside of these parameters in indicated by <LLOQ, >LLOQ, <ULOQ, >ULOQ.

Concentration % CV:

concentration percent coefficient of variation.
Excluded data is absent.

Expected concentration:

Standards for each analyte.
Excluded data is absent.

% Recovery:

(observed sample concentration/expected sample concentration) X 100%. Excluded data is faint gray.

Dilution Factor:

Dilution factor for unknowns.
Excluded data is faint gray.

Bead count:

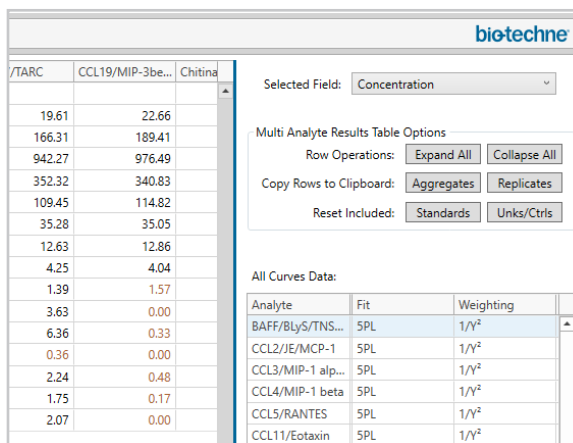
Number of beads counted in specified bead region. Excluded data is faint gray.

Note, these results are the same as those described in the Luminex xPonent software manual.

Double click on each mean to view replicates.
Double click on the mean again to hide individual replicates. Double click on individual replicates to include them or remove them.

The included column contains a check if data is included. The Included column is empty or contains a black square if data is excluded.

The black caret on the far left side of the Sample column can be toggled to view replicates as well.



A

B

C

B. The **Multi Analyte Results Table Options** section in the right window has several operations.

- Expand/collapse row operations of center table
- Copy aggregates or replicates to the clipboard
- Reset standards unknowns or controls

C. The **All Curves Data** section of the right panel contains standard curve data for each analyte including, Fit, Weighting, Recovery Range, LLOQ, ULOQ, R2, A, B, C, D, G, Units.

- A = estimated response at zero concentration
- B = slope factor
- C = mid-range concentration (C50)
- D = estimated response at infinite concentration
- G = asymmetry factor

17. Inter-assay comparison

Make inter-assay comparisons to ensure that data is consistent across assays over the long term

- To make inter-assay comparisons, import up to four CSV files. All options in the Runs control window can be simultaneously viewed across four runs. This works best on large monitors.
- Select the button containing 2 overlapping squares in the grey area at the top of the left window. When selected, the button changes color.
- To compare standard curves, select the Single Analyte Results from both assays.
- Toggle the **Analyte** button in the right windows to select the same analyte for comparison. The windows on the right are scrollable.
- Use this option to compare the following data:
 - Background signal
 - MFI
 - Curve Fit
- Inter-assay comparisons can also be made using the Multi-analyte Results
- Toggle the **Selected Field** button at the top of the right windows to view the same data field across all analytes on both runs

The center window will simultaneously visualize the standard curve and data for two runs.

- If the analyte/bead region assignments are identical, plate layouts can be copied from one plate to the next plate in the Well Assignments view using the keyboard short cuts "ctrl-c" to copy and "ctrl-v" to paste.



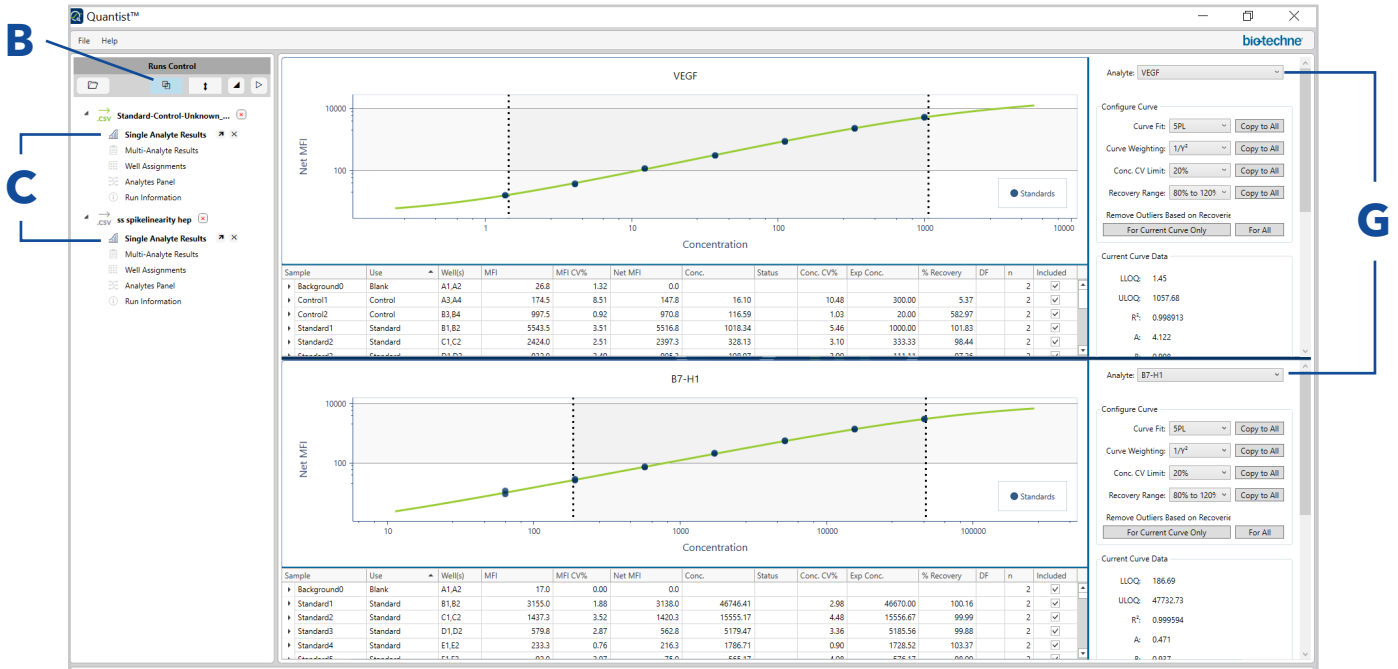
Single Analyte Results

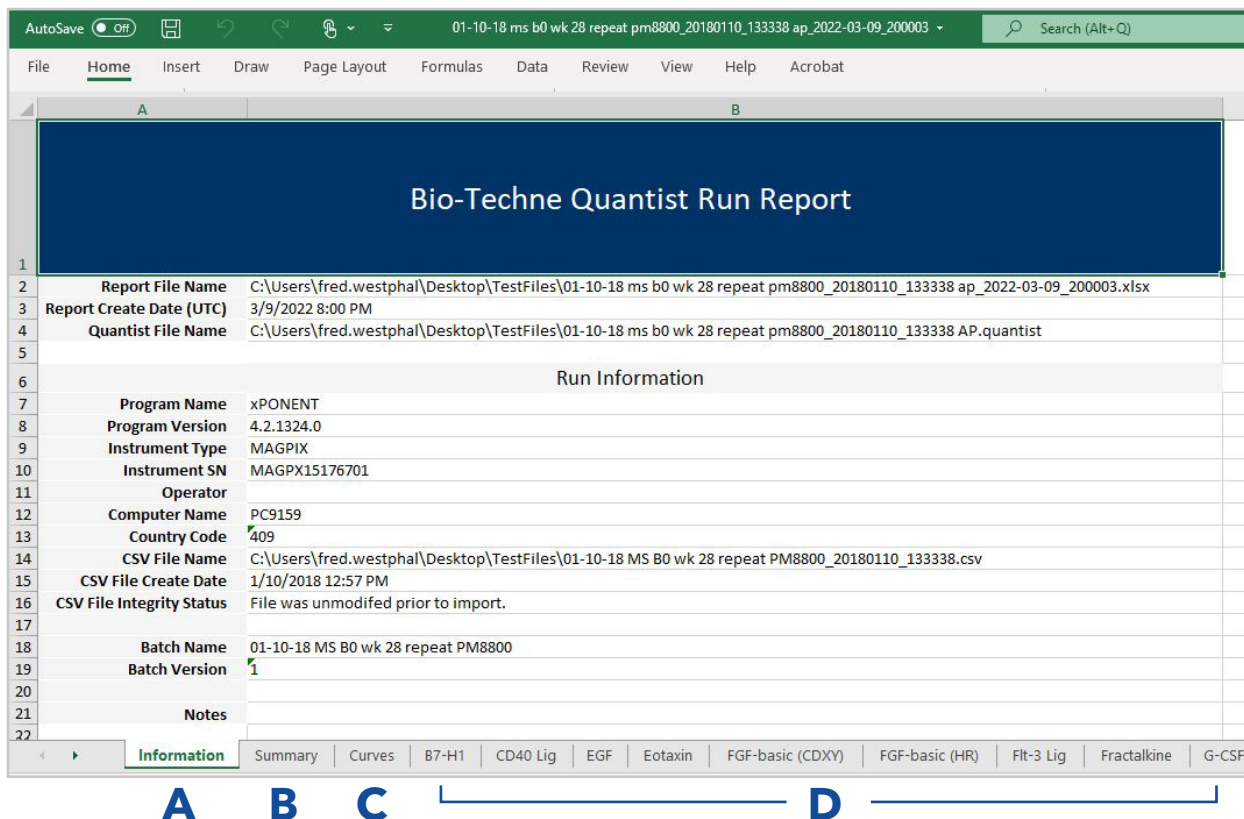


View options (right windows)

Click arrow to expand

Click X to close





19. Export data into a Quantist run report.

To export data into excel, select File/Export "filename"
 You can also simultaneously export all files to excel.
 Quantist will export a .xlsx file.

20. The Quantist Run Report contains the following information:

- A. Information Tab
 This tab contains information about the file, software, and hardware.
- B. Summary Tab
 This tab summarizes the analyte concentrations (Conc) for each well. Samples that are outside of the limits of quantitation are in a lighter color font.
- C. Curves Tab
 This tab contains standard curve plots for each analyte as well as curve fit data.
- D. Analyte Tabs
 Each analyte has its own tab that contains the following: curve plot, curve fit data, and plate data.

Plate data include:

Sample: Background, Standard, Control, Unknown

Use: Blank, Standard, Control, Unknown

Wells: Well location

MFI: Mean Fluorescent Intensity

MFI CV%: MFI coefficient of variation

Conc: Concentration

Status: Is the sample within the ULOQ and LLOQ?

Conc CV%: Concentration coefficient of variation

% Recovery: (observed sample concentration/
 expected sample concentration) X 100%

DF: Dilution Factor for unknowns

N: Number of replicates

Included: Is the sample included in the analysis

Is Aggregate: True= mean of replicates, False=
 individual replicates

Bead count: Number of beads counted in specified
 bead region

3.0 UNINSTALL QUANTIST

1. Select the windows start icon.
2. Select the settings cog in the menu.
3. Select apps in the windows settings menu.
4. Scroll down to the Quantist software icon and select it.
5. Select uninstall.

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