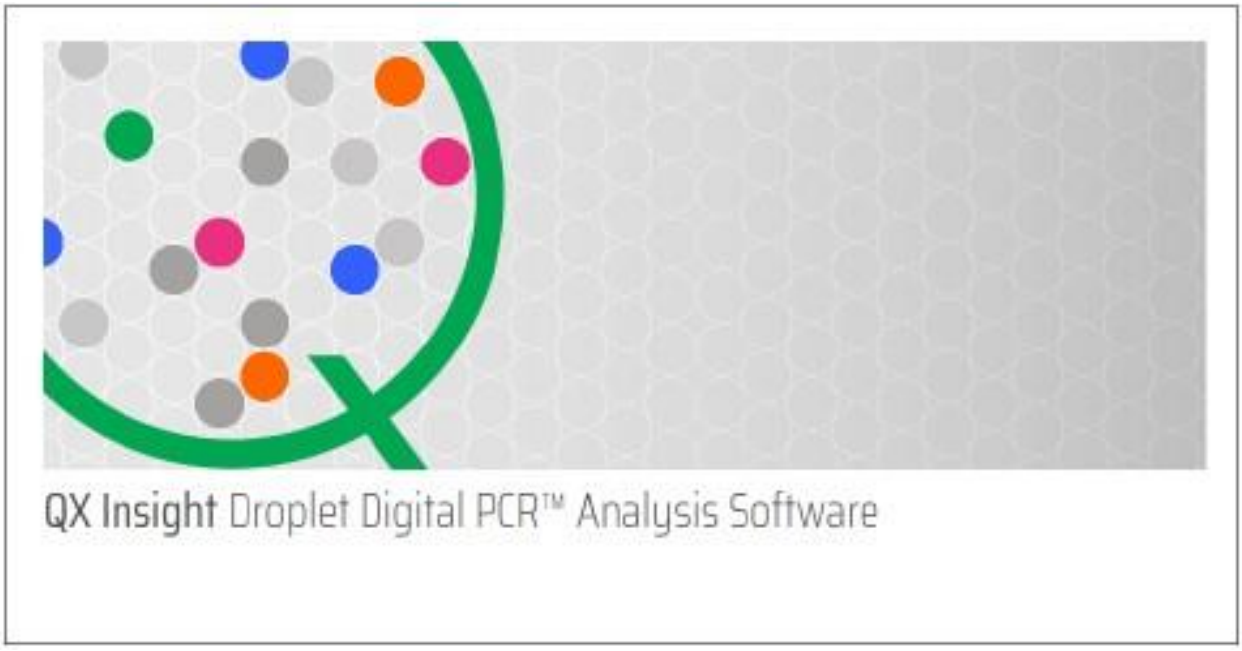
QX Insight Software

User Guide

Version 1.2



BIO-RAD

QX Insight Software

User Guide

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The Bio-Rad Technical Support department in the U.S. is open Monday through Friday, 5:00 AM to 5:00 PM, Pacific time.

Phone: 1-800-424-6723, option 2

Email: Support@bio-rad.com (U.S./Canada Only)

For technical assistance outside the U.S. and Canada, contact your local technical support office or click the Contact us link at www.bio-rad.com.

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Revision History

Document	Date	Description of Change
QX Insight Software User Guide Software Version 1.2 DIR 10000258170 Ver A	July 2026	Split the original guide into separate guides, one for instrument functionality and another for software functionality; update content for v1.2, which includes analysis capability for QX700 run data files as well as QX Continuum run data files
QX Continuum Droplet Digital PCR System and QX Insight Software Instruction Manual Software Version 1.1 DIR 10000170603, Ver C	December 2025	Update with new features in software version 1.1 (automatic software updates, analysis dashboard, new analysis features and options)

QX Continuum Droplet Digital PCR System and QX Insight Software Instruction Manual Software Version 1.0 DIR 10000170603, Ver B	August 2025	Add section on plate requirements; add instruction that wells without sample should be completely dry (no liquid filler); reword power cord text to "at least 15 amps." Add an important note to Changing Bottles and Managing Waste topics to instruct user to add bleach to the waste bottle before discarding it.
QX Continuum Droplet Digital PCR System and QX Insight Software Instruction Manual Software Version 1.0 DIR 10000170603, VerA	June 2025	User instructions for the QX Continuum ddPCR instrument and the QX Insight companion software

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Chapter 1 Droplet Digital PCR Overview

Droplet Digital™ PCR (ddPCR™) is a digital polymerase chain reaction method based on water-oil emulsion droplet technology. Using reagents and workflows that are similar to Taqman probebased design, ddPCR technology employs a combination of microfluidics and proprietary surfactant chemistries to divide each sample into a high volume of water-in-oil droplets per well.

In general, ddPCR can be performed on the instrument with all types of DNA sample. However, individual sample type compatibility for digital PCR applications might require a dedicated assay validation by the end-user. The extraction method used, as well as sample purity, can influence sample compatibility for digital PCR applications.

ddPCR Advantages

Droplet Digital PCR achieves high performance in the following areas:

- Absolute quantification of target DNA copies in samples to determine precise concentrations without using standard curves; this technique is ideal for nucleic acid target sequence measurements, viral titer concentrations, or microbial load determination
- More precise measurement of differences in gene copy number to better identify genomic alterations that indicate phenotypic variability, complex behavioral traits, and disease
- Detection of rare mutations or sequences in complex samples, such as a few tumor cells in a wild-type background
- Absolute quantification of gene expression levels, particularly involving low-abundance microRNA
- Next-generation sequencing (NGS) quantification to increase sequencing accuracy, validate variations/mutations, and reduce run repeats
- Low copy number quantification and gene expression of individual cells required by the high degree (10-fold to 100-fold) of cell-to-cell variation in gene expression and genomic content among homogeneous post-mitotic, progenitor, and stem cell populations
- Fast, precise, and cost-effective assessment of HDR (Homology directed repair) and NHEJ (non-homologous end joining) generated by CRISPR-Cas9 or other genome editing tools to detect genome edits
- Partitioning of the sample in ddPCR enables higher precision and more reliable measurement of small differences in the target DNA sequences.

- High-copy samples and background are diluted, effectively enriching the concentration in partitions with positive targets. Increased signal-to-noise ratio allows for the sensitive detection of rare targets and enables a ±10% precision in quantification.
- Error rates are reduced by removing the amplification efficiency reliance of qPCR, enabling accurate quantification of targets to near zero.
- For absolute quantification, there is no requirement for a standard curve.

ddPCR Workflow

The ddPCR process adheres to the following workflow:

- Experiment prep — samples are prepared by combining DNA or RNA with primers, probes dye, and Bio-RadddPCR supermix.
- Droplet generation — samples are fractionated into thousands of droplets in each well, with target and background DNA distributed randomly into the droplets during the partitioning process.
- Thermal cycling — following droplet generation, the droplets are run through the thermal cycler, which performs PCR amplification of the nucleic acid target in each individual droplet.
- Droplet reading — Each droplet is scanned (read) to determine the fraction of positive droplets in the original sample. Poisson statistical formulas are used to determine the absolute quantity.

Note: Positive droplets containing at least one copy of the target DNA molecule exhibit increased fluorescence, as compared to negative droplets. Poisson statistical formulas are used to determine the absolute quantity.

Finding Out More

You can visit bio-rad.com to download product information, technical notes, videos, and user manuals related to ddPCR instruments and technology. You can also click the tiles in the QX Insight Software Home page to locate and purchase assays, supermixes, and consumables for runs on the QX Continuum

Table of Contents

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Chapter 3 Introduction to QX Insight Software

QX Insight Software provides fast and reliable setup and analysis functionality for ddPCR experiments, as follows:

- If your lab uses the QX Continuum™ ddPCR System, you can ☐ Create a robust library of plate and thermal profile templates for your ddPCR experiments ☐ Establish a connection from QX Insight Software to any QX Continuum in your lab using the instrument IP address; this allows you to directly upload plate layouts from QX Insight to the instrument, and enables the automatic transfer of in-progress or completed run data from the instrument to the QX Insight computer
  - ☐ Add runs to a QX Insight workspace ☐ Save the run in the workspaces folder (.qxi format)
- If your lab uses a QX700™ ddPCR System model, you can ☐ Open a completed QX700 run file (.niodata) in a QX Insight workspace from a shared location
  - ☐ Save the .niodata file in the workspaces folder; the file automatically saves in .qxi format and the source file remains in its original location, unaltered.

For both systems, after the run data appears in a workspace, you can use the software's robust analysis features to evaluate the data.

See Table 1 on page 12 for available functionality by instrument.

Table 1. Functions by instrument

Instrument	Function
QX Continuum only	Use the Connectivity dialog in Settings to specify instrument IP addresses for connections between QX Insight and QX Continuum Important: Only one live connection at a time is allowed.
QX Continuum only	Define thermal profiles and plate templates in QX Insight to create a robust library for reuse on the QX Continuum Note: QX700 plates are defined in the QX700 System Control Software, which is installed on the QX700 instrument model.
QX Continuum only	Directly upload configured plates with assigned thermal profiles from the QX Insight Plates view to your QX Continuum
QX Continuum only	Automatically transfer in-progress or completed run data from a connected instrument to a QX Insight analysis workspace Note: You can add up to 10 QX Continuum runs to a workspace.

QX700	Open a .niodata run file in QX Insight from a QX700 storage location or a USB drive
QX Continuum QX700	Use a variety of workspace features to analyze ddPCR data collected from runs on the QX Continuum and the
QX Continuum QX700	Save run data files in the .qxi format.

Note: If you need to install or update QX Insight Software on one or more computers, see Appendix A, Installing QX Insight Software.

About the Compatible Instruments

In QX Insight Software, you can analyze run data collected on the QX Continuum Droplet Digital PCR System and all models of the QX700 Droplet Digital PCR System. Both instruments use an integrated workflow that is automated to perform the sequential droplet generation (partitioning), thermal cycling (amplification), and droplet reading (imaging) processes. The all-in-one systems provide accurate results without risk of contamination from moving a plate or cartridge set from instrument to instrument.

Important: The instruments cited herein are intended for research use only by laboratory personnel who have completed the appropriate training provided by Bio-Rad™ Laboratories, Inc. *Do not use the instrument or software for diagnostic procedures.*

QX Continuum Droplet Digital PCR System

To identify target molecules in up to four detection channels, the QX Continuum processes configured wells in a 96-well plate, in the default or user-defined order.

The QX Continuum connects via wireless or LAN to QX Insight Software, which allows a logged-in user to upload configured plates to the QX Continuum from QX Insight and accept processed run data from the QX Continuum into a QX Insight workspace. After a run is added to the workspace, you can analyze the data in a variety of analysis charts. For more information on the instrument, see the QX Continuum Droplet Digital PCR System Instrument Guide (catalog no. 10000258167).

QX700 Droplet Digital PCR System

To identify target molecules in up to seven detection channels, the QX700 ddPCR System processes the configured chambers in a cartridge plate containing up to three cartridges. The QX700 is available in three models

(E for essential functionality, S for standard functionality, and HT for high throughput capability) and users can open the .niodata files created on any QX700 model in a QX Insight workspace, and then analyze the run data in a variety of analysis charts. For more information on the instrument, see the QX700 Droplet Digital PCR System Instrument Guide, Standard Edition (catalog no. 10000258608).

Important: The QX700 can connect to the organization's LAN but does not connect to QX Insight. To access QX700 run files, you can copy them to a USB drive or open them from a network storage folder.

QX Insight Software Views

QX Insight Software features the functional views briefly described in Table 2.

Important: The QX Insight Thermal Profiles, Plates, and Runs views apply to QX Continuum users only. QX700 users must configure thermal profiles and cartridge layouts in the QX700 ddPCR System Control Software. Run files must be stored in a location that can be accessed by the QX Insight computer (network or USB).

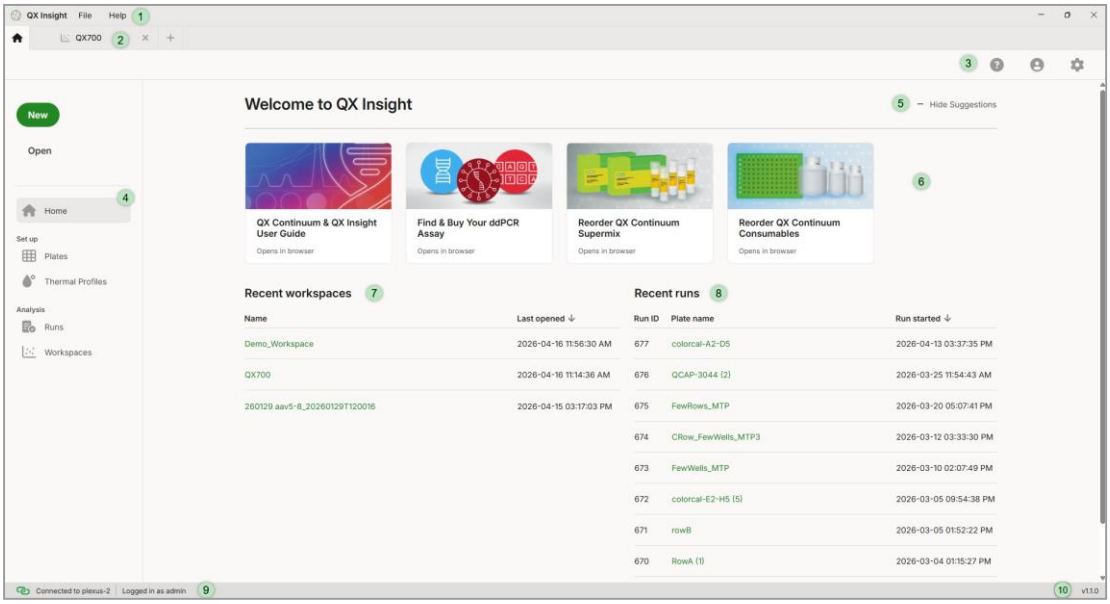
Table 2. QX Insight Software views

View	Description
Home	View and open the ten most recent saved workspaces; add any of the ten most recent runs to a workspace (if connected to a QX Continuum); access tiles that direct you to instrument and ddPCR product information. For more information, see Home View on page 15.
Thermal Profiles	Create and save thermal profiles; view all thermal profiles that you created; add, edit, and delete thermal profiles; filter the list of thermal profiles For more information, see Thermal Profiles View on page 16.
Plates	Create and save plates; view all plate configurations that you created; add, edit, or delete plates; filter the list of plates For more information, see Plates View on page 18.
Runs	View the list of QX Continuum runs that can be added to a workspace for analysis; filter the list of runs. For more information, see Runs View on page 19. Note: QX700 run files automatically open in a workspace.
Workspaces	Create and save workspaces for QX Continuum or QX700 run data analysis; add, edit, or delete workspaces; filter the list of workspaces. For more information, see Workspaces View on page 22.

Home View

The Home view appears by default when you open QX Insight Software, and displays an array of quick-access tiles, as well as recent saved workspaces and runs. Up to 10 saved workspaces for both instruments appear under Recent workspaces, but runs appear only for the QX Continuum, and only after you are connected and logged in. The software does not connect to the QX700, so there are no corresponding runs displayed.

For more information, see Setting Up Connections in QX Insight on page 25 and Logging Into QX Continuum From QX Insight on page 28.



LEGEND

1	Menu bar
2	Open tabs ■ Click the X to close a tab ■ Click the icon to display the *Create new* options (plate, thermal profile, or workspace)
3	Toolbar (user assistance, user profile, settings)
4	Navigation pane (appears next to all list views)

LEGEND , CONTINUED

5	Toggle link to show or hide the quick access tiles

6	Quick access tiles that are linked to the user assistance and compatible product information
7	List of up to ten most recent saved analysis workspaces for QX Continuum and QX700
8	List of up to ten most recent QX Continuum in-progress and completed runs
9	Information bar showing connected QX Continuum instrument, and logged-in user
10	QX Insight software version

Thermal Profiles View

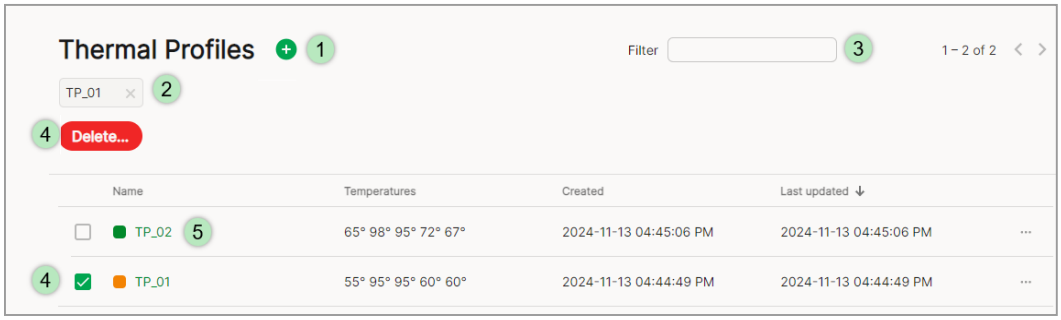
Note: This section applies to QX Continuum only.

When you click Thermal Profiles in the navigation pane, the Thermal Profiles view appears and displays a list of the thermal profiles you have created in QX Insight Software.

Important: You must create at least one thermal profile before you can create and save plate layouts. For information, see Thermal Profile Configurations on page 31. From the Thermal Profiles view, you can

■ Create, edit, and save thermal profiles ■ View thermal profiles ■ Filter the list of thermal profiles ■ Scroll the list (if the list extends beyond the allotted space, a scroll bar appears on the right ■ Delete thermal profiles

Each functional area is highlighted in the following graphic:



LEGEND

1	Click the plus icon to create a thermal profile.
2	Tabs for open or selected thermal profiles appear above the Delete button.
3	Enter characters to filter the list of thermal profiles. The selection narrows as you type applicable characters.

4	To enable the Delete button, select the checkbox for the thermal profile.
5	To open the thermal profile, click the file name.

Each column in the Thermal Profiles view is briefly described in Table 3.

Table 3. Thermal Profiles view

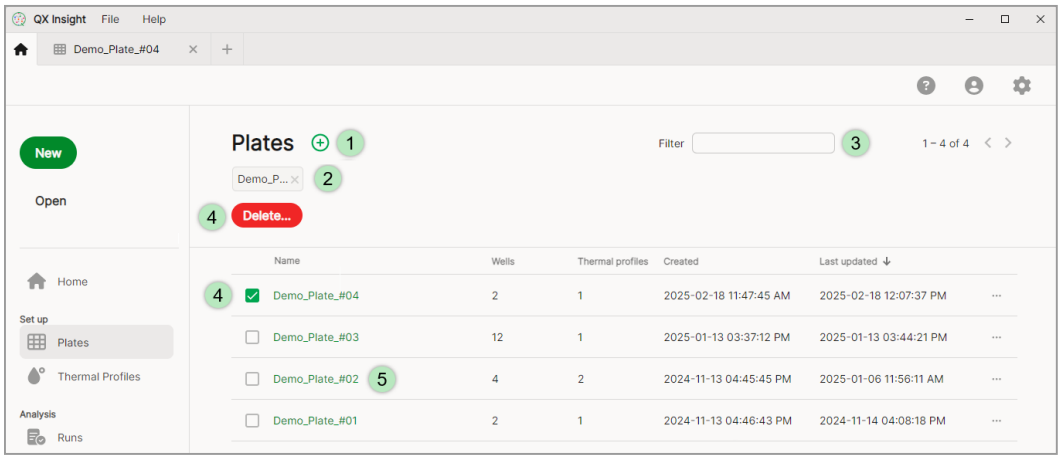
Column name	Contents
Name	Name you entered for the thermal profile
Temperatures	Temperature specified for each of the startup and thermal cycling zones
Created	Date and timestamp when the thermal profile was created
Last updated	Date and timestamp when the thermal profile was edited and saved

Plates View

Note: This section applies to QX Continuum only.

When you click Plates in the navigation pane, the Plates view appears and displays a list of the plate layouts you have created in QX Insight Software. From the Plates view you can ■ Create and edit plates ■ Open the plate layout ■ Filter the list of plates

■ Scroll the list (if the list extends beyond the allotted space, a scroll bar appears on the right ■ Delete plates (QX Insight enables the Delete button after you select a checkbox) Each functional area is highlighted in the following graphic:



LEGEND

1	Click the plus icon to create a plate.
2	Tabs for open or selected plate appear above the Delete button.
3	Enter characters to filter the list of plates. The selection narrows as you type applicable characters.
4	To enable the Delete button, select the checkbox for the plate.
5	To open the plate, click the file name.

Each column in the Plates view is briefly described in Table 4.

Table 4. Plates view

Column name	Contents
Name	Name you entered for the plate
Wells	Number of wells defined in the plate layout
Thermal profiles	Number of thermal profiles assigned to wells in the plate
Created	Date and timestamp when the plate was created
Last updated	Date and timestamp when the plate was last edited and saved

Runs View

Note: This section applies to QX Continuum only.

When you click Runs in the navigation pane, the Runs view appears and displays a list of your completed QX Continuum runs (and one in-progress run, if applicable), *provided your instance of QX Insight is connected to the QX Continuum and you are logged into the software using your QX Continuum credentials.*

From the Runs view, you can

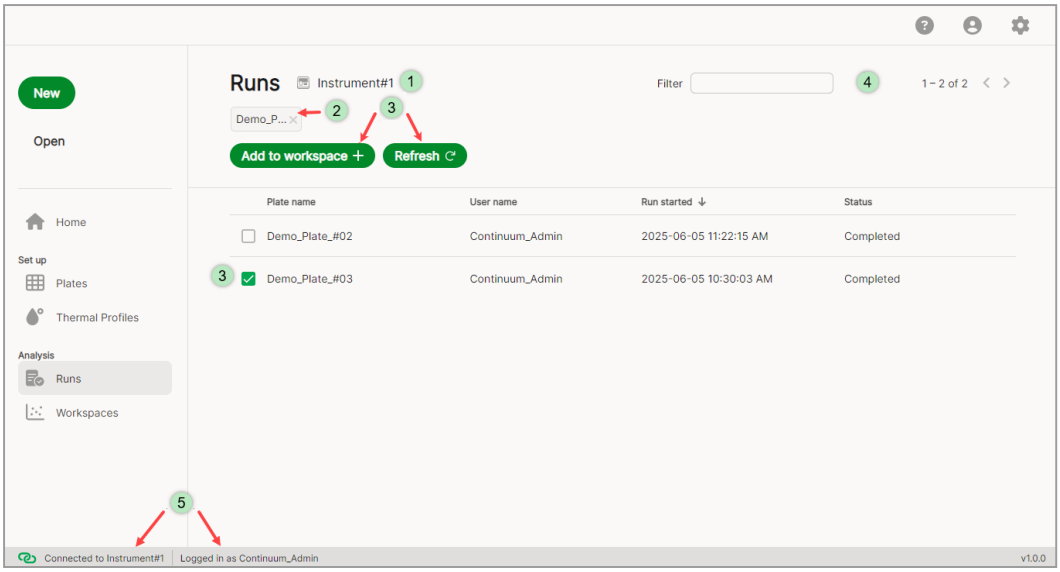
■ View all completed runs and a run in progress ■ Add a run to an analysis workspace

■ Filter the list of runs

■ Scroll the list (if the list extends beyond the allotted space, a scroll bar appears on the right

■ Refresh the run list ■ QX Insight Software.*QX Insight does not have a Delete option for runs*.

Each functional area is highlighted in the following graphic:



LEGEND

1	Instrument identifier
2	Selected run label

■ Add to workspace button is enabled when you select a checkbox ■ Refresh button is always enabled to add a run to, or delete a run from, the display

3 Note: You must click the Refresh button to remove a deleted run from the Runs view. Runs are automatically transferred from the instrument while you are logged in from QX Insight Software, but you might need to refresh the view.

4	Enter all or part of a run name to filter the list.
5	You must be connected and logged in to see your runs

Important Notes:

- You must be connected to the QX Continuum from QX Insight Software to view your list of runs and add them to workspaces. Administrators can access all runs, while standard users can access only the runs that were performed while the user was logged into the instrument.
- You can add up to 10 runs to a QX Insight workspace. For more information, see Adding QX Continuum Runs to a Workspace on page 65.
- You must delete runs on the QX Continuum touch screen, and then refresh the Runs page in QX Insight
  - The Runs view is NOT available for QX700 run (.niodata) files. You must open the file from the storage folder, which automatically adds the run to a QX Insight workspace.

Each column in the Runs view is briefly described in Table 5.

Table 5. Runs view

Column name	Contents
Plate Name	Name you entered for the plate during plate setup
User Name	Your user name; if you are an administrator, all users who have performed runs are listed
Run Started	Date and timestamp when the run started on the QX Continuum
Status	Status of the run (in progress or completed)

Workspaces View

The QX Insight Software workspace is the functional area in which you can analyze your run data, as follows:

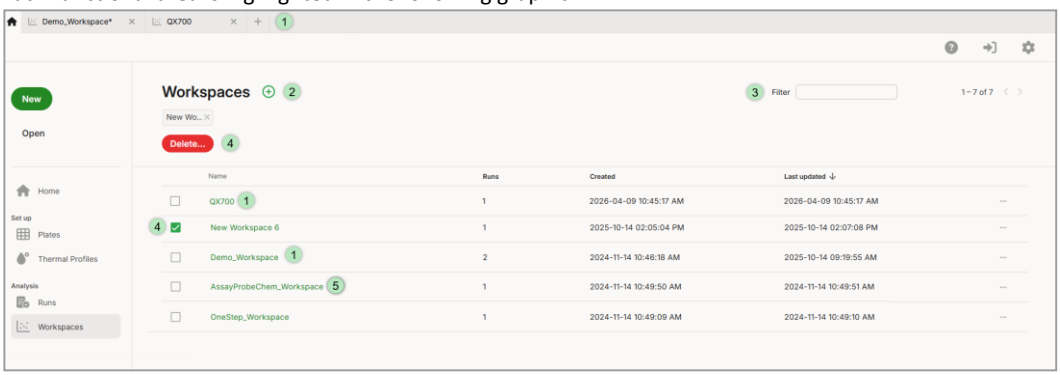
- If you are using a QX700 model, you can open the completed run data file (.niodata) in a workspace.
- If you are using a QX Continuum, the instrument retains the provisional run data in its storage location until you log into the instrument from QX Insight. After the login is verified, you can create and populate a workspace with up to 10 QX Continuum runs, one of which can be in progress. In-progress data is transferred incrementally as the processing for each well concludes, while data for completed runs is transferred to the workspace immediately.

The Workspaces view contains a list of the saved workspaces, as well as options to do the following:

- Create and save a workspace
- Filter the list of workspaces

■ Scroll the list (if the list extends beyond the allotted space, a scroll bar appears on the rightl ■ Select and open saved workspaces for analysis ■ Add up to 10 runs to a workspace ■ Delete a workspace

Each functional area is highlighted in the following graphic:



LEGEND

1	Open workspaces appear in separate tabs
2	Click the plus icon to create a workspace
3	Enter characters to filter the list of workspaces; the selection narrows as you type applicable characters.
4	Select the checkbox to ■ Display the label for selected or open workspaces above the Delete button ■ Enable the Delete button
5	Click the file name to open the workspace

Each column in the Workspaces view is briefly described in Table 6

Table 6. Workspace view columns

Column name	Contents
Name	Name you entered for the workspace
Runs	Number of runs added to the workspace
Created	Date and timestamp when the workspace was created

Last updated	Date and timestamp when the workspace was edited and saved

For more information on individual analysis workspaces and how to use them, see Chapter 9, Droplet Data Analysis.

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Chapter 4 Connecting and Logging In

To enable direct data and file access capabilities, the computers on which QX Insight Software is installed must be connected to the customer network.

Additionally, to enable direct plate uploads and run data transfers between the QX Continuum ddPCR System and QX Insight, connections can be defined in the QX Insight settings using the QX Continuum IP addresses.

For access to QX700 ddPCR System cartridge layouts and run data files, the instrument and computer can be connected to the customer network and the user can navigate to the shared locations using the QX Insight Open option.

For environments without network connections, you can transfer files using a USB drive.

Setting Up Connections in QX Insight

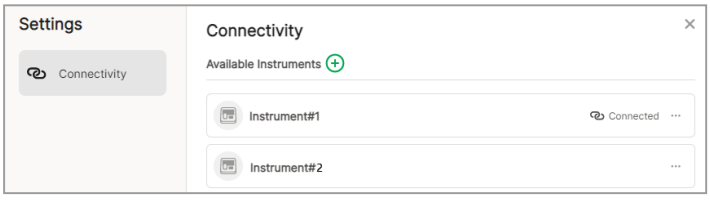
Note: *This section applies to the QX Continuum only.* Although the QX700 can be connected to the customer LAN to facilitate shared access to file storage locations, the instrument does not connect directly to QX Insight Software.

From the Connectivity page in the QX Insight Software Settings menu, you can ■ Define

connections to your QX Continuum instruments ■ Connect to an instrument

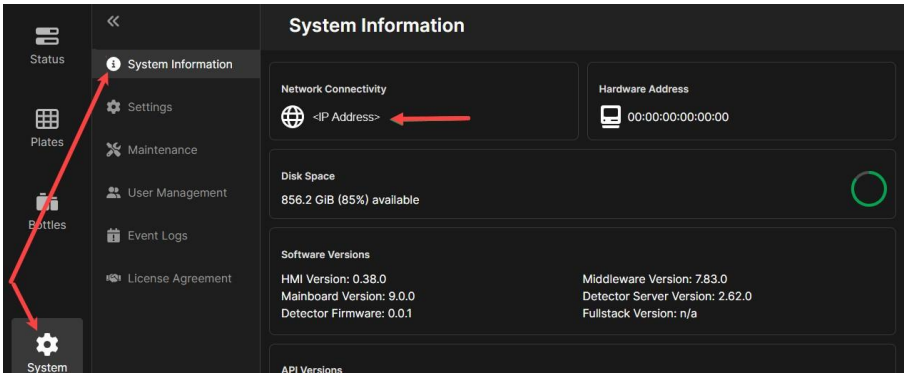
- Disconnect from one instrument and connect to another
- Delete the instrument from the Connectivity list

The wireless connection between the QX Continuum ddPCR System and QX Insight Software enables the direct upload of plate configurations to the instrument and the automatic transfer of run data to a QX Insight workspace for analysis. You can configure multiple instrument connections in the Connectivity dialog and quickly switch between them, but only one connection at a time can be active.



To create a connection

1. In the touch screen, tap System in the navigation pane, and then tap System Information.

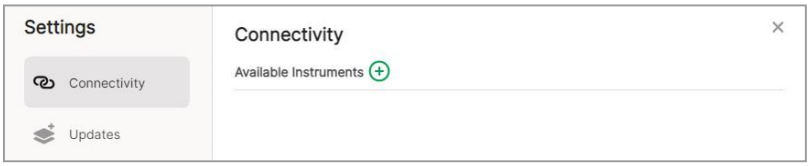


2. Write down the IP address specified under Network Connectivity.

3. Open QX Insight on the computer. The default view appears, and the status in the lower-left corner appears as *Not connected to machine*.

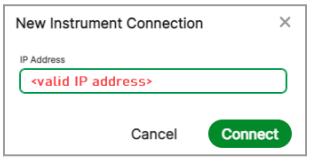
4. Click *Select an instrument to continue*.

5. When the Connectivity dialog appears, click the ⊕ icon next to Available Instruments.



Setting Up Connections in QX Insight

6. When the New Instrument Connection dialog appears, enter the IP address that you copied from the instrument.

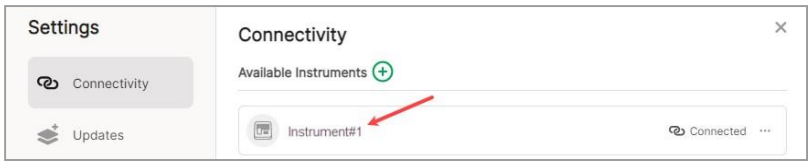


When the IP address is recognized as valid, the Connect button is enabled.

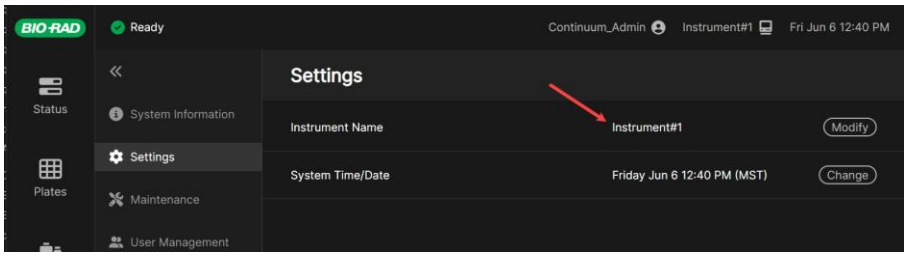
Note: If you receive an error message, check the IP address. If the problem persists, contact your system administrator or Bio-Rad Technical Support.

7. Click Connect.

The instrument is added to the Connectivity dialog and is automatically connected to QX Insight.



The instrument name shown in QX Insight is populated from the QX Continuum System Settings screen.



The instrument appears as Connected in the lower-left corner of QX Insight. You can use the Connectivity dialog to easily connect and disconnect different QX Continuum instruments to and from QX Insight.

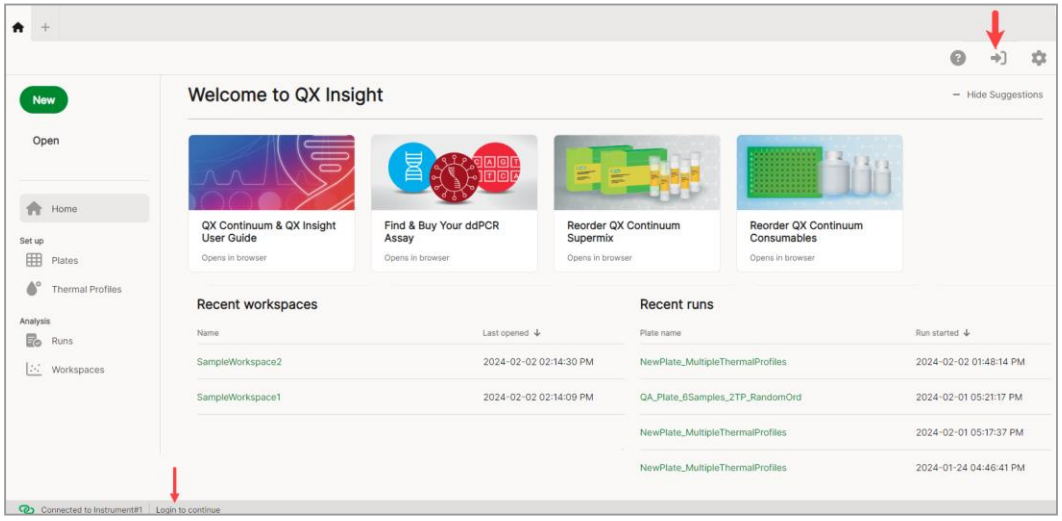
8. To log into the connected QX Continuum, see Logging Into QX Continuum From QX Insight on page 28.

Logging Into QX Continuum From QX Insight

Note: *This section applies to QX Continuum users only*. You do not need to log into QX Insight to open and analyze the .niodata files that are produced by the QX700 ddPCR System.

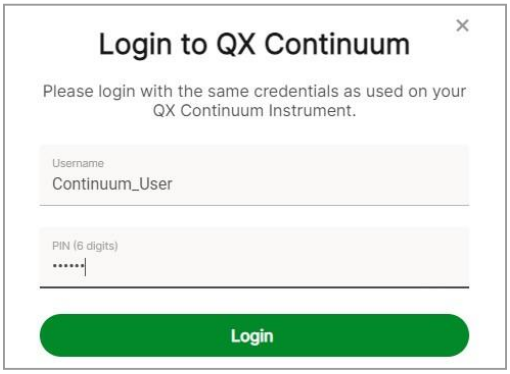
In order to directly upload configured plates to a QX Continuum, or transfer run data from the QX Continuum to a QX Insight workspace, you must log into the QX Continuum from QX Insight using your QX Continuum user ID and PIN.

1. Open QX Insight and click the Login icon ( ) in the upper-right corner or click *Login to continue* in the lower-left corner.



Logging Into QX Continuum From QX Insight

2. When the Login dialog appears, enter your QX Continuum user ID and six-digit PIN.



Note: If you have forgotten your user ID or PIN, contact your system administrator. The admin can communicate your user ID and create a new PIN.

3. Click Login.

The confirmation appears in the lower-left corner.

After QX Insight confirms your sign in information, the icon in the tool bar changes to .

Important: If you close QX Insight, you are automatically logged out and you must log in again after you reopen the application. The connected instrument remains connected.

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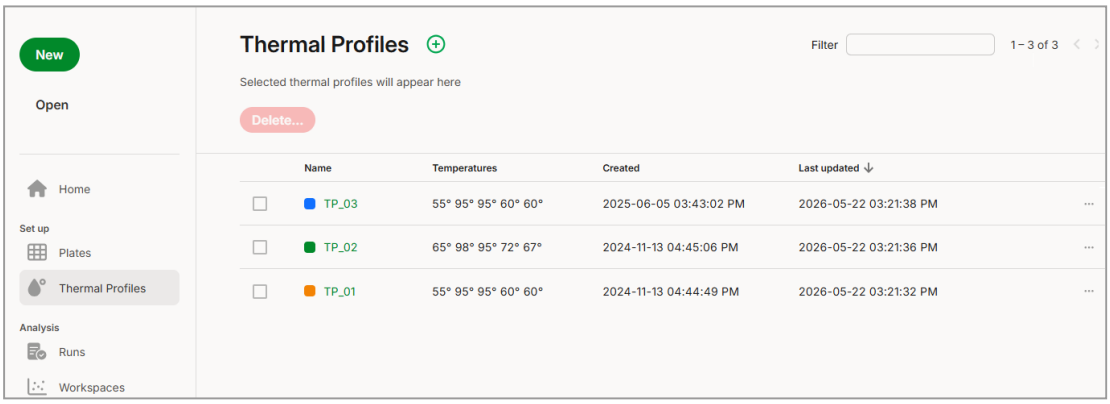
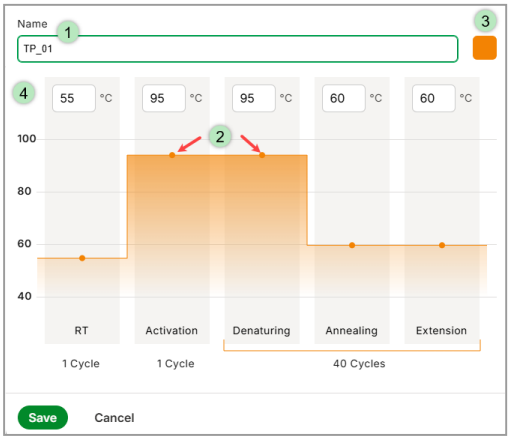
Chapter 5 Thermal Profile Configurations

Note: This chapter applies to the QX Continuum only.

When you click Thermal Profiles in the navigation pane, the Thermal Profiles view appears and displays the list of thermal cycling protocols you have created in QX Insight Software.



Thermal profile configurations contain the temperature settings for the repeated heating and cooling segments.



LEGEND

1	Default or modified thermal profile name
2	Temperature drag-and-drop
3	Color palette
4	Temperature field

Note the following:

- You must create at least one thermal profile for assignment to a plate before you can complete the plate configuration and upload the plate to the QX Continuum.
- You can create and edit a thermal profile only in QX Insight Software. ■ The New Thermal Profile and Edit Thermal Profile dialogs have identical layouts.
- You can color-code your thermal profiles for efficient organization and easy identification of multiple profiles in the plate.
- The thermal profile template opens to the default temperature ranges for all five process stages.
- You can manually adjust the temperatures for each stage within the allowed ranges.
- The denaturing, annealing, and extension process stages are configured for 40 cycles, which cannot be changed.
- The extension zone cannot be more than 5° lower than the annealing zone.
- A run on a full plate with one thermal profile assigned finishes in ~6 hrs (1.5 hrs for the first well and ~2.5 min for each subsequent well).
- You can apply up to eight thermal profiles, each specifying a different set of temperature ranges, to different wells or well sets in a plate; not available for the QX700.

  Important: Assigning multiple thermal profiles to the plate extends the processing time. A full plate with eight thermal profiles assigned can take ~16 hours to complete.

Creating or Editing Thermal Profiles

This section explains how to create a new thermal profile, or edit an existing thermal profile, in QX Insight Software.

1. Open QX Insight Software and click Thermal Profiles in the navigation pane.
2. Do one of the following:

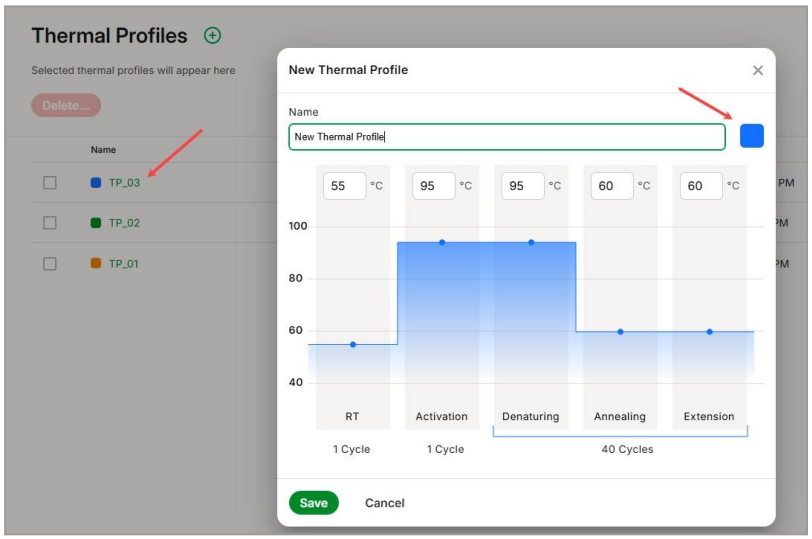
■ Click New, and then click the + Thermal Profile tile. ■ Click the icon to open the New Thermal Profile dialog. ■ Click a thermal profile in the list to open a thermal profile for editing.

Tip: In Plate Setup, you can also select one or more wells and click the icon in the Well Data dialog.

The New Thermal Profile or Edit Thermal Profile dialog opens.

Note: The New and Edit dialogs are identical.

If you have previously created thermal profiles, the dialog shows the color code applied to the last thermal profile you created. Otherwise, the dialog displays the default color and shows the QX Continuum's five thermal cycling zones.



3. Change the default thermal profile name.

4. To change the array of temperatures within each established range, enter a new temperature or click the dot at the top of each temperature indicator and drag up or down. You cannot drag above or below the preset temperature limits.

Table 7 contains the operational temperature ranges and number of cycles performed for each thermal cycling stage.

Table 7. Thermal profile cycles and temperature ranges

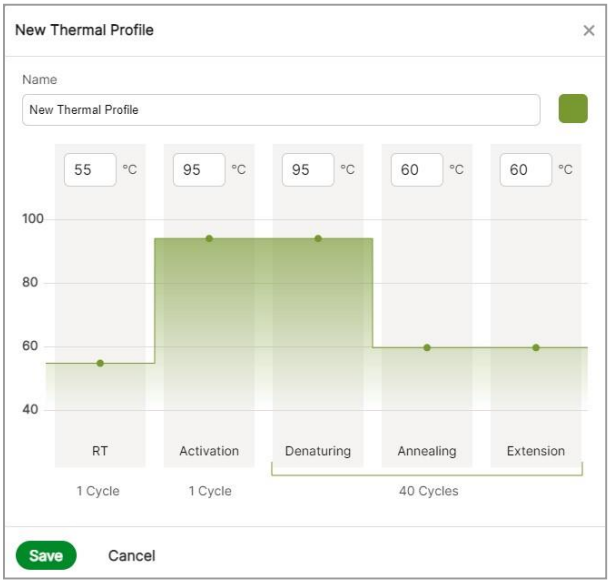
Thermal cycling stages	No. of cycles	Temperature: Suggested	Default	Max	Min
Zone 1 Reverse Transcription (RT)	1	52° C	55° C	65° C	45° C
Zone 2 Activation (preheating)	1	96° C	95° C	98° C	90° C

Zone 3 Denaturing (heating to separate double-stranded DNA)	40	95° C	98° C	90° C
Zone 4 Annealing (decreased heat to loosen the nucleotides)	40	60° C	72° C	50° C
Zone 5 Extension (using the loosened nucleotides to recreate the double-stranded DNA from each single strand)	40	60° C	72° C	50° C

5. To assign a color to the thermal profile, click the color palette icon and select a color.

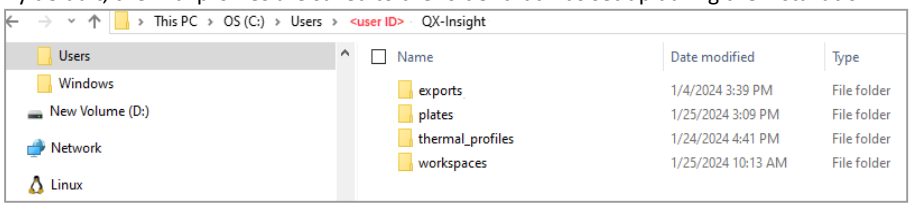
   Tip: This feature can assist with organizing your thermal profiles categorically, and identifying thermal profiles when more than one is used in a plate.

The display changes color.

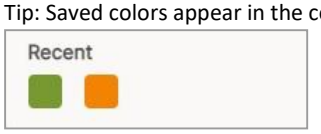


6. To save the thermal profile, click Save or Save as and follow the prompts.

   By default, thermal profiles are saved to the folder that was set up during the installation.



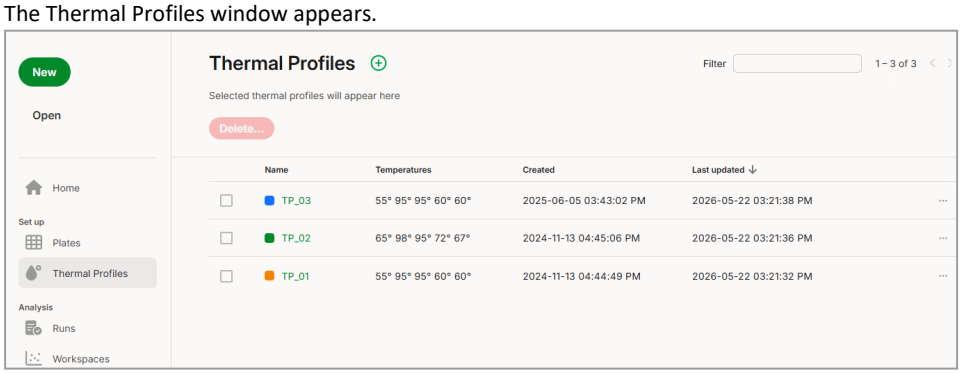
   Tip: Saved colors appear in the color palette under Recent.



Filtering Thermal Profiles

1. Open QX Insight Software and click Thermal Profiles.

   The Thermal Profiles window appears.



2. In the Filter field, enter identifying text for the thermal profile.

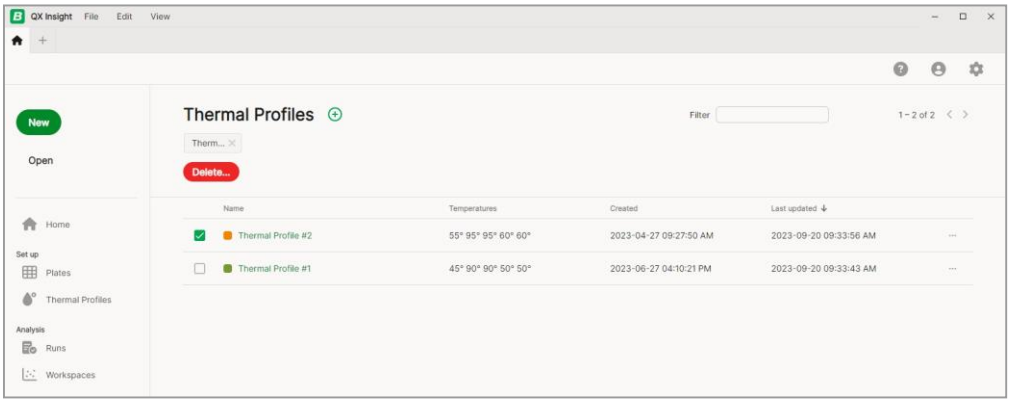
   As you enter characters, the list of thermal profiles narrows to those matching your entry.

Deleting a Thermal Profile

Deleting a Thermal Profile

1. Open QX Insight Software and click Thermal Profiles.

   The Thermal Profiles window appears.



2. Select the checkbox for the thermal profile to be deleted.

   QX Insight enables the Delete button.

   Note: You can select multiple checkboxes to delete the corresponding thermal profiles as a group.

3. Click Delete.

4. When the Delete Confirmation dialog appears, click Delete to permanently delete the thermal profile.

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Chapter 6 Plate Configurations

Note: This chapter applies to the QX Continuum only. Plate configurations for the QX700 are created in the QX700 ddPCR System Control Software. For information, see the QX700 Droplet Digital PCR System User Guide, Standard Edition (catalog no. 10000258608).

When you click Plates in the navigation pane, the Plates view appears and displays the list of plates you have created in QX Insight Software.

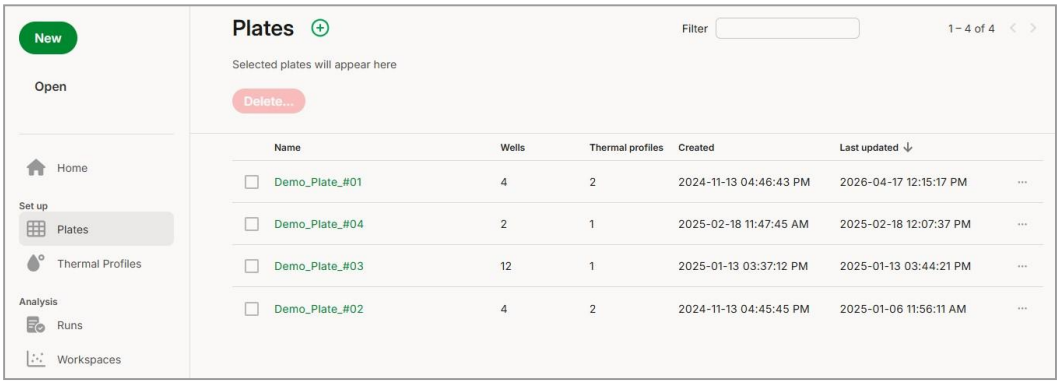
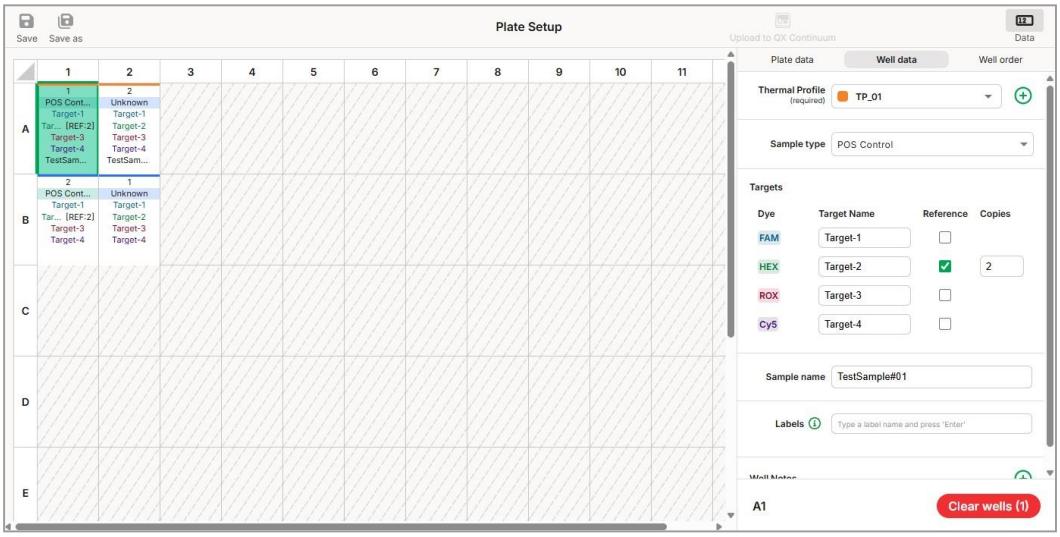
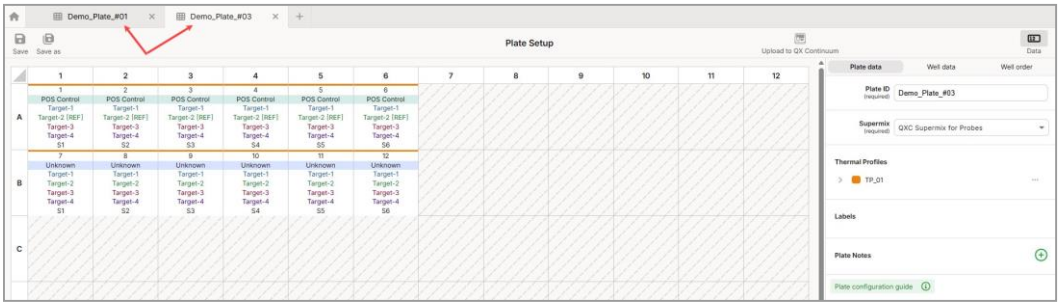


Plate configurations contain the settings for the entire plate (Plate data tab). selected wells (Well data tab), and the well processing order (Well order tab).



Note the following:

- You must create at least one thermal profile to assign to the plate before you can save the plate and upload it to the QX Continuum.
- You can
  - ☐ Open multiple plate layouts simultaneously in QX Insight
  - ☐ Configure up to 96 wells in each plate layout to match the physical plate setup ☐ Assign up to eight thermal profiles to wells in the plate layout (one profile per well) ☐ Change the order in which wells are processed
- When you open a plate, a corresponding tab appears above the user interface and you can easily navigate between tabs to create and edit plates.



- The changes are retained in each tab when you save the configurations.

  Important: Bio-Rad recommends that you save frequently in case of power loss or accidental closure of the application. In both scenarios, your changes are lost if your work was not saved. For information, see Saving Files on page 57.

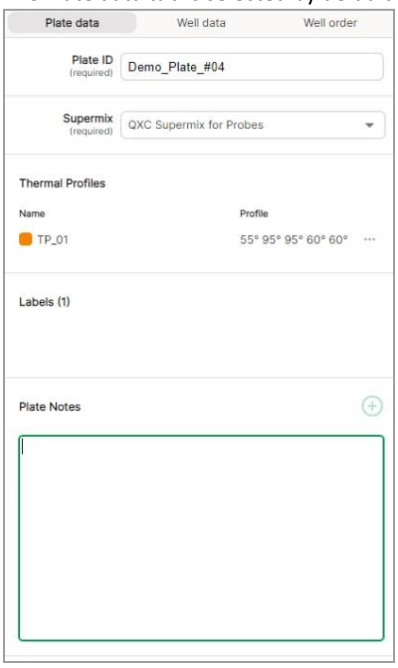
- After the configuration is complete, you can upload the plate to a connected QX Continuum where it remains available for selection until it is deleted.

Creating or Editing Plates

You can create a robust library of plate templates for your ddPCR experiments that will be run on the QX Continuum. This section explains how to create and save a new plate or edit an existing plate.

1. Open QX Insight Software and click Plates in the navigation pane.
2. Do one of the following:
   - Click New, and then click the + Plate tile. ■ Click the ⊕ icon to open the blank Plate Setup view.
   - Click Plates, and then click a plate to display its configuration in the Plate Setup view.

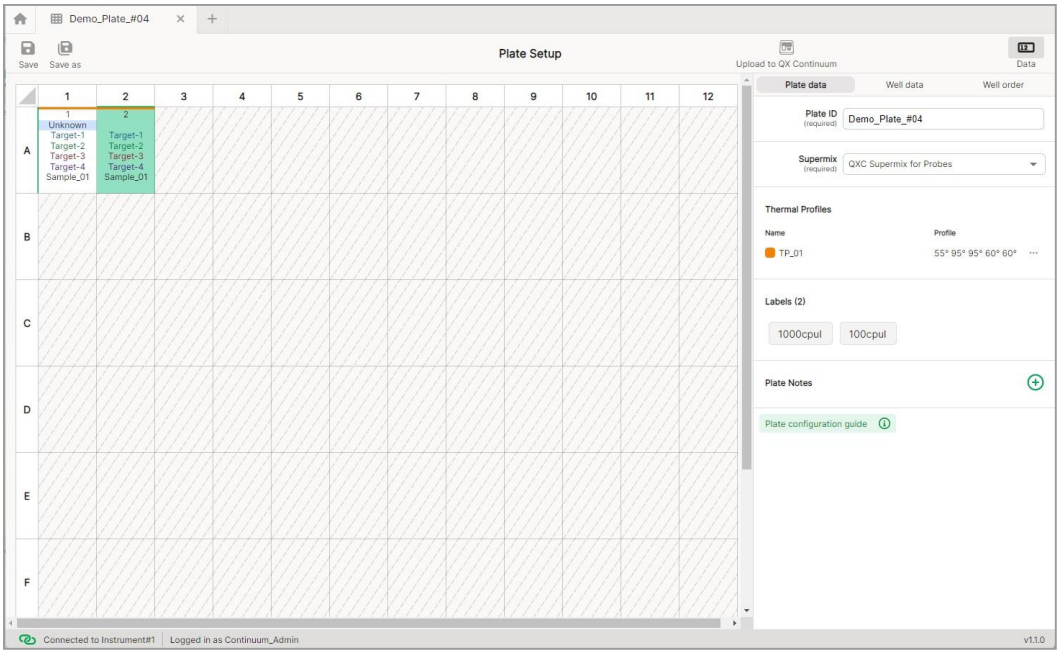
The Plate data tab is selected by default.



Defining Plate Data

Use the Plate data tab to name the plate, select a supermix, and add plate notes. The Thermal Profiles and Labels sections are automatically populated after you enter or select information on the Well data tab. The thermal profile name and temperature array appear under Thermal Profiles and your defined labels appear under Labels.

1. Enter or change the plate ID.



2. Select a supermix from the dropdown list.

   Important: Use ONLY the supermixes from Bio-Rad that are specifically designed and approved for use with the QX Continuum Droplet Digital PCR System. Do NOT use other supermixes, including QX supermixes, since they can harm the instrument.

   The Thermal Profiles and Labels sections are blank until you assign them in the Well Data tab.

3. (Optional) Click the ⊕ icon next to Plate Notes and add or change pertinent information about the plate. For information, see Adding Plate Notes and Well Notes on page 48.

4. Select the Well data tab and continue to the next section.

Defining Well Data and Well Order

After you select the Well data tab, you can select a well or well group, and then do the following: ■ Assign a thermal profile

■ Identify the target genes (one per dye/up to four per well)

■ Specify a descriptive sample name ■ Create and assign labels for

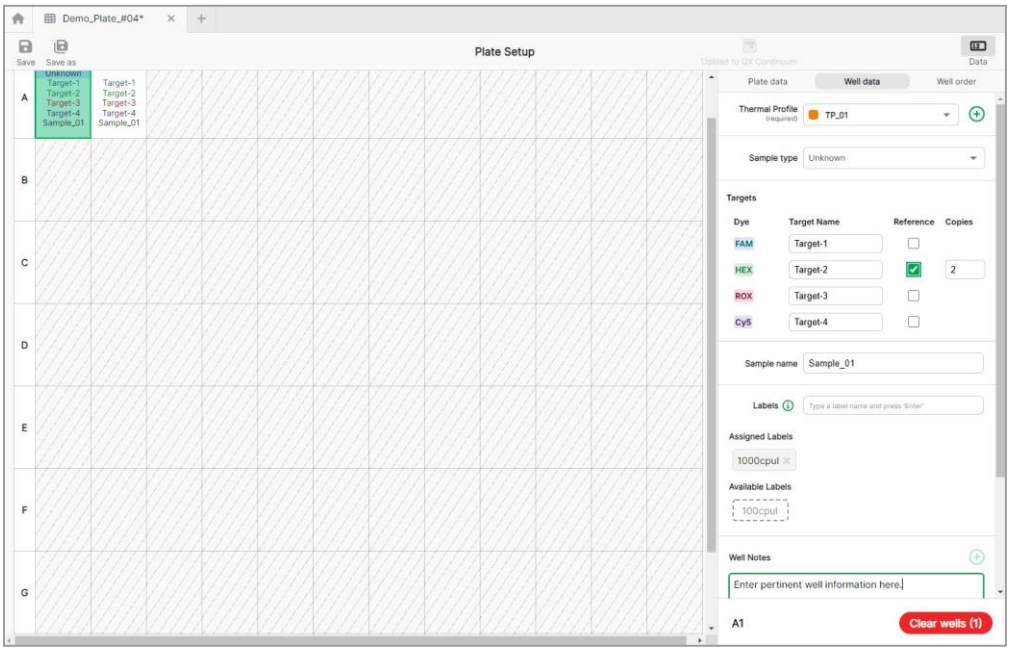
additional identification ■ Add applicable notes in the well notes

field

Important: Ensure the configured plate layout matches the physical plate. Empty wells should be dry and should remain excluded.

To create or edit well data

1. Select one or more wells in the Well Selector and then select the Well Data tab.



2. Assign a thermal profile to the selected well or well group.

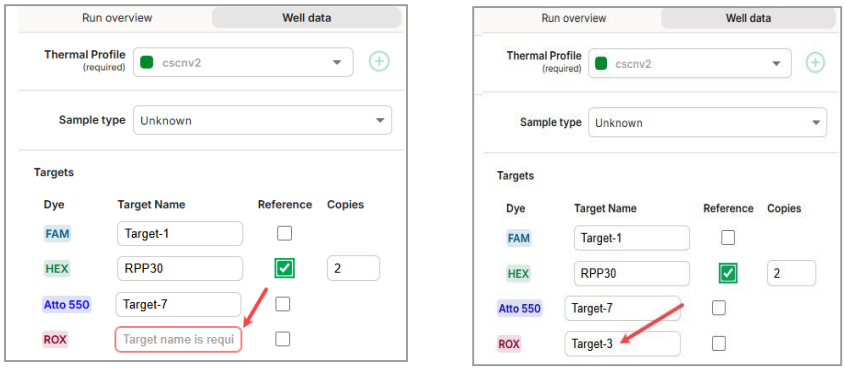
   You can assign a single thermal profile to all wells, or assign different thermal profiles to different wells or well groups. You can assign up to eight thermal profiles in the plate.

   Important: Assigning more than one thermal profile to the plate extends the plate processing time. For information, see Assigning Multiple Thermal Profiles on page 45.

   When you assign a thermal profile to one or more wells, the Targets section is enabled and contains default entries for the four fluorescent dyes.

3. Change the target names to descriptive entries that identify the target genes of interest.

   The Target Name field cannot be empty. If you delete the current entry without a replacement, the software restores the entry before saving.



4. To specify a particular target as a *reference* gene, select the checkbox on the right.

   When you select the Reference checkbox, the reference gene is used in determining the copies per cell of the gene of interest. A reference target is required to calculate Copy Number, Ratio, and Fractional Abundance data points. The default number of copies is 2. Follow ddPCR application guidelines to determine the gene most appropriate to use as a reference in your experiment.

5. Select the sample type from the dropdown list. You can choose from Unknown, POS Control, NEG Control, and NTC (no template control).

6. Enter or change the sample name, which can serve as a unique identifier for a well or group of wells.

   Note: If you enter a different sample name for each well, the sample name shows Mixed Content when you select the wells as a group.

7. (Optional) Add labels. For information, see Using Well Identification Labels on page 46.

8. (Optional) Enter or change notes regarding the well. For information, see Adding Plate Notes and Well Notes on page 48.

9. To change the default order of well processing, see Reordering Wells and Thermal Profiles on page 49.

10. When you are done, click Save or Save as and follow the prompts to save the plate layout. Assigning

Multiple Thermal Profiles

As shown in the following graphics, you can assign a different thermal profile to each well in your plate, *up to a total of eight (8) thermal profiles* for the entire plate. Full-plate processing time is extended when you add multiple thermal profiles to the plate (*up to 16 hours for the maximum of eight thermal profiles*).

Fig. 1: Thermal profile assigned to well A1

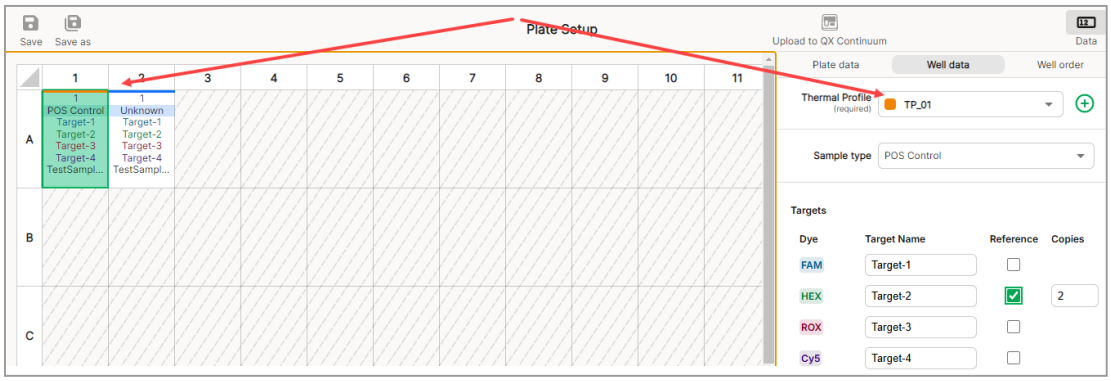
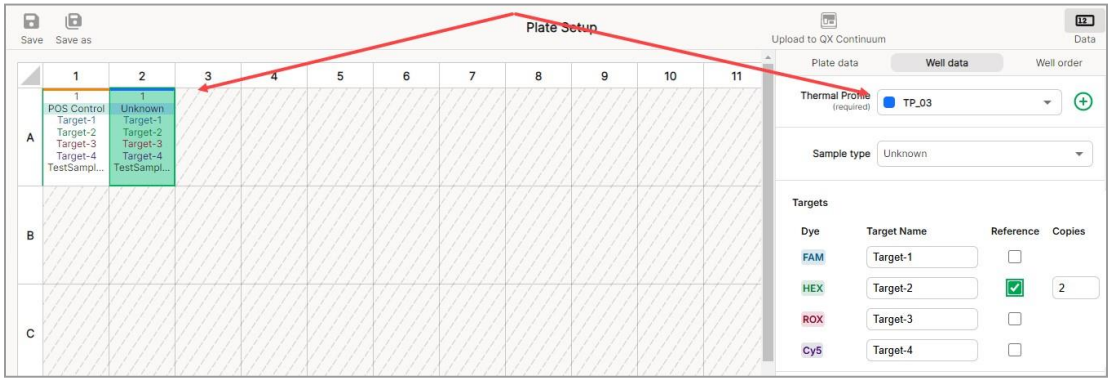


Fig. 2: Thermal profile assigned to well A2



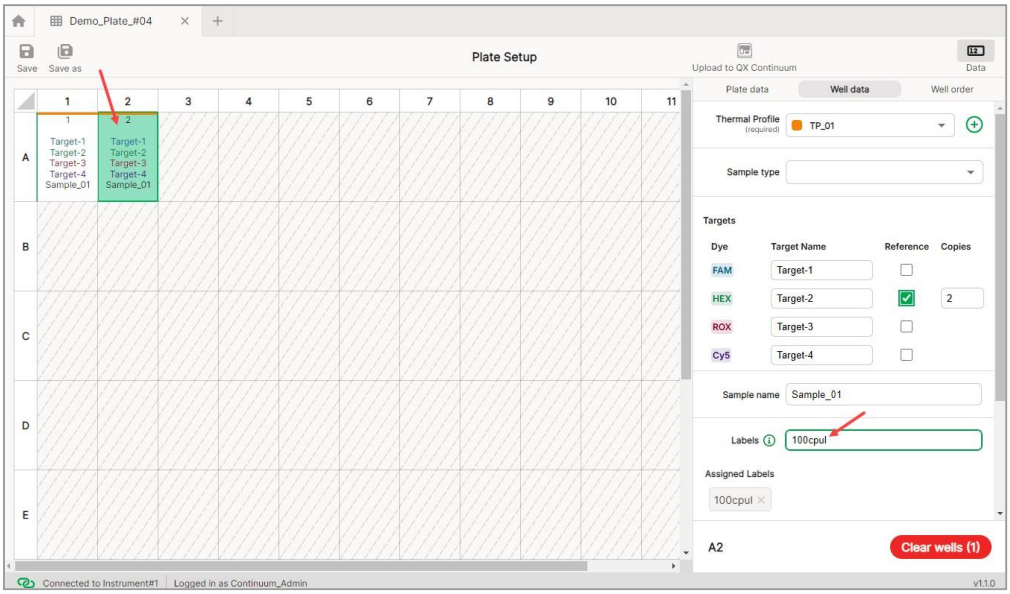
Using Well Identification Labels

Labels allow you to categorize wells into certain groups using a common identifier. You can create labels during plate setup or at any time in Edit mode. Labels are not case-sensitive. You must select a well to add a label and the label you create is automatically applied to the well.

You can add up to 10 labels, each consisting of up to 16 characters. After your labels are defined, you can click a label to select the wells to which it is assigned. Run files include the labels so you can easily select particular well groups.

To create and apply a label

1. Select a well to which the label will be assigned.



2. On the right, select the Well data tab and then type the applicable label in the Labels field and press Enter.

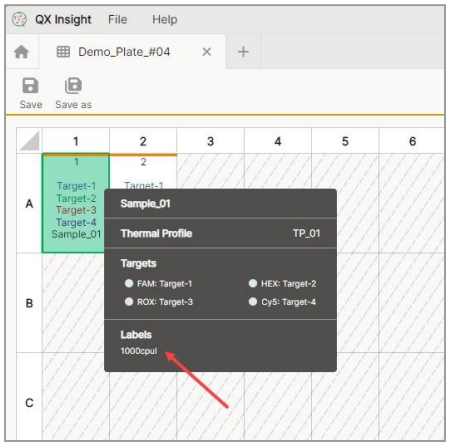
   The label is added to the well data.

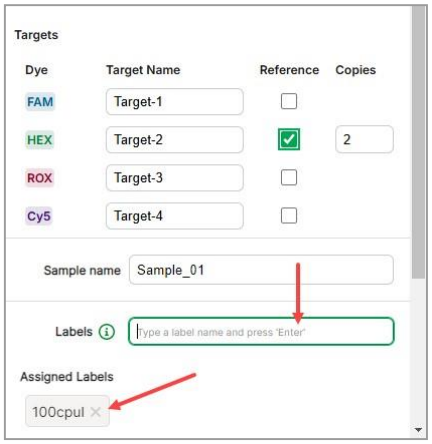
3. Repeat to create another label.

4. To add a label to another well, select the well and then click in the Labels field and click a label.

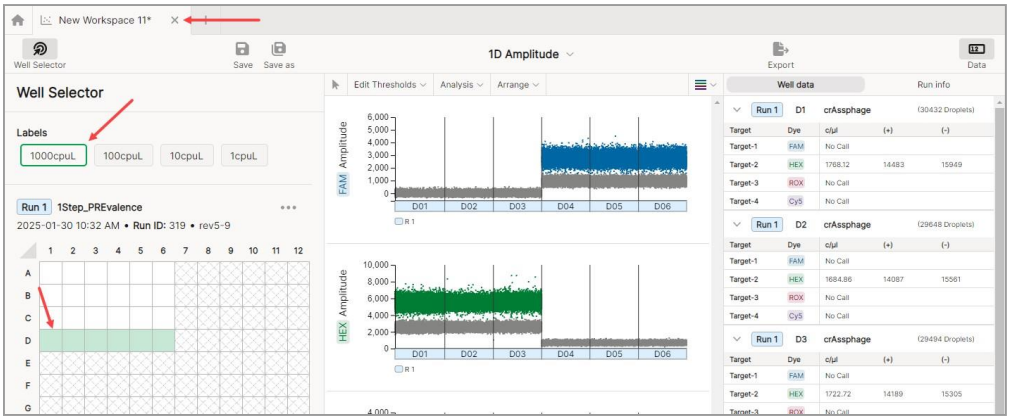
Note the following:

- When you press Enter, the Labels field clears and the label is automatically assigned to the selected well.
- In the Plate Setup view, the label appears in the tool tip when you pause on a well and also in the Assigned Labels section of the Well data tab.





- After you run the plate on the QX Continuum and open the run for analysis in a QX Insight workspace, the assigned labels appear above the plate grid in the Well Selector.
- When you click a label, QX Insight highlights the applicable row and the corresponding well data appears in the chart view and in the Well data tab.



5. You can double-click an available label to quickly assign it to a given well.

To remove a label

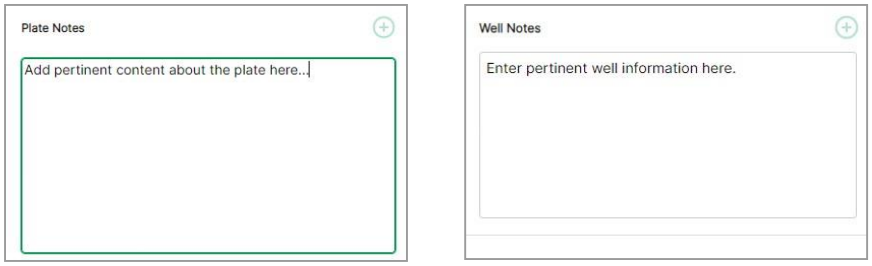
▶ Click the X on the right side of the label.

Adding Plate Notes and Well Notes

You can use the Plate Notes field to add descriptive information about the plate in general, and the Well Notes field to add descriptive information about a selected well or group of wells. You can use up to 512 characters (including spaces) in both fields.

1. Click the ⊕ icon next to Plate Notes (Plate data tab) or Well Notes (Well data tab) to expand the field.

2. Click in the field and enter pertinent information for the plate or well.



The notes are saved when you save the plate.

Reordering Wells and Thermal Profiles

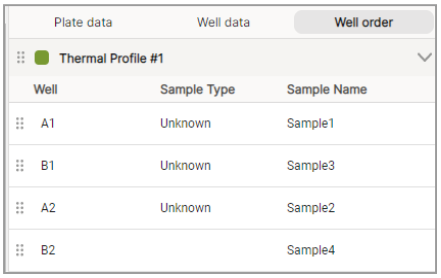
By default, wells are processed in rows (for example, A1, A2 followed by B1, B2) for each thermal profile assigned. When you set up wells and assign multiple thermal profiles, the first profile you assign to a well assumes the initial processing position in the Well order tab. Subsequent wells and thermal profiles follow in the order

When you change the well processing order, the thermal profile processing order also changes. The default well processing order is by well and by row, so the thermal profile assigned to the first well or set of wells is processed first, followed by the thermal profile assigned to the next well or set of wells, and so forth.

To change the processing order, select the Well Order tab and do one of the following:

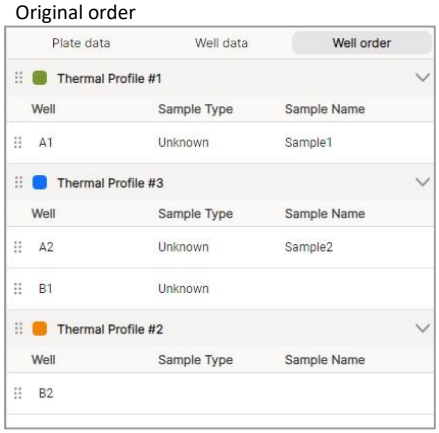
- One thermal profile — click and drag a well row to a different position. For example, Move well A2 to the position below well B1).

Original order Revised order



- Multiple thermal profiles — Click and drag a well to a different position. The thermal profile changes to the thermal profile in that group. For example, move well B2, which was originally assigned Thermal Profile #2, to the position beneath well A1. Well B2 is now assigned Thermal Profile #1.

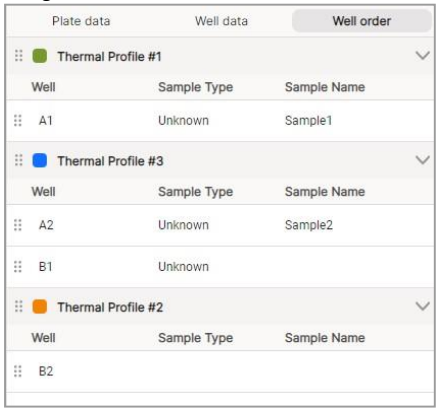
Original order



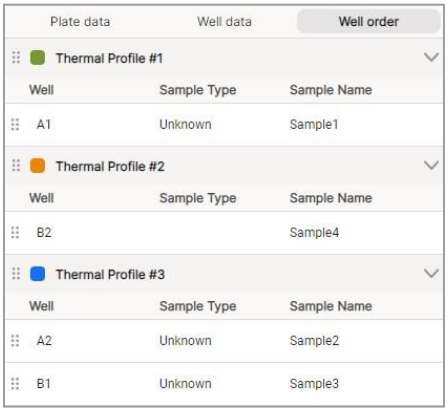
Revised order

- Click and drag a thermal profile to a different position (move Thermal Profile #2 to the position below Thermal Profile #1). This does not change the original thermal profile assignments.

Original order



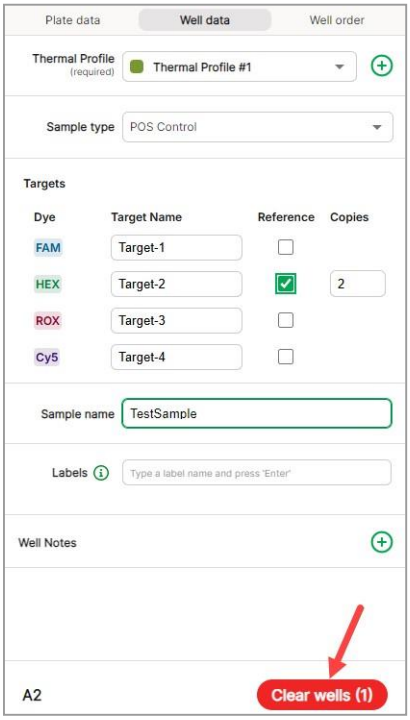
Revised order



Clearing and Reconfiguring Wells

1. To clear well content and start over, select the wells to clear in the Well Selector.
2. In the Well Data tab on the right, click Clear wells.

   Note: The number of wells to be cleared appears in the Clear wells button.



Uploading a Plate Layout to QX Continuum

If you are logged into a connected instance of QX Continuum from QX Insight Software, you can upload new or edited plates directly to the instrument from QX Insight using the Upload to Continuum option in the plate setup view

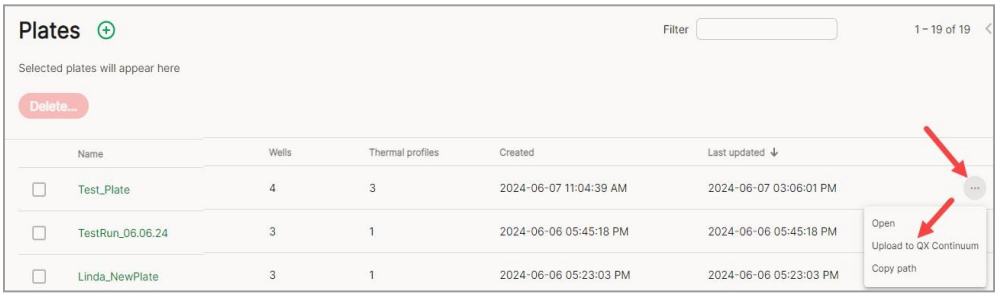
If you are not connected and logged in from QX Insight, you can copy the plates to a USB drive, and then upload them to the instrument. For information, see Transferring Files Using a USB Drive on page 61.

Important: QX Insight plate layouts are unique to QX Insight and QX Continuum and are not transferable to other applications.

You cannot edit plates in the QX Continuum touch screen. You must make your changes in QX Insight, and then upload the plate to the instrument again. A copy number is appended to the plate name and the existing plate is not overwritten. You must manually delete it if you no longer need it.

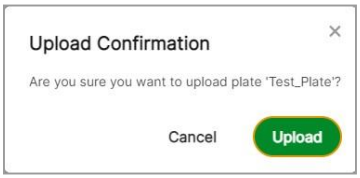
1. Do one of the following:

- In the Plates view, click the three-dot icon on the right to display the pop-up menu, and then click Upload to QX Continuum.



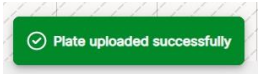
- In the Plate Setup view, click Upload to QX Continuum.

A confirmation message appears, asking you to confirm the upload.

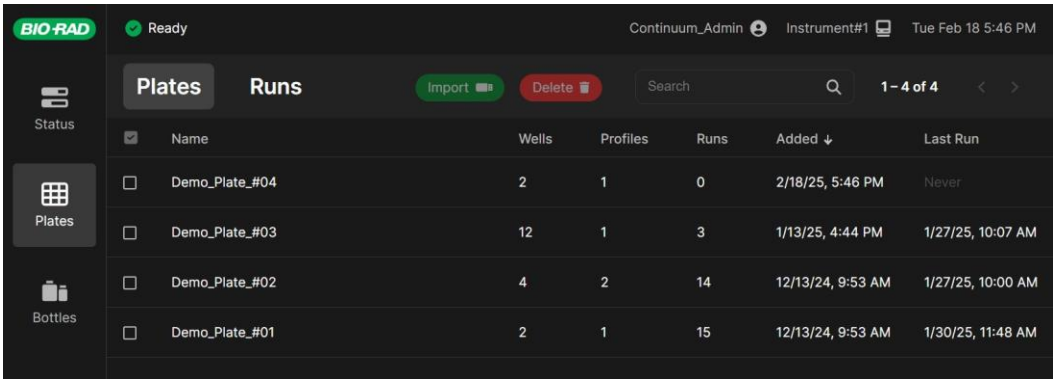


2. Click Upload.

A successful upload message appears at the bottom of the QX Insight view.

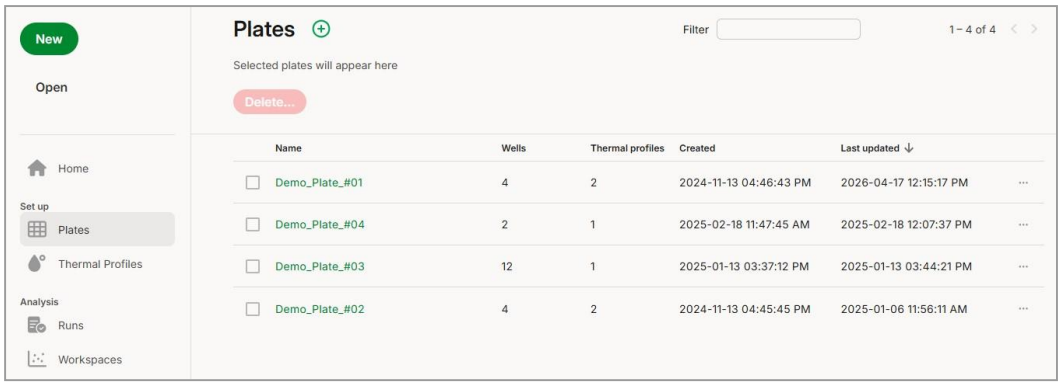


The plate appears in the Plates screen of the connected QX Continuum.



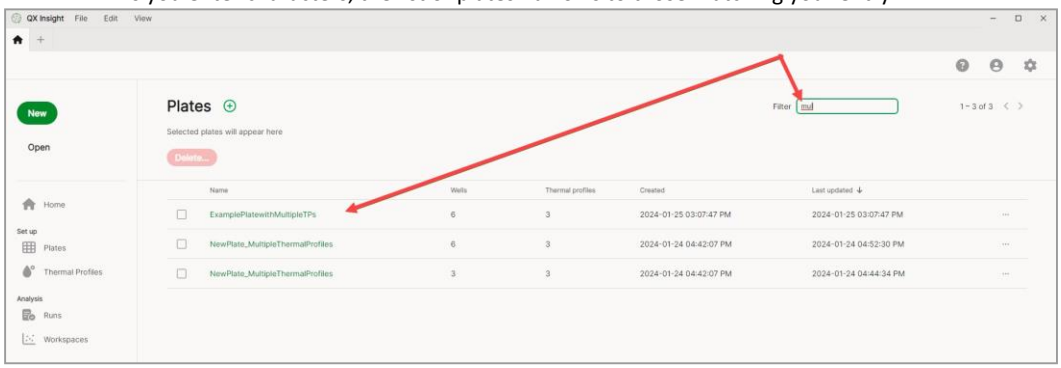
Filtering the Plate List

1. In QX Insight, click Plates to display the Plates view.



2. In the Filter field, enter a plate name.

As you enter characters, the list of plates narrows to those matching your entry.

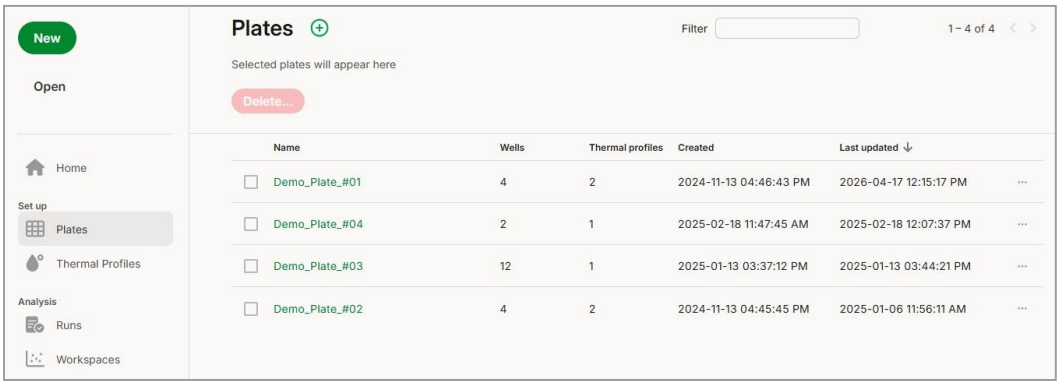


Deleting Plates

Deleting Plates

1. Open QX Insight Software and click Plates.

The Plates window appears.



2. Select the checkbox for the plate to be deleted.

   QX Insight enables the Delete button.

   Note: You can select multiple checkboxes to delete the corresponding plates as a group.

3. Click Delete.

4. When the Delete Confirmation dialog appears, click Delete to permanently delete the plate.

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Chapter 7 File Management

The following sections contain information on saving files, sharing files with other users, and transferring files using a USB drive.

Saving Files

In QX Insight Software, you can save the following file types:

Table 8. File types

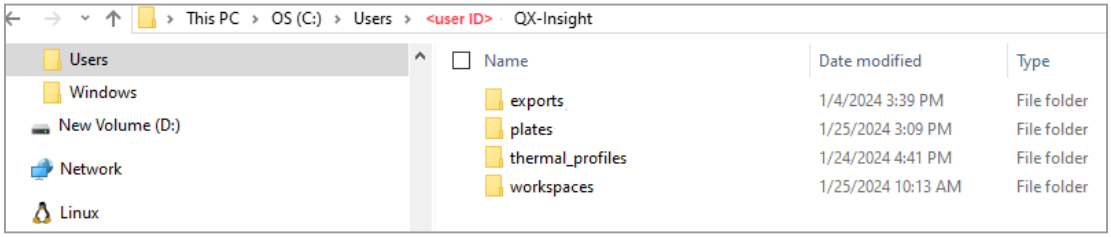
For this instrument...	You can save these files...
QX Continuum	■ Thermal profiles ■ Plate layouts ■ Analysis workspaces
QX700	■ Analysis workspaces

Saved files appear in the Thermal Profiles, Plates, and Workspaces views. Saved workspaces (up to 10) also appear in the Home view. All runs opened in QX Insight workspaces are saved as .qxi files. When saving a QX700.niodata file, the native file remains unaltered in its original location.

Important: If you close a workspace without saving it, you must add the run to a new workspace in order to continue analyzing the data.

You can use the Save option to save files in their current or default locations or you can use the Save as option to navigate to a new location before saving the file.

The following graphic identifies the default folders that are created as part of the. QX Insight Software installation.



To save a plate or thermal profile, do one of the following:

- Click Save to automatically save the file with its current file name and in the default location. ■ Click Save as to change the file name or storage location, and then click Save.

To save a workspace ▶ In the top menu bar, do one of the following:

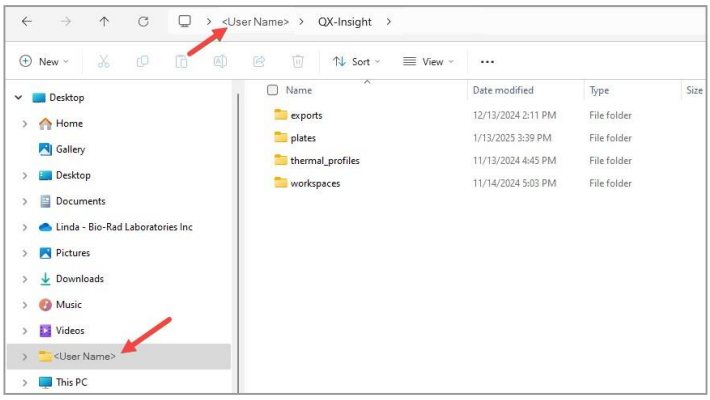
- Click Save to save an existing .qxi file with its current name.
- Click Save as to save the workspace as a .qxi file. You can keep the default name or enter a new name, and then click Save.

Sharing Files

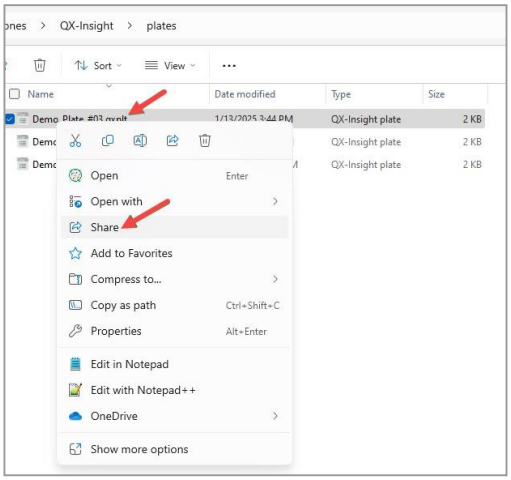
Provided you are connected to your organization's network, you can share, with other users, exports, plates, thermal profiles, and workspaces from your Windows personal file folders.

1. Navigate to and open your QX-Insight folder.

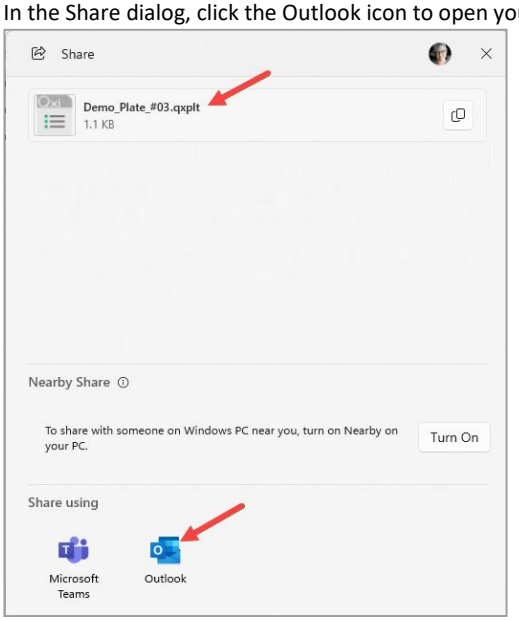
   Tip: On Windows systems, the file path is C:\Users\<user name or ID>.



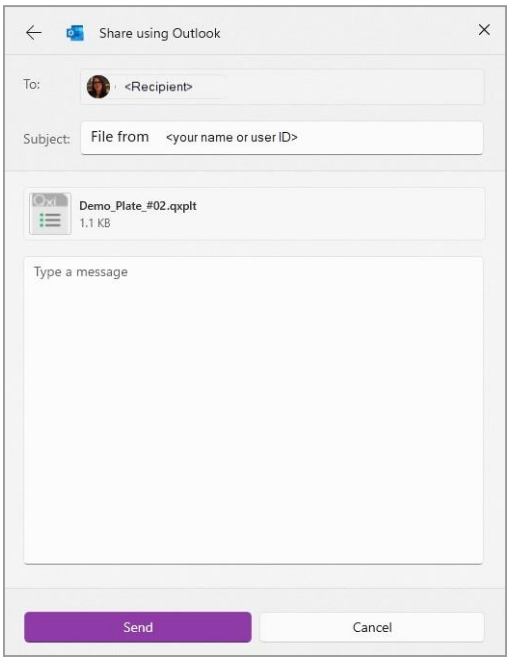
2. Open a subfolder (such as plates, as shown below), select a file and right-click, and then select Share.



3. In the Share dialog, click the Outlook icon to open your list of Contacts.



4. Select a user to open the Share using Outlook dialog.



5. Type a brief message (optional), and then click Send to share the file.

Transferring Files Using a USB Drive

QX Continuum instruments can connect to the customer network using an Ethernet cable to a network port or a wireless connection. The QX700 can connect to the customer network (LAN) using an Ethernet cable only.

Only QX Continuum instruments can connect to QX Insight using the QX Insight Connectivity dialog, which allows plate uploads and run data transfers between the instrument and software. For information on the QX Continuum, refer to the QX Continuum Droplet Digital PCR System Instrument Guide (catalog no. 10000258167).

The QX700 does not connect to QX Insight, but you can access .niodata run files from their storage location as long as they are in a network folder and there is a LAN connection. For information on the QX700, refer to the QX700 Droplet Digital PCR System Instrument Guide, Standard Edition (catalog no. 10000258608).

You can use a USB drive to move files from one location to another in the following circumstances:

- Your QX Continuum instrument IS NOT connected to a LAN or wireless network, so files stored on the instrument or connected computer are not accessible
- Your QX Continuum instrument IS connected to a LAN, but is not connected to QX Insight, so files cannot be transferred directly to or from the instrument.

- Your QX700 is not connected to a LAN.

To transfer plate files

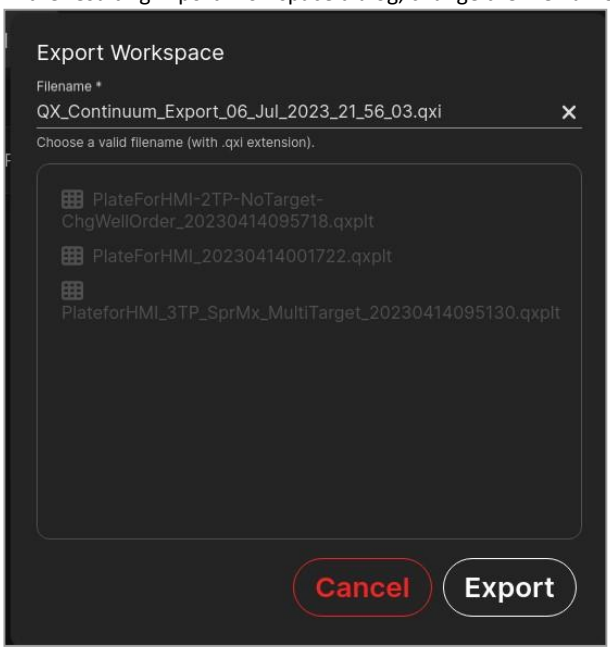
1. Copy the file from the Plates storage location on the QX Insight computer to the USB drive.
2. Insert the USB drive into the USB port on your QX Continuum.
3. In the left pane of the touch screen, click Plates.
4. When the Plates screen appears, click Import.

   The contents of the USB drive appear in the QX Continuum File Browser.
5. Click the plate file to be imported and then click Import.

   The file is imported and appears in the QX Continuum Plates list.

To transfer run files

1. Insert a USB drive into the USB port on your instrument.
2. In the left pane on the QX Continuum touch screen, click Plates, and then select the Runs tab.
3. Click the checkbox next to the run and then click Export.
4. In the resulting Export Workspace dialog, change the file name (optional) and click Export.



   The file is copied to the USB drive.
5. Remove the USB drive from the QX Continuum and insert it into the QX Insight computer.
6. Navigate to the workspaces folder and copy the data from the USB.

Chapter 8 Analysis Workspaces

A workspace is a QX Insight Software view in which you can analyze run data collected on the QX Continuum ddPCR System and the QX700 ddPCR System. A workspace contains the following elements:

- Well selector layout that matches your instrument
- Chart or view containing graphical interpretations of the ddPCR configurations and run data
  - Tabs containing plate data, well data, run information, and well processing order[1]

When you create a workspace, QX Continuum data is automatically transferred from the instrument to a connected instance of QX Insight. When you manually open a QX700 run data (.niodata) file from a USB or network location, it opens in a workspace automatically. Workspaces are always saved in .qxi format.

This chapter describes the general workspace elements. For more information on each chart view and the corresponding analysis capabilities, see Chapter 9, Droplet Data Analysis.

[1] Processing order applies to QX Continuum only.

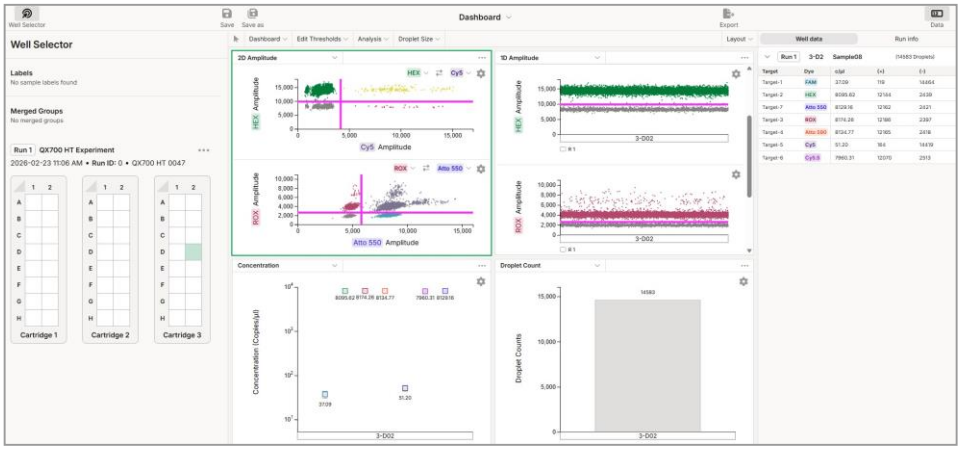
Opening a QX700 Run File in a Workspace

To analyze an experiment that was run on the QX700 ddPCR System, you can navigate to a .niodata file and open it in a workspace. After the file is saved as a .qxi workspace file, you can open it from the workpaces folder or click the file name in the Home or Workspaces view.

Important: You can open only one .niodata run file in a workspace. Files are large and might take a couple of minutes to open. Each .niodata file is saved in the workspaces folder as a .qxi file. The .niodata file remains in the source storage folder and is not updated.

1. Open QX Insight Software and click Open in the left pane.
2. In the Open file dialog, navigate to and select the file, and then click Open.

   The file opens in the workspace and the Dashboard appears by default.



Note the following:

- To retain the transferred run data in the workspace format (.qxi), you must save the workspace in QX Insight. For information, see Saving Files on page 57.
- The workspace is always saved to the workspaces folder, no matter where the .niodata file originates. .
- Up to 10 saved workspaces appear in the Home view and all saved workspaces appear in the Workspaces view.
- If you close the workspace without saving it, QX Insight discards the transferred run data and you must re-add the run to a new or existing workspace to continue analyzing the data.

Adding QX Continuum Runs to a Workspace

To analyze run data collected on the QX Continuum Droplet Digital PCR System, you must sign into the connected QX Continuum from QX Insight Software, and then add the run to an analysis workspace. You can add runs to a workspace from multiple views.

Important: Run data is not available until it is added to a workspace and the data provided is *provisional* until the run is completed.

To view a run in a workspace, you can do any of the following:

- Click a run in the Home view to automatically open it in a new workspace
- Create a new workspace in the Workspaces view, and then add up ten runs (see Creating a QX Continuum Workspace and Adding Runs on page 66) ■ Select up to ten runs in the Runs view and then create a workspace (see See Selecting QX Continuum Runs and Creating a Workspace) ■ Add additional runs to an existing workspace (Adding QX Continuum Runs Within a Workspace on page 70)

After a run is added to a workspace, the run data is transferred as follows:

- If you add an in-progress run to a workspace, run data is transferred to the workspace as processing of each well is completed. *Provisional* run data is collected and displayed in the workspace while the run is in progress; if the run fails, the data is discarded.
- If you add one or more completed runs to a workspace, successful run data is transferred immediately for analysis.

  Important: A red X in the Well Selector identifies a failed well with no corresponding data. If a run fails completely (all wells have a red X), perform a system flush and then rerun the plate.

After the workspace is created, populated, and saved, you can analyze your data without being connected and logged into the instrument.

Important: *You must save the workspace in QX Insight to retain the transferred run data*. Up to 10 saved workspaces appear in the Home view and all saved workspaces appear in the Workspaces view. If you close the workspace without saving it, QX Insight discards the transferred run data and you must re-add the run to a new or existing workspace to continue analyzing the data.

Creating a QX Continuum Workspace and Adding Runs

To add a QX Continuum run to a workspace, you must be connected to the instrument and logged in from QX Insight.

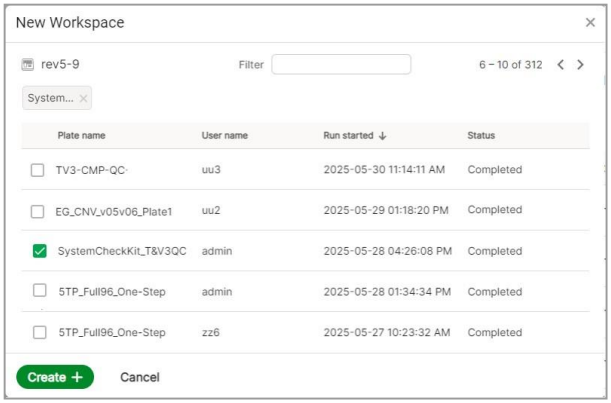
1. Do one of the following:

   - Click New, and then click the + Workspaces tile. ■ Click the  icon to open the New Workspace dialog.

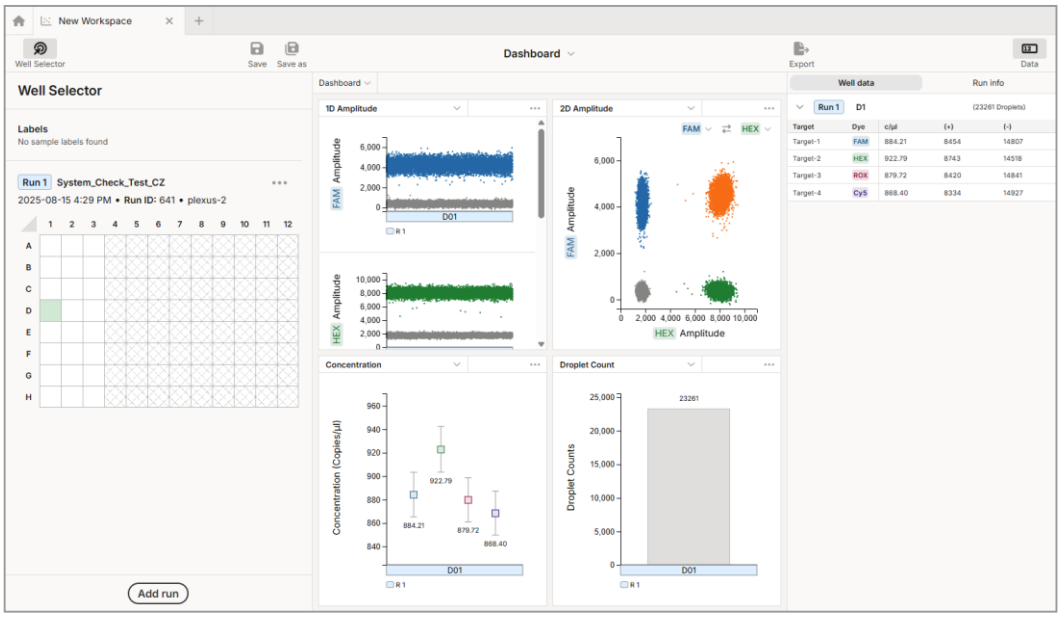
- Click a workspace in the list to open a workspace for editing.

2. In the New Workspace dialog, select up to 10 runs and then click Create.

   Note: Click the > icon to advance to the next set of runs in the dialog and < to go back.



   QX Insight opens the workspace and shows each plate on the left. The charts are populated when you select one ore more wells.



3. To save the workspace, click Save or Save as and follow the prompts.

   QX Insight saves the workspace to the Workspaces library and the link appears on the Workspaces page. You can keep the same run name and overwrite the existing information, or rename the data file to keep both workspaces.

Selecting QX Continuum Runs and Creating a Workspace

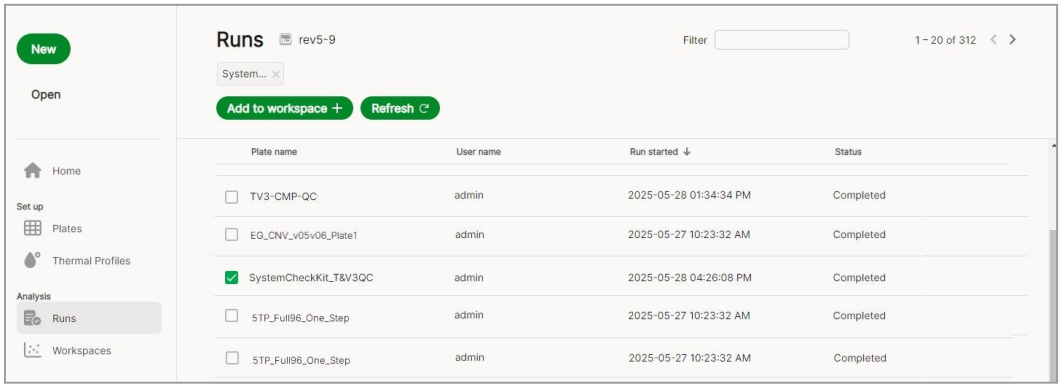
You can click a run in the Home view to automatically open it in a workspace or you can select one or more runs in the Runs view and then add them to a workspace.

To add a QX Continuum run to a workspace, you must be connected to the instrument and logged in from QX Insight.

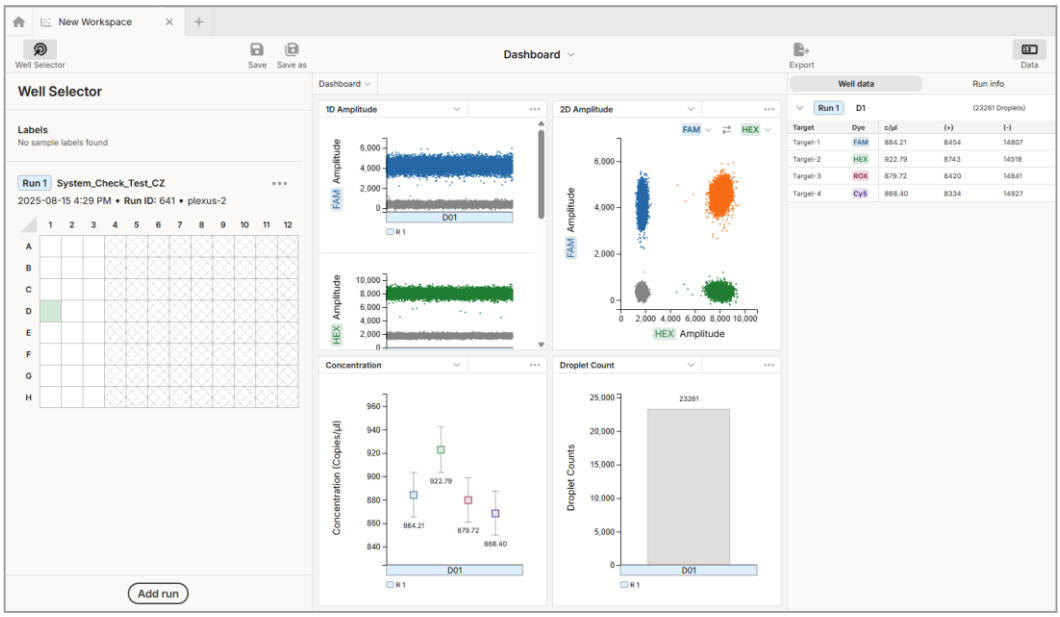
1. Do one of the following:

   - From the Home view, click a run in the Runs column. QX Insight Software creates and opens the workspace immediately. To add more runs, see Adding QX Continuum Runs Within a Workspace on page 70
   - From the navigation pate, click Runs and continue to Step 2.

2. In the Runs view, select the corresponding checkbox for each run to be added (up to 10), and then click Add to Workspace.



The run is added. A blank Dashboard view opens by default and you must select at least one well in the Well Selector to populate the charts.

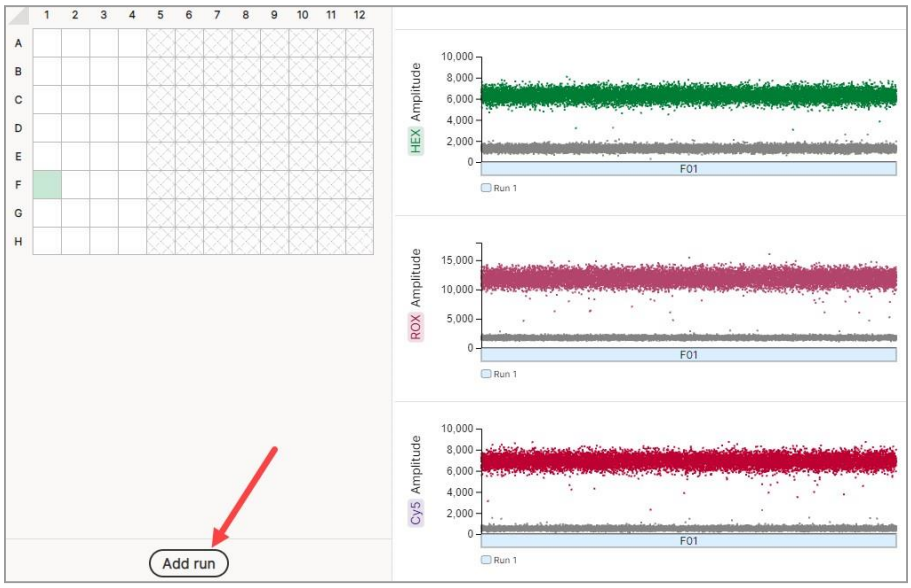


3. To save the workspace, click Save or Save as and follow the prompts.

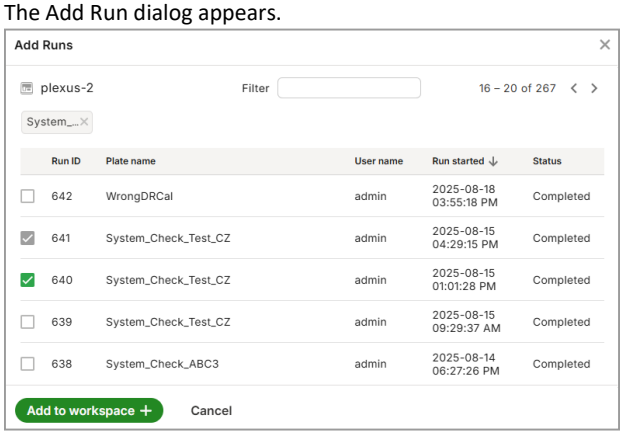
   QX Insight saves the workspace to the Workspaces window. You can keep the same run name and overwrite the existing information, or rename the data file to keep both workspaces.

Adding QX Continuum Runs Within a Workspace

1. Click Add run to open the Add runs dialog.

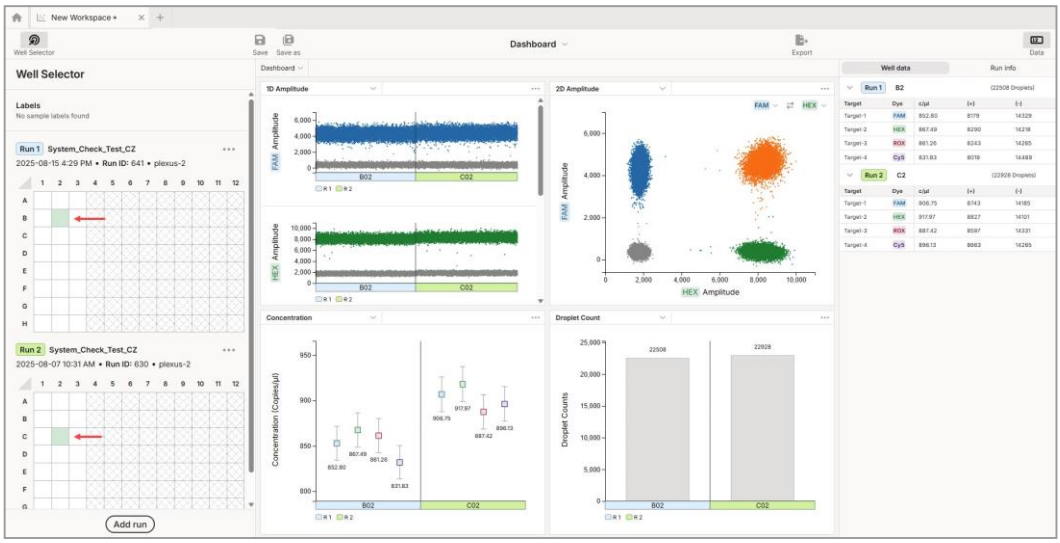


The Add Run dialog appears.



2. Select the checkbox for the run to be added, and then click Add to workspace.

   Note: QX Insight recognizes the runs you added previously, and adds the new run.



3. To save the workspace, click Save or Save as and follow the prompts.

   QX Insight saves the workspace to the Workspaces window. You can keep the same run name and overwrite the existing information, or rename the data file to keep both workspaces.

Well Selector

The Well Selector appears on the left and automatically displays the configuration for the applicable instrument:

■ 96-well layout that represents a run from the QX Continuum (Fig. 3) ■ Three-cartridge

layout that represents a run from any QX700 model (Fig. 4)

When you open the run in a workspace, the correct plate layout appears by default and shows the wells configured for the run. Instrument and run metadata appear above the plate or cartridge layout.

For information on selecting wells in each layout, see Selecting Wells on page 109.

Fig. 3: QX Continuum plate layout

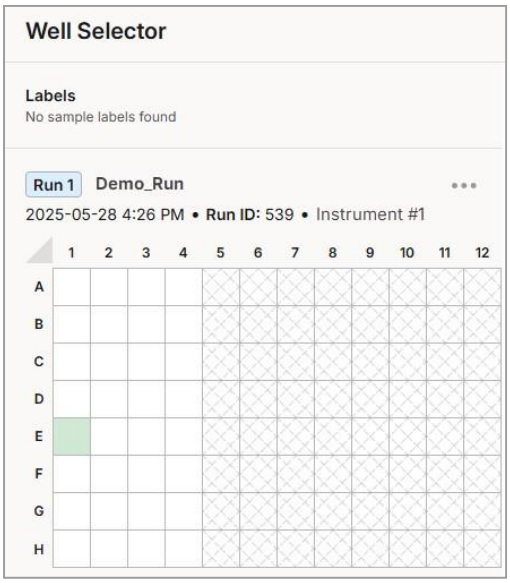


Fig. 4: QX700 cartridge layout

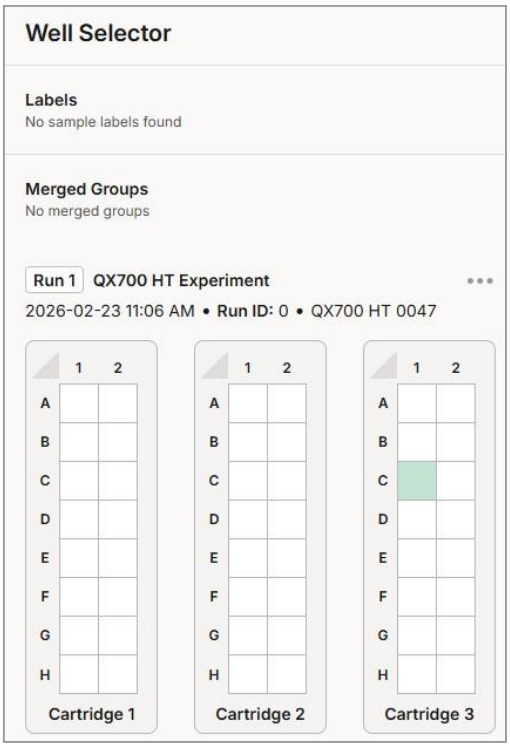
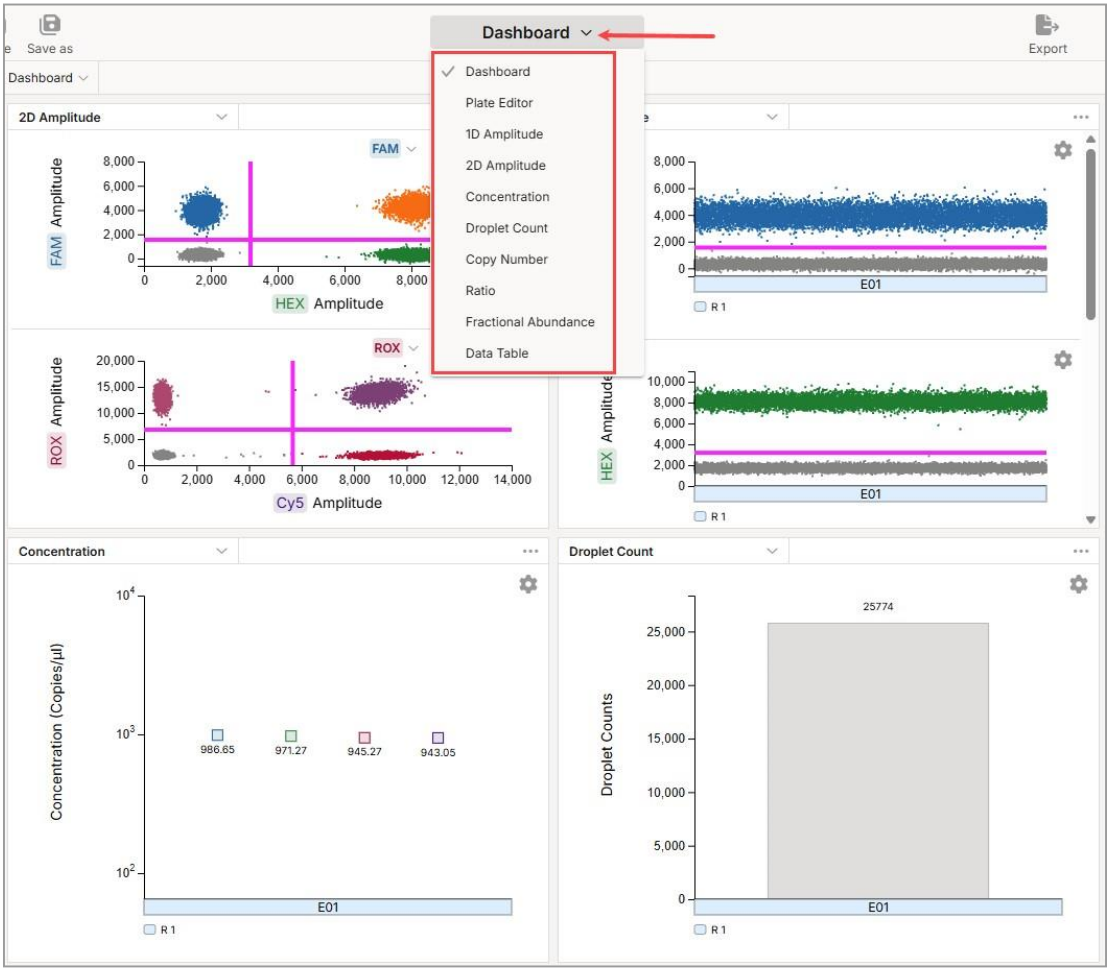


Chart View

The chart view, which illustrates the data in dot plots and data points, is the middle portion of the workspace. The Dashboard is the default view but you can select other charts from the dropdown list.

Note: If you are viewing a QX700 data file, the Spillover option also appears in the list. For information, see Spillover Compensation on page 105.



Supported Fluorophores

The QX Continuum Droplet Digital PCR System can read droplet fluorescence in up to 4 channels: ■ FAM ■ HEX ■ ROX ■ Cy5

The QX700 Droplet Digital PCR System can read droplet fluorescence data in up to 7 channels:

- FAM ■ HEX
- Atto-550
- ROX
- Atto-590
- Cy5
- Cy5.5

Run, Well, and Data Tabs

In the QX Insight workspace, the pane on the right displays the tabs available in the selected analysis views.

Note: The pane is hidden when you display the Data Table and Spillover views.

Table 9. Run, well, and data tabs

Tab	Description
Well data	For the selected wells, displays the well ID, total droplets, targets, fluorophores, concentration, and number of positive and negative droplets This tab appears to the right of the dashboard, amplitude dot plots, data point charts; the well data is modified if you change the thresholding.
Run info	For the selected wells, displays the well ID, sample information, thermal profile information, and target identifiers This tab appears to the right of the dashboard, amplitude dot plots, data point charts, and displays reference targets defined in the Plate Editor.
Plate Editor Run overview	Displays the following information for the relevant instrument: ■ For QX Continuum runs, contains run details such as the run date, user, run ID, plate ID, supermix and instrument name; also contains thermal profile names and temperatures, and labels and plate notes ■ For QX700 runs, contains run details such as the run date, user ID, instrument serial number, and RDG16 cartridge information; also contains thermal profile names and temperatures, and labels and plate notes This tab appears only in the Plate Editor view.
Plate Editor Well data	Displays the thermal profile assigned to each well, sample type, targets (including reference targets), sample name, labels, and well notes. This tab appears only in the Plate Editor view. You can edit all entries except for thermal profile.

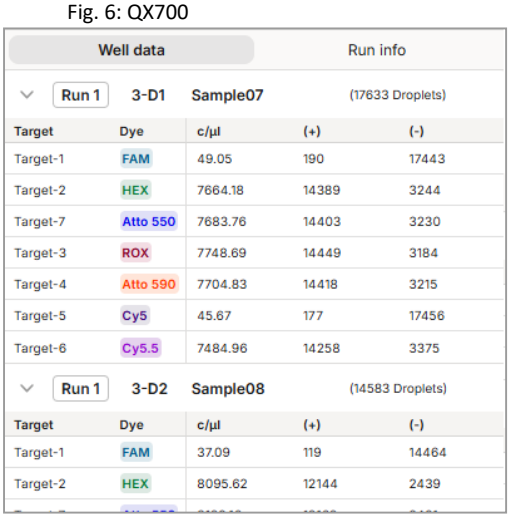
Plate Editor Well order	Displays a list of wells in the order they were processed; information includes the well ID, sample type, and sample name. This tab appears only in the Plate Editor view, and only for the QX Continuum.

Well Data Tab

For each well, QX Insight calculates and displays the concentration per µL, total droplets, and positive and negative droplet counts per target and dye.

Fig. 5: QX Continuum

Fig. 6: QX700



Run Info Tab

As shown in Fig. 7 and Fig. 8, well and thermal profile configuration details appear in the Run Info tab. QX Continuum wells are identified with the row letter, followed by the column number (A1) and QX700 wells are identified by cartridge and then by well number (1-A1).

Fig. 7: QX Continuum

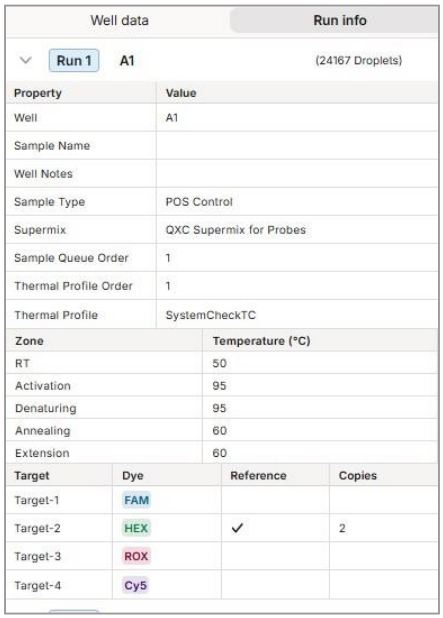
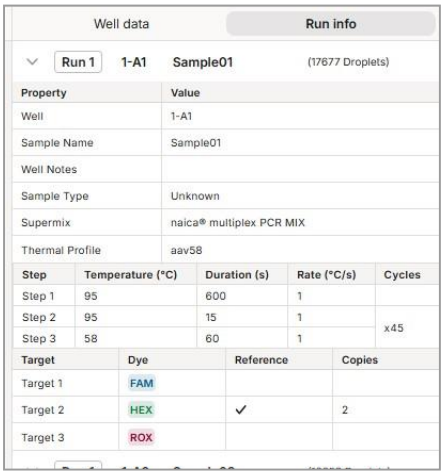


Fig. 8: QX700



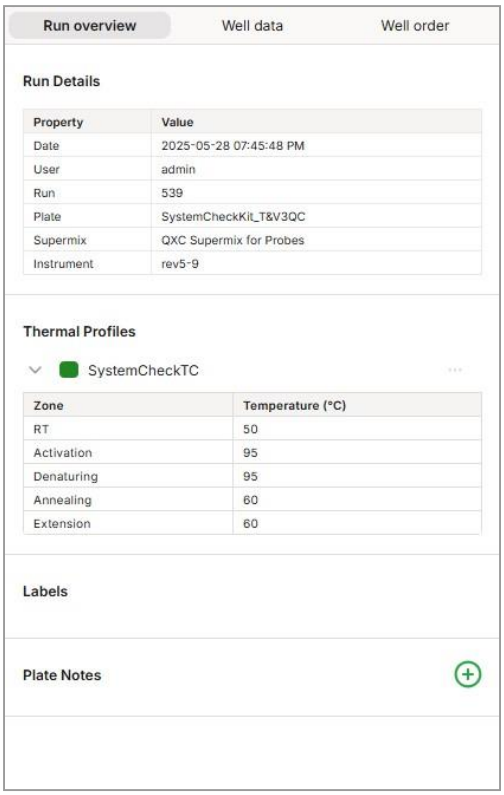
Run Overview Tab

The Run Overview tab appears when you open the analysis Plate Editor and contains the following:

- Identifiers for the run, user, instrument, and altitude (at or below 500 m is low, above 500m is high)
- Thermal profile identifier and configured temperatures
- Assigned labels
- Plate notes

Fig. 9: QX Continuum

Fig. 10: QX700



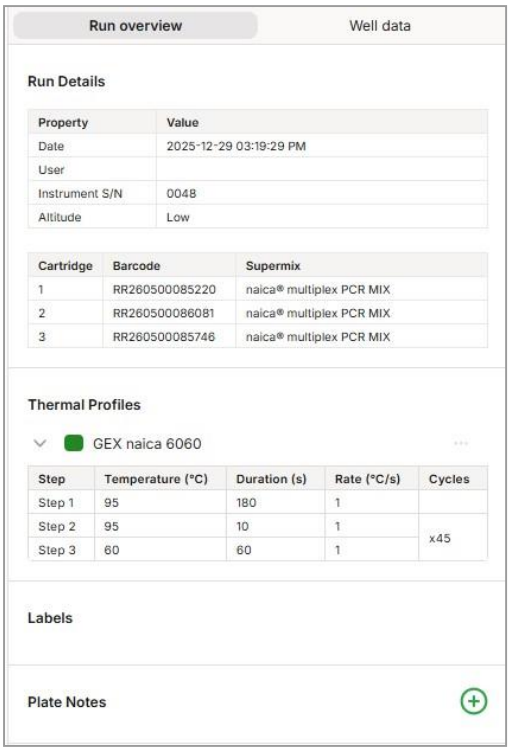


Plate Editor Well Data Tab

In the Plate Editor Well data tab, you can select one or more wells and change the sample type, rename targets, select reference targets and change the number of copies, add or change sample names, assign labels to group samples, and add well notes.

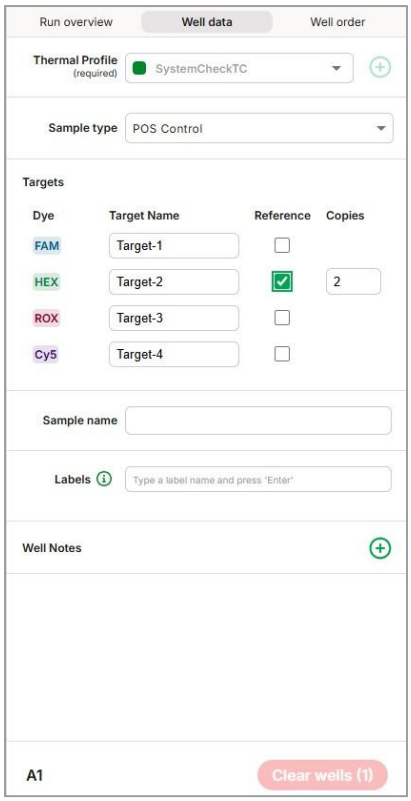
When you select the Reference checkbox, the reference gene is used in determining the copies per cell of the gene of interest. A reference target is required to calculate Copy Number, Ratio, and Fractional Abundance data points. The default number of copies is 2. Follow ddPCR application guidelines to determine the gene most appropriate to use as a reference in your experiment.

Tip: Each well must have at least one non-reference target. You can select the checkbox under Reference for the remaining fluorophores.

Note: Selected wells are identified in the lower-left corner (A1, D1). If you select more than one well, the well range is displayed (A1:A4, D1:D4, and so forth).

Fig. 11: QX Continuum

Fig. 12: QX700



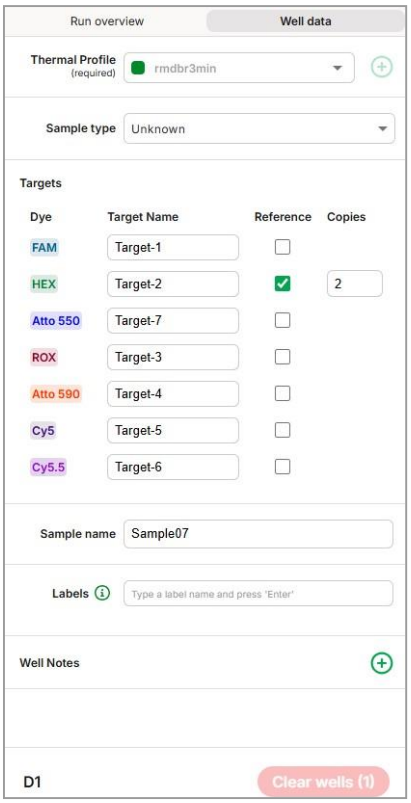
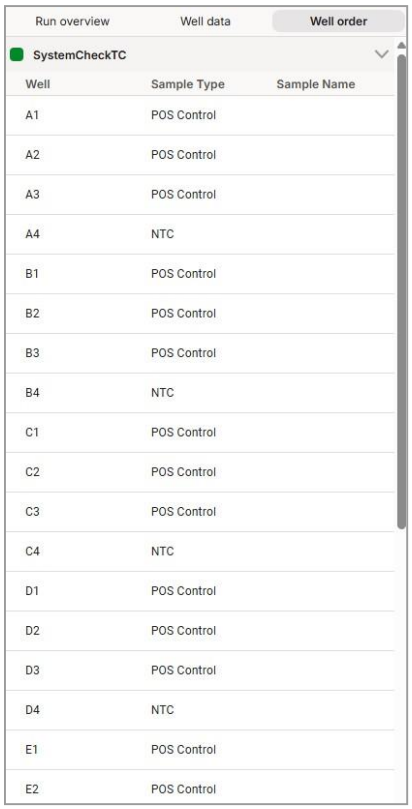


Plate Editor Well Order Tab

Note: This tab applies to QX Continuum runs only.

The well processing order is specified in QX Insight Software when the plate is configured. For information on configuring a new processing order, see Reordering Wells and Thermal Profiles on page 49.

In the Plate Editor Well order tab, you can see the order in which the wells were processed on the QX Continuum. The well number appears on the left, followed by the sample type, and the sample name, if applicable.



Well	Sample Type	Sample Name
A1	POS Control	
A2	POS Control	
A3	POS Control	
A4	NTC	
B1	POS Control	
B2	POS Control	
B3	POS Control	
B4	NTC	
C1	POS Control	
C2	POS Control	
C3	POS Control	
C4	NTC	
D1	POS Control	
D2	POS Control	
D3	POS Control	
D4	NTC	
E1	POS Control	
E2	POS Control	

Chapter 9 Droplet Data Analysis

QX Insight Software provides the following views and features for analysis of the ddPCR data collected on your QX Continuum ddPCR System or QX700 ddPCR System:

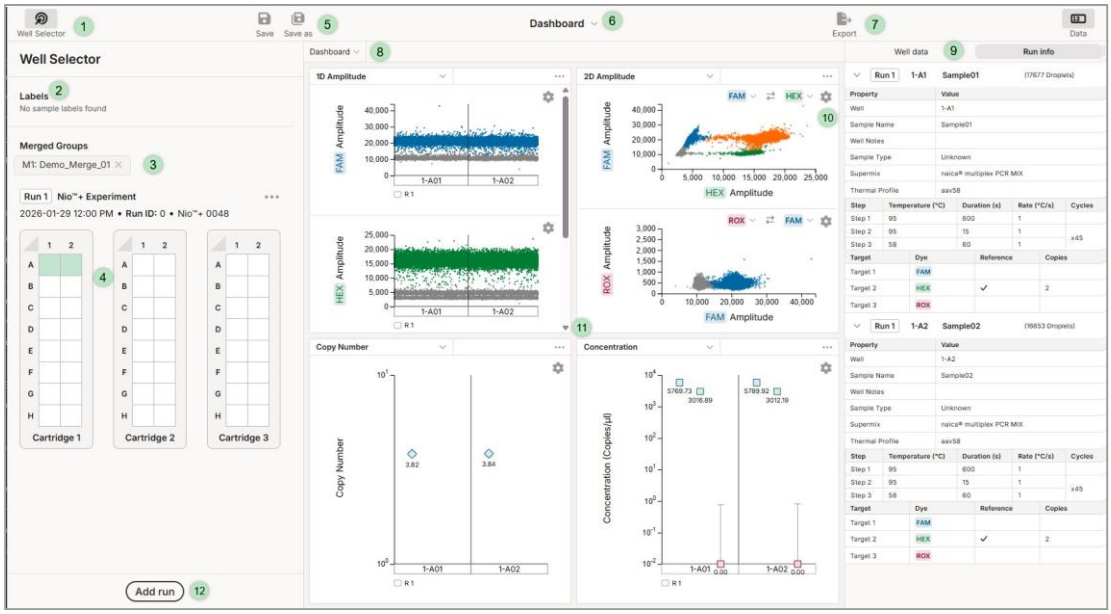
■ Configurable dashboard, where you can display multiple analysis views simultaneously ■ A variety of display and recalculation analysis options ■ Droplet data distributed in dot plots and calculated as data points ■ Data table containing comprehensive data ■ Post-run plate editor that matches your instrument ■ Fluorescence spillover compensation matrix (QX700 only)

When you open a QX700 run data file in, or add a QX Continuum run data file to, a QX Insight workspace, the applicable analysis features are enabled.

After a run opens in a workspace, the Dashboard view appears by default. The initial display is blank, but the dashboard is populated after you select one or more wells in the plate or cartridge layout, as shown in Fig. 13 and Fig. 14 on page 82.

Fig. 13: Analysis workspace for a QX Continuum run

Fig. 14: Analysis workspace for a QX700 run



LEGEND

1	Show/hide toggle icon for the Well Selector on the left and the data and configuration tabs on the right
2	Labels that you defined and assigned to wells ■ Click a label to select the corresponding wells and display the details
3	Merged well groups ■ Click a merged group to select the merged wells and display the consolidated details
4	Well selector ■ The well selector displays QX Continuum runs in a 96-well plate layout and QX700 runs in a three-cartridge (48-well) layout ■ A separate layout appears for each run that you add to the workspace. ■ Successfully processed wells appear as solid white and failed wells appear with a ⊗; unused wells appear with a pattern; *a run is considered successful even if some wells fail* ■ You can add up to ten runs to a workspace; *You cannot remove a run after adding it*.
5	Save options

6	View selection ■ Click the arrow to display a different view. Note: If you select Ratio, Fractional Abundance, or Copy Number, data appears only when you select a reference target.
7	Export option (choose from droplet amplitudes, cartridge data, and chart images) Note: QX Insight exports data for all runs that were added to the workspace.
8	Analysis options for the selected chart
9	Data and configuration tabs; separate tables appear for each selected well in each added run Note: If the table does not appear when you select the well, click the well identifier (A1, B2, and so forth)
10	Settings icon; navigate to the dialog containing axis values
11	Graphical display for selected charts
12	Add run button; click to add a run. You can add up to 10 runs to a workspace.

Analysis Charts and Views

QX Insight Software provides the views described in Table 10 to display your ddPCR data in selected wells for analysis.

Table 10. Workspace views

View	Description
Dashboard	Opens by default when you open the run in a workspace; simultaneously displays default or selected views of the analysis data and you can customize the dashboard layout in a preferred design and save it as the new default view See Analysis Dashboard on page 85 (default display).
Plate Editor	Plate or cartridge layout with named targets and well position in the processing order See Analysis Plate Editor on page 86.
Amplitude plots	One-dimensional and two-dimensional amplitude dot plots See 1D Amplitude on page 88 and 2D Amplitude on page 90

Data point charts	Concentration, ratio, fractional abundance, and copy number data point charts *(ratio, fractional abundance, and copy number data appears only if a reference is specified in the Run Info tab)* See Concentration on page 91, Copy Number on page 94, Ratio on page 97, and Fractional Abundance on page 100.
Droplet Count	Bar chart showing the total droplet count, with positive and negative droplet counts specified in the chamber data table See Droplet Counts on page 103.
Data Table	Contains all plate setup information and run data from the processed run See Data Table on page 104.
Spillover	QX700 only; load a file or manually adjust for fluorescence spillover See Spillover Compensation on page 105.

Analysis Dashboard

The Dashboard in QX Insight Software appears by default when you open a run file in an analysis workspace, and provides a consolidated view of multiple analysis windows. The display remains blank until you select one or more wells. As you select wells in the Well Selector grid, the dot plot and data point charts, as well as the well data on the right, adjust accordingly. You can add, remove, or change the charts in the central display, and you can and set a new default layout at any time. For information, see Dashboard Options on page 121.

Fig. 15: QX Continuum ddPCR run

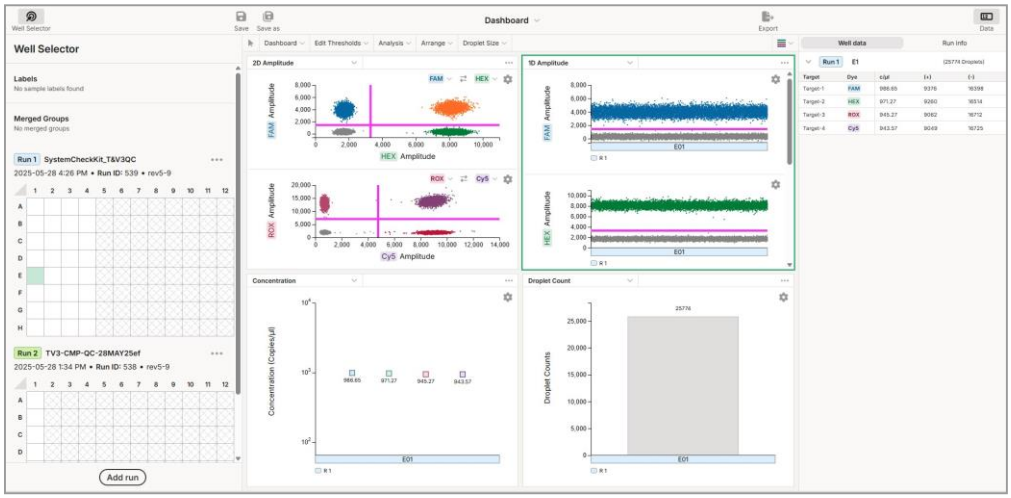
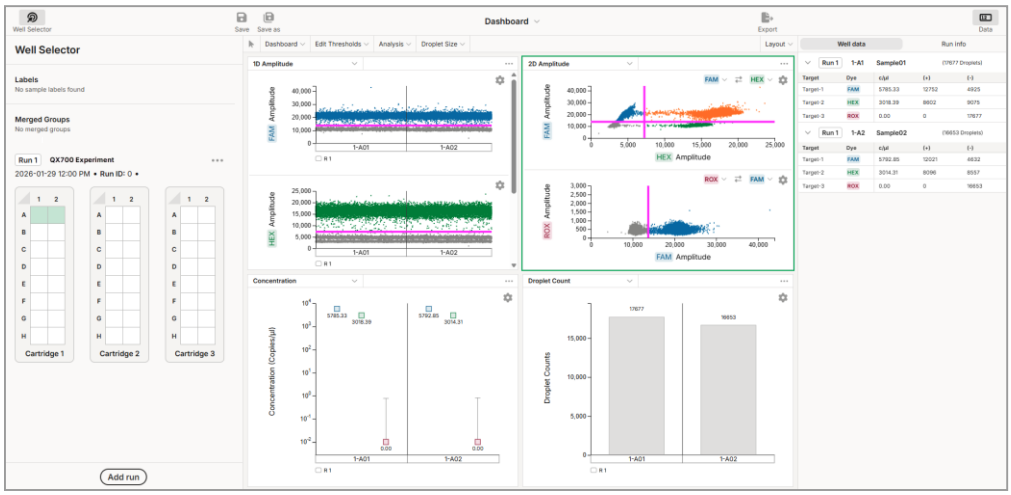


Fig. 16: QX700 ddPCR run



Analysis Plate Editor

The analysis Plate Editor view in the workspace shows the post-run layout as a 96-well plate for the QX Continuum or a three-cartridge (48-well) layout for the QX700. Tabs on the right display information about the run and the wells for both instruments, and the well processing order for the QX Continuum.

Fig. 17: QX Continuum Plate Editor

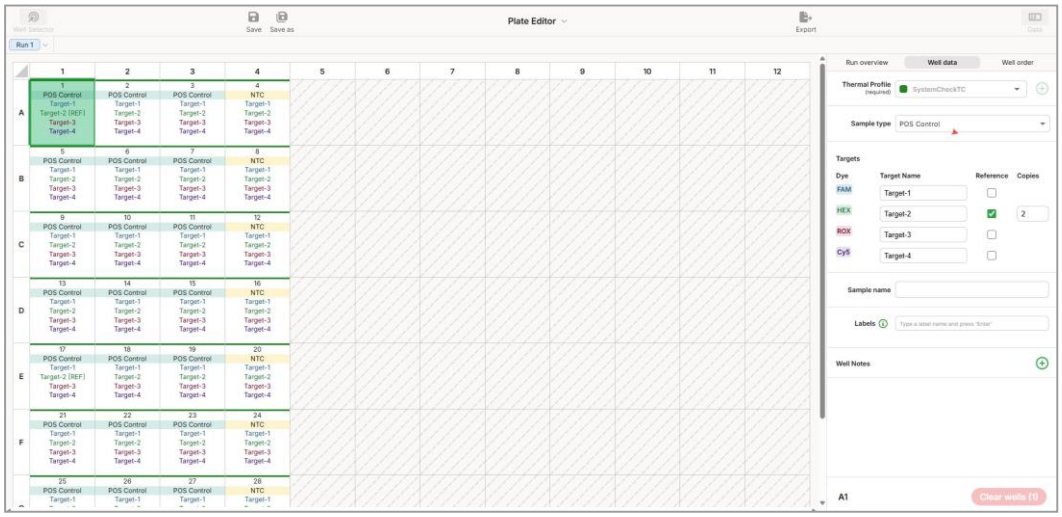
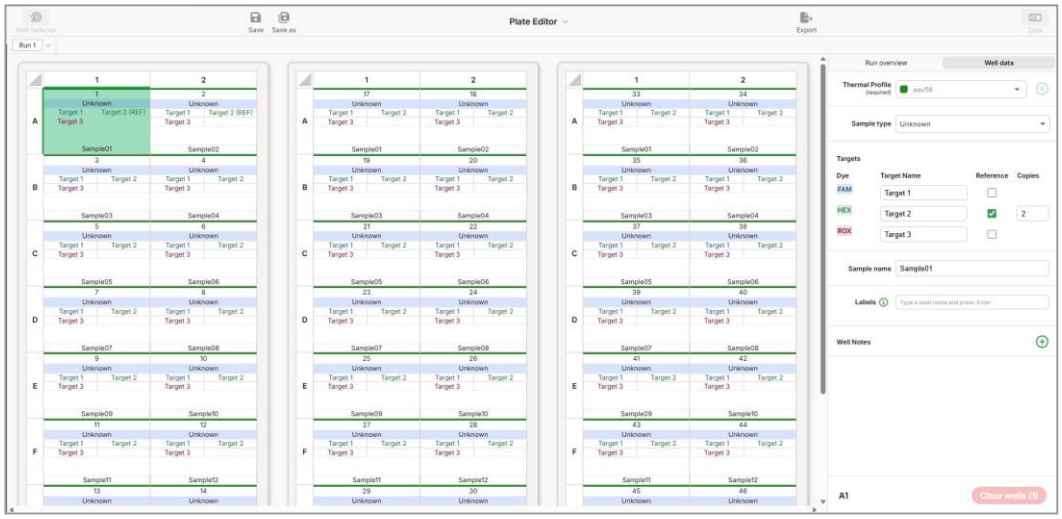


Fig. 18: QX700 Plate Editor



Each tab contains the following:

- The Run overview tab contains the run details, thermal profiles assigned (including temperatures), labels applied, and plate notes.

- The Well data tab contains the thermal profile name, sample type, targets (including reference), sample name, labels, and well notes.
- The QX Continuum layout also shows the well processing order in a table containing the well identifier, sample type, and sample name.

You can also see the wells with reference targets in the plate or cartridge layout, as shown in the following graphics:

Fig. 19: Reference target in QX Continuum Plate Editor

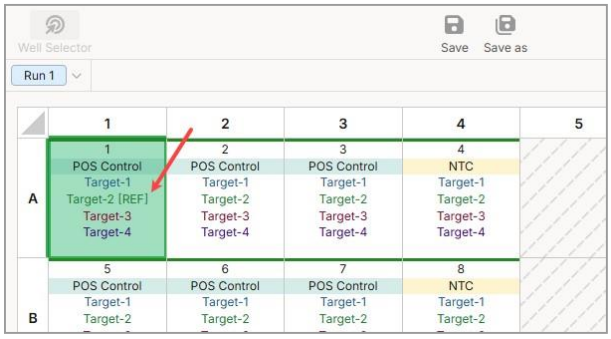


Fig. 20: Reference target in QX700 Plate Editor

From the Plate Editor, you can also export the plate data and droplet amplitudes.

Tip: If you pause on a well, a tool tip containing more information appears. For information, see Viewing Tool Tips on page 120.

1D Amplitude

Use the 1D Amplitude analysis window to view droplet dot plots for each selected well. The plots illustrate all accepted droplets, with a visual representation of the positive and negative droplets.
Positive droplets appear in the fluorescence color and negative droplets displayed in gray.

Fig. 21: QX Continuum 1D Amplitude

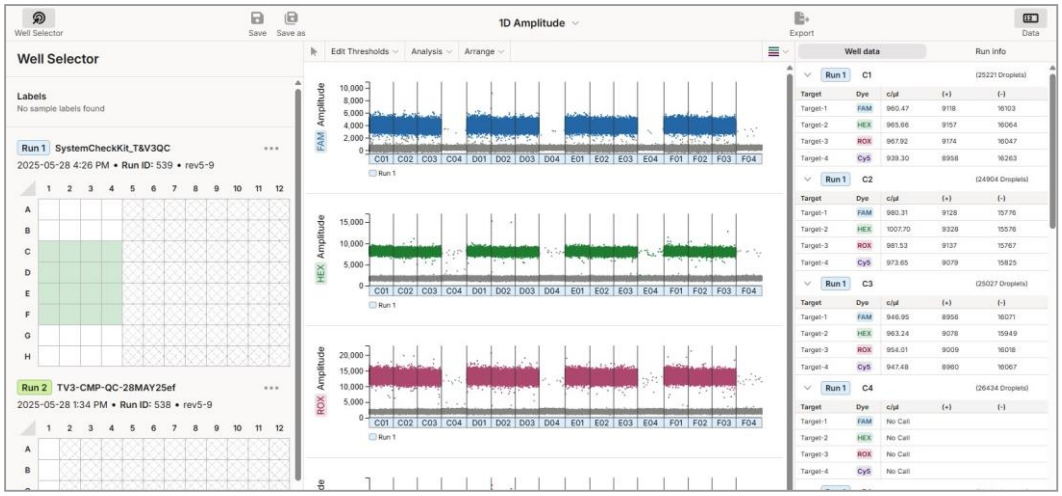
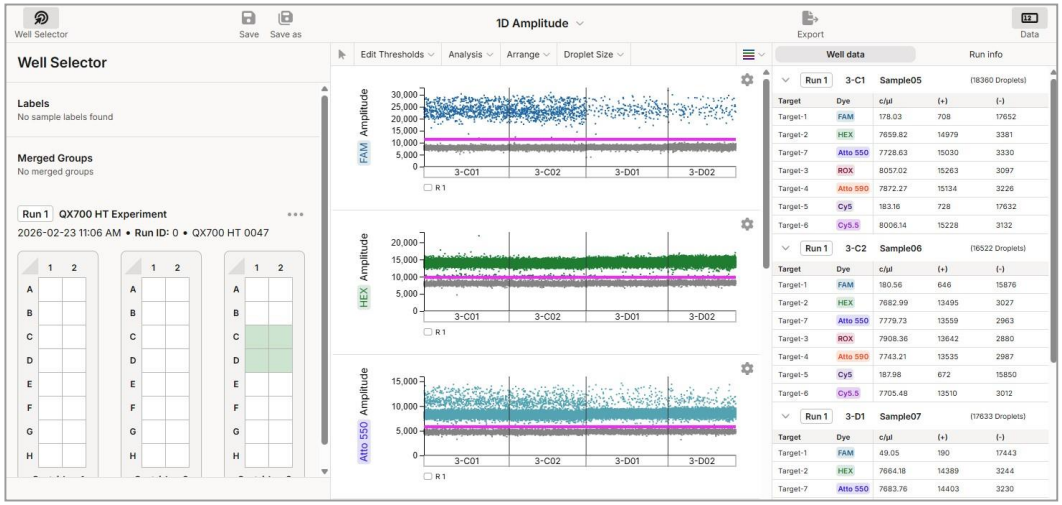
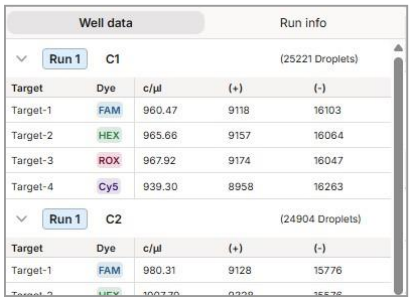


Fig. 22: QX700 1D Amplitude



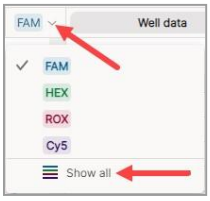
In the Well Data tab on the right, QX Insight calculates and displays the positive and negative droplet counts and concentration per µl for each dye in each well.



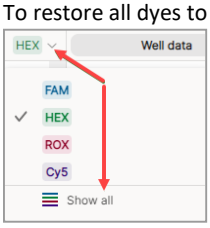
Plot data for all dyes appears by default, but you can select one dye at a time to display the corresponding plot data.

To display a different dye

1. Click the dropdown arrow to display the list of dyes, and then select a dye to display the corresponding plot data.



2. To restore all dyes to the display, click the icon in the upper-right corner and select Show all.



Note: You can save a new channel configuration in the workspace, which appears when you open the workspace again.

The 1D Amplitude tab also provides options to add manual thresholds for one or more wells, restore the automatic calculations, and arrange the well data by collection time, row processing order or column processing order. For information, see Analysis Options for Amplitude Displays on page 127.

2D Amplitude

For selected wells, the 2D Amplitude view illustrates fluorescence and individual cluster representations for positive droplets with two dyes, droplets containing both targets, and droplets containing neither target. Plot data appears for fluorophore combinations as follows:

- If you are using the QX Continuum, plot data for two dye combinations (FAM/HEX and ROX/Cy5) appears by default, but you can select one dye at a time to display the corresponding plot data.

- If you are using the QX700, plot data appears for the fluorophore channels selected for the experiment. Channels that remained unused in the ddPCR run are disabled.

Fig. 23: QX Continuum 2D Amplitude

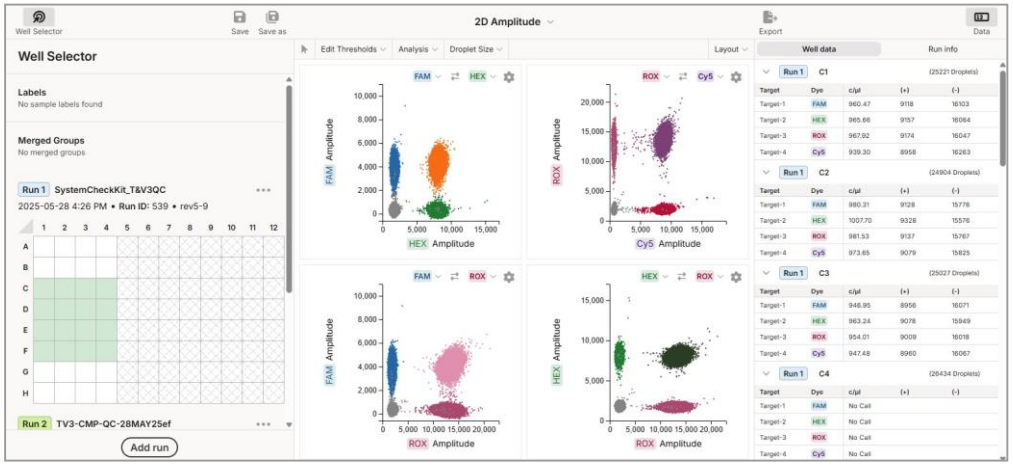
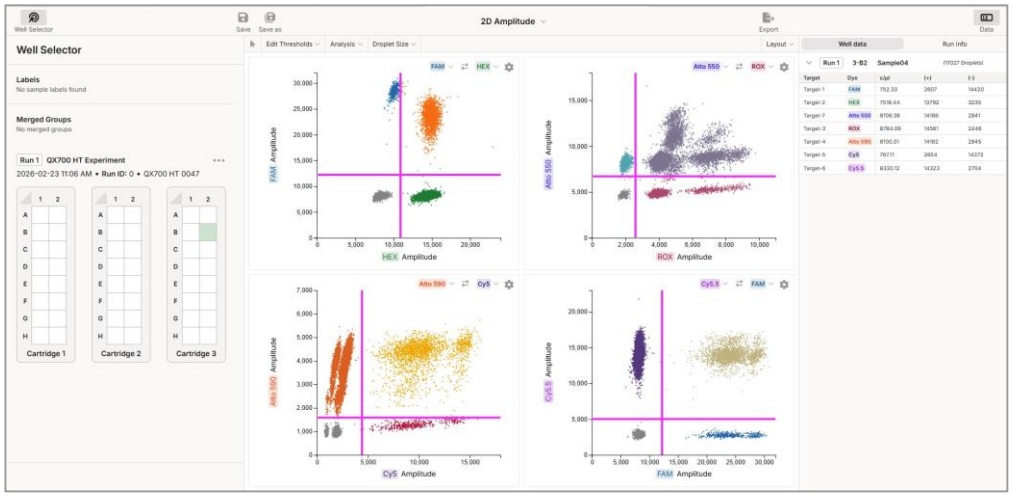


Fig. 24: QX700 2D Amplitude



Concentration

Concentration measures the number of target molecule copies in each µl of sample. QX Insight Software uses the Poisson error model algorithm and plots the concentration for each target as a data point, as shown in Fig. 25 and Fig. 26. Concentration values for each channel are calculated using the droplet volume.

You can set new axis boundaries and you can use the Error Model dropdown to recalculate your results based on a different confidence interval (CI) of either None, 95% (default), or 68%. When you change the confidence interval, each data point is updated. For information, see Analysis Options for Data Point Displays on page 135.

Fig. 25: QX Continuum concentration data

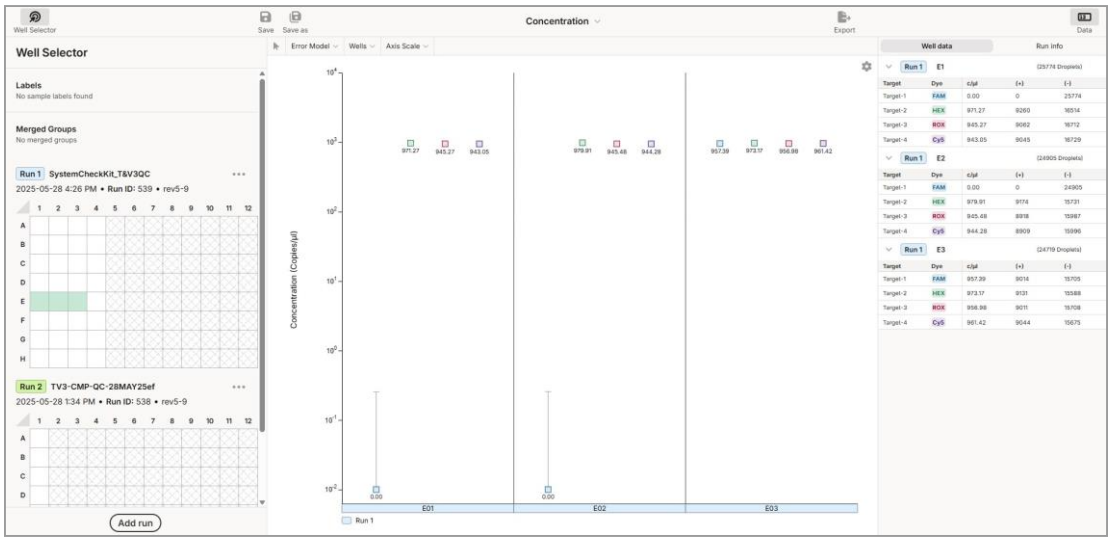
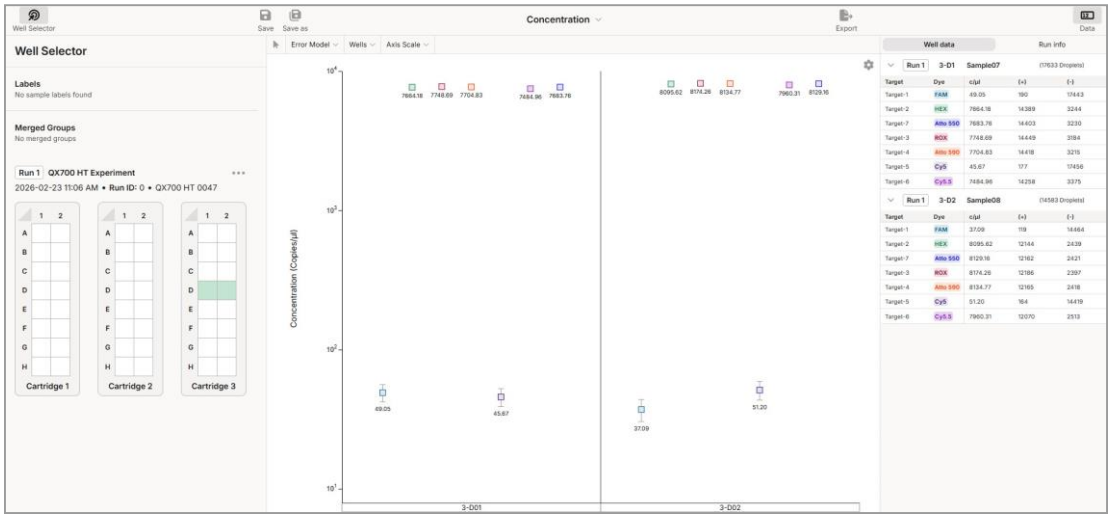


Fig. 26: QX700 concentration data



Tip: If you pause on a data point, a tool tip containing more information appears. Concentration Calculation The software uses the calculations shown below.

$$c = -\ln(\frac{N_{neg}}{N}) / V_{dropletUnits}$$

Where:

c	Concentration
– ln	Negative natural logarithm
N	Total number of accepted droplets
N_{neg}	Number of negative droplets
$V_{dropletUnits}$	Droplet unit volume = number of accepted droplets/input volume

Confidence Interval Calculation

The confidence interval is also calculated and appears in the Poisson columns in the Data Table view.

The software uses the calculations shown below.

$$p = \frac{N_{neg}}{N}$$

$$\partial_{N_{neg}} = \sqrt{Np(1-p)}$$

$$N_{neg,\min} = N_{neg} - 1.96 * \partial_{N_{neg}}$$

$$N_{neg,\max} = N_{neg} + 1.96 * \partial_{N_{neg}}$$

$$c_{max} = -ln\left(\frac{N_{neg,min}}{N}\right) \div V_{dropletUnits}$$

$$c_{min} = -ln\left(\frac{N_{neg,max}}{N}\right) \div V_{dropletUnits}$$

Where:

p	p-value (probability of finding the target in the sample)
N	Total number of droplets
N_{neg}	Negative droplets counted
$N_{neg,min}$	Minimum number of negative droplets (95% CI)
$N_{neg,max}$	Maximum number of negative droplets (95% CI)
∂	Delta
c_{min}	Minimum concentration, (95% CI)
c_{max}	Maximum concentration (95% CI)
– ln	Negative natural logarithm

Important Notes:

- If you select a 68% confidence interval, the multiplier is changed from 1.96 to 1.00.
- Because Concentration uses a negative log (a decreasing function), the max negative-droplet count maps to the min concentration, and vice versa.
- The software uses a different calculation for low (less than 100) pos or neg droplet counts.

Copy Number

Copy number quantifies the target gene relative to a reference gene, allowing calculation of the number of target copies per genome. Copy number variation measures mutations that increase or reduce the number of copies of a particular DNA segment due to duplicate or deleted DNA sequences. QX Insight Software uses the Poisson error model algorithm and plots the copy number variation for each target as a data point, as shown in Fig. 27 and Fig. 28.

You can set new axis boundaries and you can use the Error Model dropdown to recalculate your results based on a different confidence interval (CI) of either None, 95% (default), or 68%. When you change the confidence interval, each data point is updated. For information, see Analysis Options for Data Point Displays on page 135.

Fig. 27: QX Continuum copy number variation data

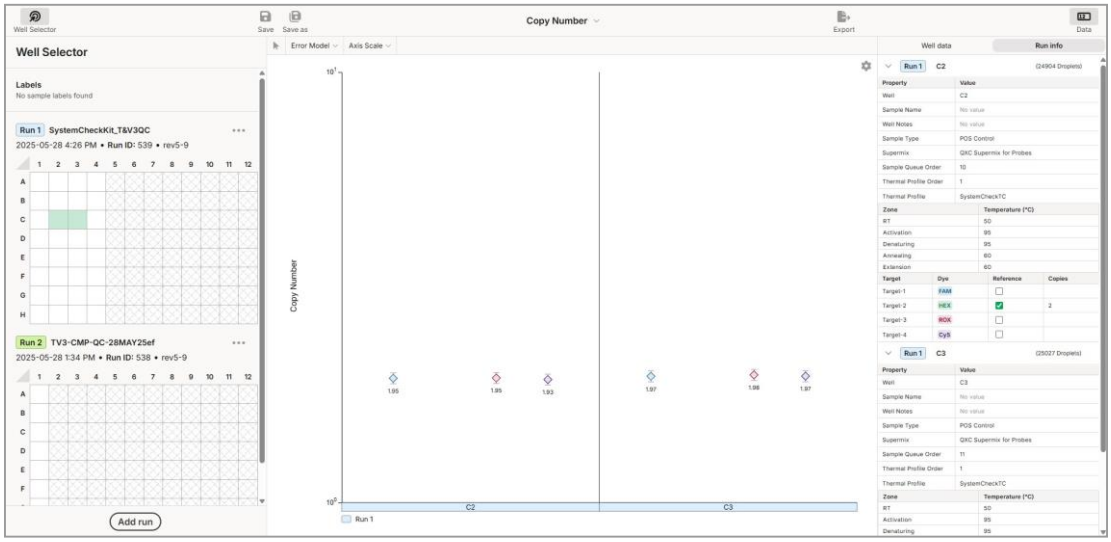
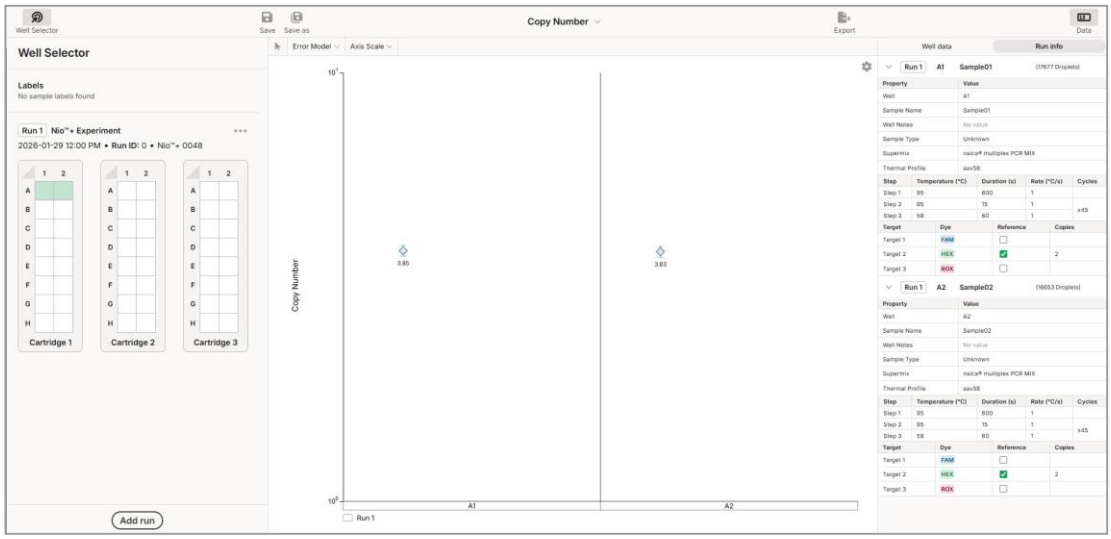


Fig. 28: QX700 copy number variation data



Note the following:

- QX Insight calculates the data when the non-reference and reference targets have concentration data in the results.
- There must be at least one *non*-reference target concentration.
- You can select up to three reference targets, but you must select at least one to display the data. Reference targets are selected in the Run Info tab on the right.
- If you select multiple reference targets, the geometric means of the variable values are used in the calculations.
- You can display the calculated values in linear or logarithmic format. ■ You can pause on a data point to display a tool tip with more information.

Copy Number Variation Calculation

The software uses the calculations shown below.

$$CNV = \frac{A}{B} N_b$$

Where:

A	Target concentration
B	Reference concentration

Nb	Number of reference copies in the genome (defined during plate or cartridge setup)

Confidence Interval Calculations

The confidence interval is also calculated and appears in the Poisson columns in the Data Table view.

The software uses the calculations shown below.

$$CI_r = N_b \frac{A}{B}\sqrt{\frac{CI_A^2}{A^2}+\frac{CI_B^2}{B^2}}$$

$$(CNV_{low\_95\%}, CNV_{hi\_95\%}) = (CNV-(1/2)CI_{CNV}, CNV+(1/2)CI_{CNV})$$

Where:

A	Channel 1 target concentration
B	Channel 2 target concentration
CIA	Confidence interval of Channel 1 target (95% max - 95% min)
CIB	Confidence interval of Channel 2 target (95% max - 95% min)
Nb	Number of reference copies in the genome (defined during plate or cartridge setup)

Ratio

Ratio determines the quotient of two target molecules (target vs reference) in each µL of sample based on the default settings (T1:T2). QX Insight Software uses the Poisson error model algorithm and plots the ratio for each target as a data point, as shown in Fig. 29 and Fig. 30.

You can set new axis boundaries and you can use the Error Model dropdown to recalculate your results based on a different confidence interval (CI) of either None, 95% (default), or 68%. When you change the confidence interval, each data point is updated. For information, see Analysis Options for Data Point Displays on page 135.

Fig. 29: QX Continuum ratio data

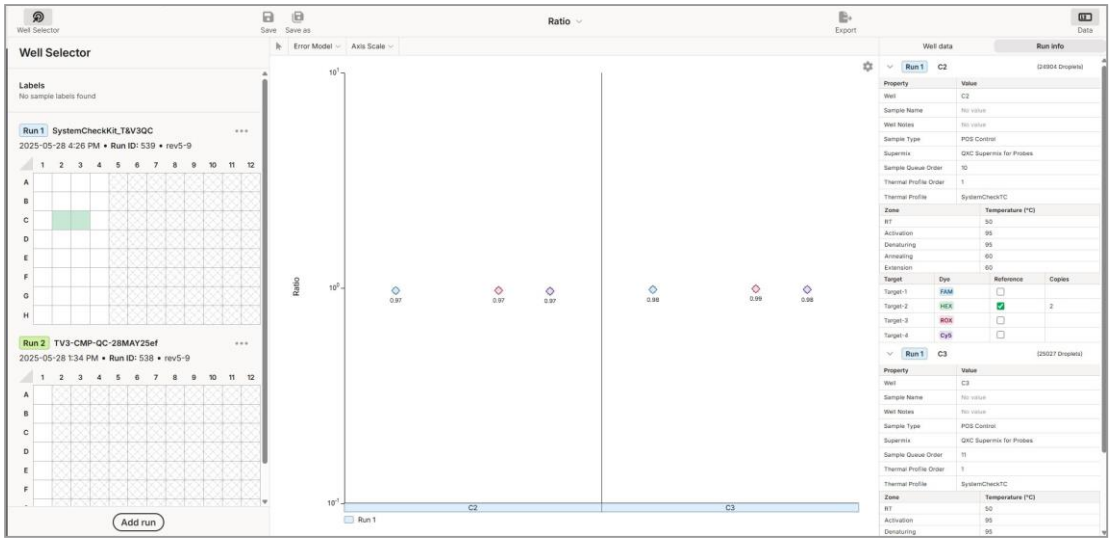
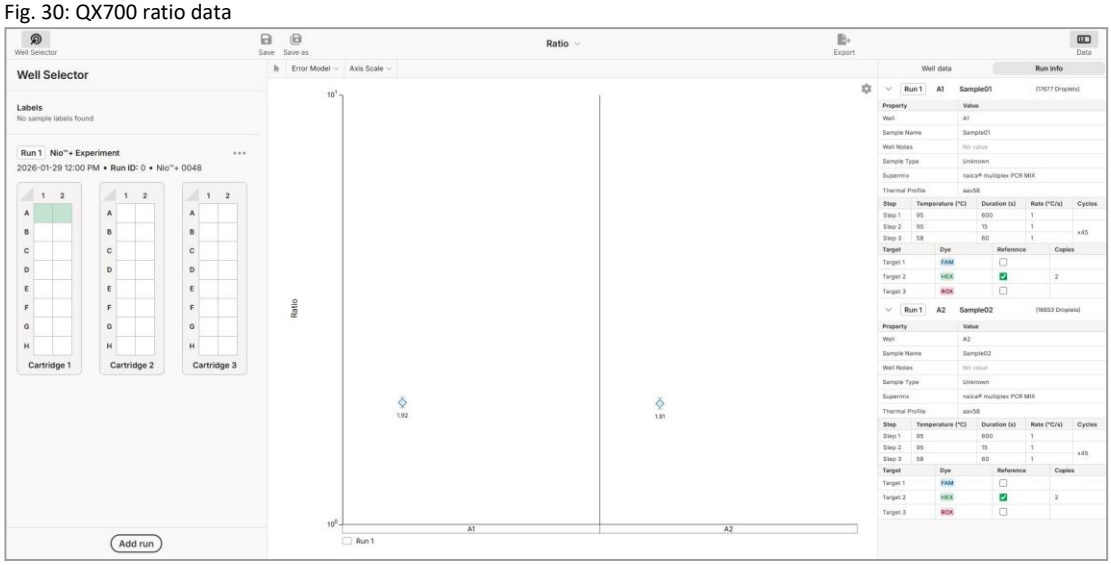


Fig. 30: QX700 ratio data



Note the following:

- QX Insight calculates the data when the non-reference and reference targets have concentration data in the results.
- There must be at least one *non*-reference target concentration.
- You can select up to three reference targets, but you must select at least one to display the data. Reference targets are selected in the Run Info tab on the right.

- If you select multiple reference targets, the geometric means of the variable values are used in the calculations.
- You can display the calculated values in linear or logarithmic format. ■ You can pause on a data point to display a tool tip with more information.

Ratio Calculation

The software uses the calculations shown below.

$$r = \frac{A}{B}$$

Where:

r	Ratio
A	Target concentration
B	Reference concentration

Confidence Interval Calculations

The software uses the calculations shown below.

$$CI_r = N_b \ \frac{A}{B}\sqrt{\frac{CI_A^2}{A^2}+\frac{CI_B^2}{B^2}}$$

$$(r_{low\_95\%}, r_{hi\_95\%}) = (r-(1/2)CI_r, r+(1/2)CI_r)$$

Where:

r	Ratio
A	Channel 1 target concentration
B	Channel 2 target concentration
CI_A	Confidence interval of Channel 1 target (95% max - 95% min)
CI_B	Confidence interval of Channel 2 target (95% max - 95% min)

Fractional Abundance

Fractional abundance can determine rare mutations by precisely determining the proportion of a given target DNA molecule (such as a mutant) as part of the total molecules (such as mutant plus wild-type) in each µL of sample. QX Insight Software uses the Poisson error model algorithm and plots the fractional abundance for each target as a data point, as shown in Fig. 31and Fig. 32.

You can set new axis boundaries and you can use the Error Model dropdown to recalculate your results based on a different confidence interval (CI) of either None, 95% (default), or 68%. When you change the confidence interval, each data point is updated. For information, see Analysis Options for Data Point Displays on page 135.

Fig. 31: QX Continuum fractional abundance data

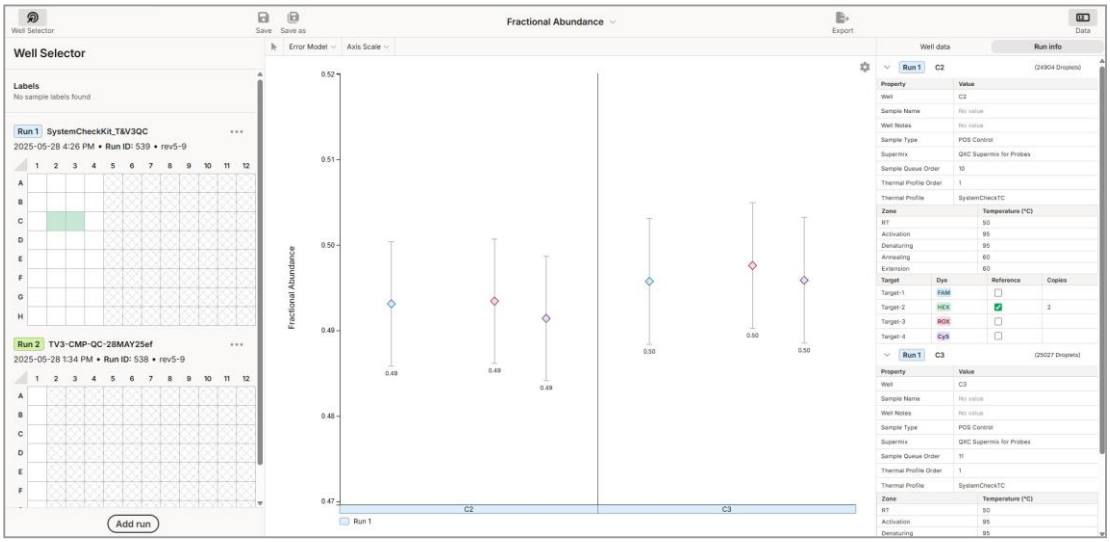
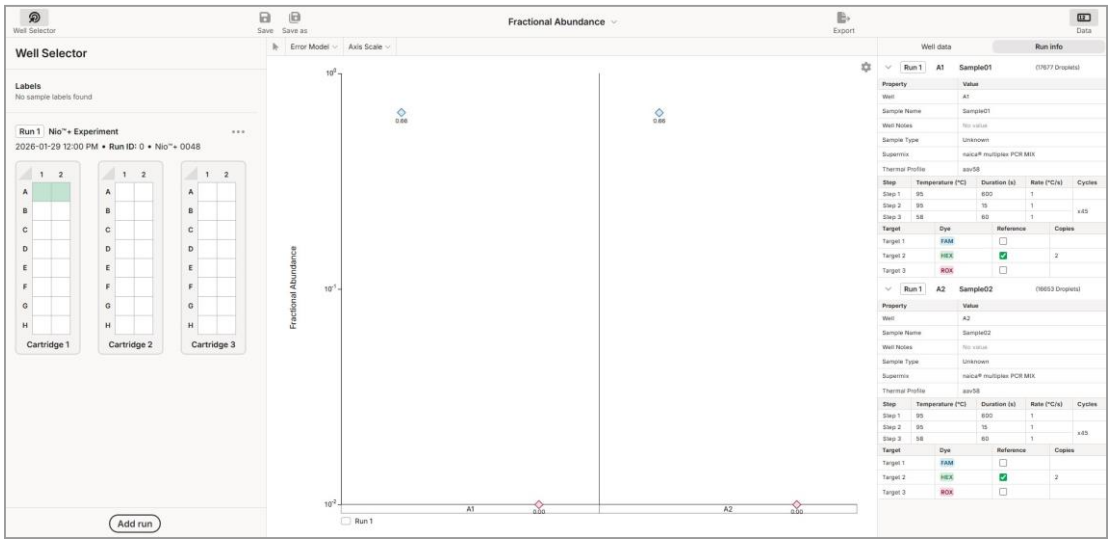


Fig. 32: QX700 fractional abundance data



Note the following:

- QX Insight calculates the data when the non-reference and reference targets have concentration data in the results.
- There must be at least one *non*-reference target concentration.
- You can select up to three reference targets, but you must select at least one to display the data. Reference targets are selected in the Run Info tab on the right.
- If you select multiple reference targets, the geometric means of the variable values are used in the calculations.
- You can display the calculated values in linear or logarithmic format. ■ You can pause on a data point to display a tool tip with more information.

Fractional Abundance Calculation

The software uses the calculations shown below.

$$f = \frac{A}{A+B}$$

Notes: You can multiply by 100% to create a percentage (50%) or use the above formula to show the decimal calculation (.499, or .50 if rounding).

Where:

f	Fractional abundance
A	Target concentration (mutant)
B	Reference concentration (wild-type)

Confidence Interval Calculations

The software uses the calculations shown below.

$$CI_f = \frac{1}{(A+B)^2}\sqrt{B^2 CI_A^2 + A^2 CI_B^2}$$

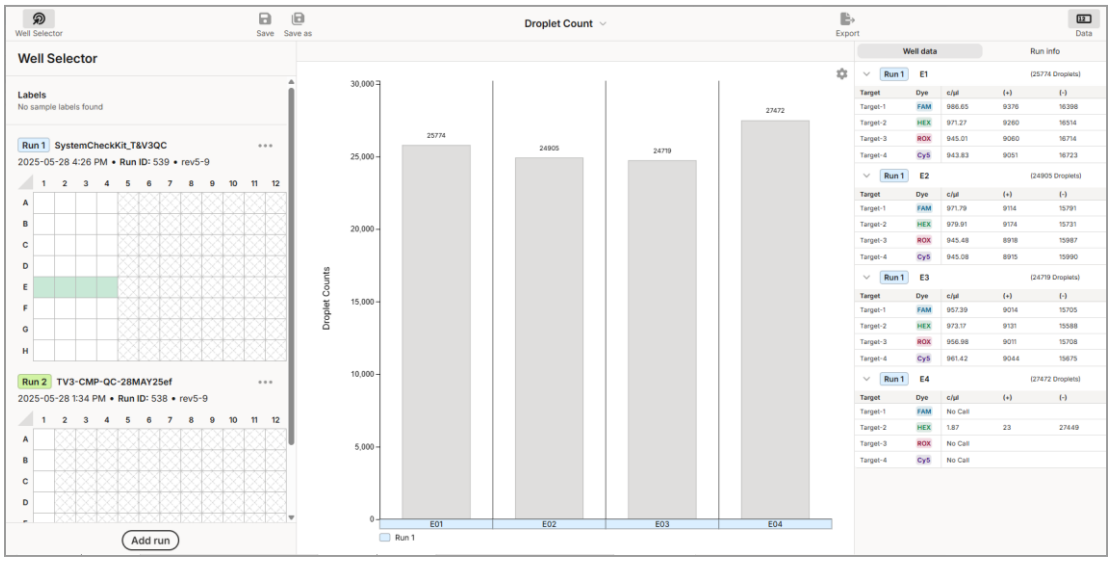
$$(f_{low\_95\%}, f_{hi\_95\%}) = (f - (1/2)CI_f, f + (1/2)CI_f)$$

Where:

f	Fractional abundance
A	Channel 1 (FAM) target concentration
B	Channel 2 (HEX) target concentration
CI_A	Confidence interval of Channel 1 target (95% max - 95% min)
CI_B	Confidence interval of Channel 2 target (95% max - 95% min)

Droplet Counts

The Droplet Counts tab displays a bar chart showing the total number of droplets that were read in each of the selected wells. In the Well data tab on the right, the positive and negative droplet counts, as well as the droplet concentration, are shown for each dye in each well.

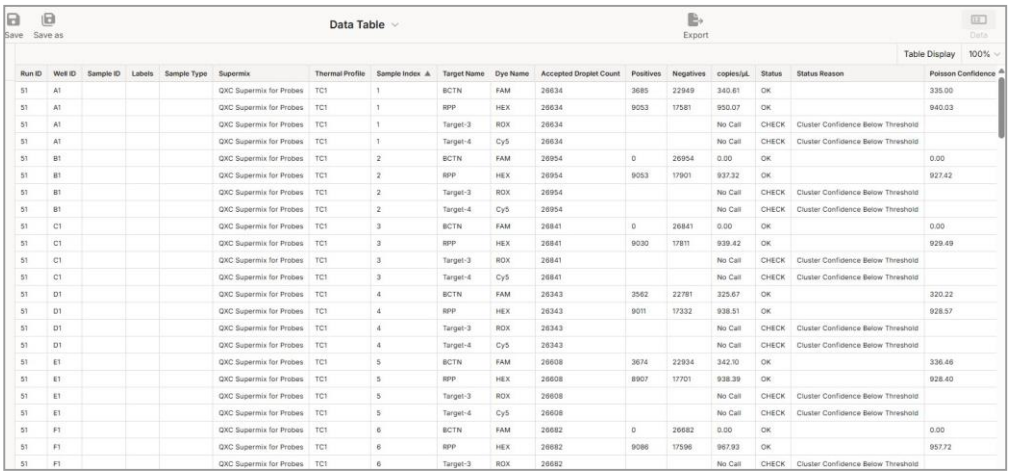


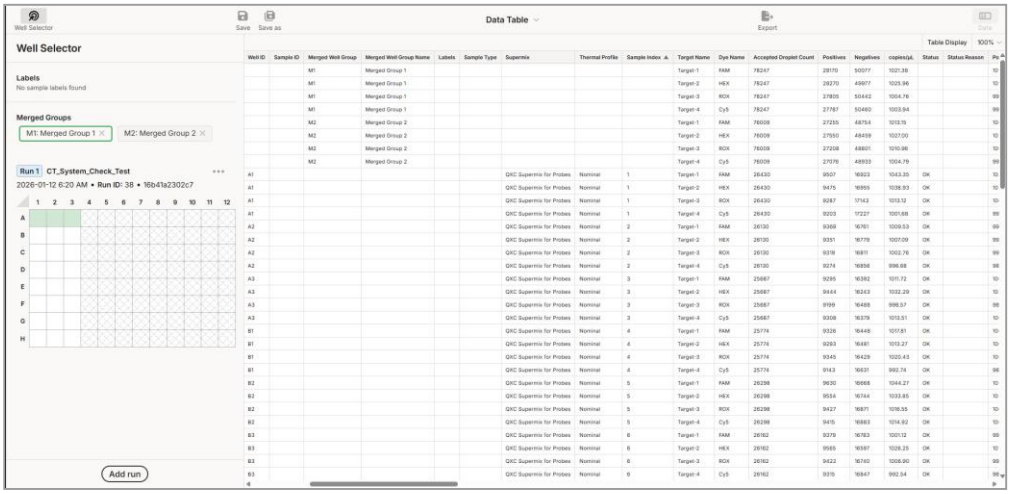
To populate the bar chart ▶ Select one or more wells from the experiments displayed in the Well Selector pane on the left.

Data Table

From the dropdown list in the Workspace view, you can select the Data Table option to display the full range of analysis data from the ddPCR run. Data for all wells appears in the table regardless of the wells selected in the Well Selector. Wells with no calculated copies per µL appear in the table as No Call, with a status of CHECK (this occurs when a well fails or when the cluster confidence interval is below the automatically applied threshold).

The default display shows all available columns, but you can show or hide columns in the Table Display dialog to customize the view according to your needs. The view changes when you click Apply. See Data Table Options on page 147.





Spillover Compensation

Important: Spillover compensation applies to QX700 run data files only.

Spectral overlap can sometimes occur between fluorophores in two or more QX700 detection channels, which can affect the accuracy of target quantification in multiplex experiments. For example, FAM in one detection channel can also produce a signal in one or more other channels, such as HEX.

To offset the overlap, you can apply a spillover compensation matrix (.ncm or .qx700cm) file that adjusts the excitation matrix coefficients where each fluorophore intersects with the remaining fluorophores (see Fig. 34). You can use an existing matrix or create a new matrix.

Important: You can also manually enter and apply spillover coefficients in a matrix, but Bio-Rad does not recommend using this method unless you are an expert user, proficient in ddPCR workflows and calculations.

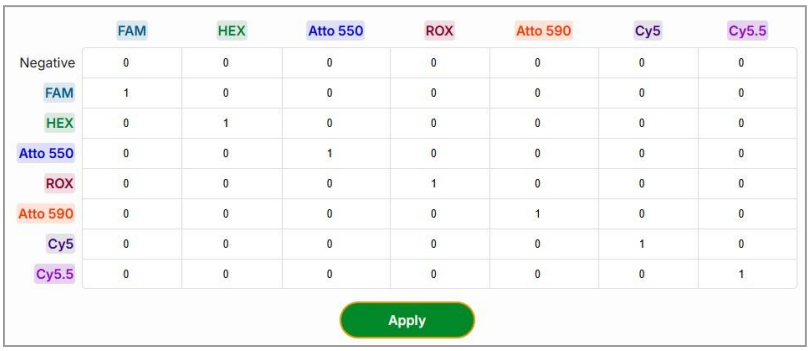
A compensation matrix corresponds to specific thermal cycling conditions and a specific multiplex assay composed of oligonucleotides (probes and primers) and target sequence DNA. You should create a new compensation matrix if either or both of the following changes are made to the experiment before the wells are processed:

- Primers, probes, or DNA target sequences are modified (such as a change in primer or probe sequence or concentration)
- The thermal cycling program is modified

The following bullet points describe the Spillover Compensation dialog as it appears in QX Insight:

- In an *uncompensated* experiment, the default grid is called an Identify Matrix, and is populated with a 1 at each point where the fluorophore intersects, and zeroes in all other fields, as shown below.

  Fig. 33: Uncompensated experiment



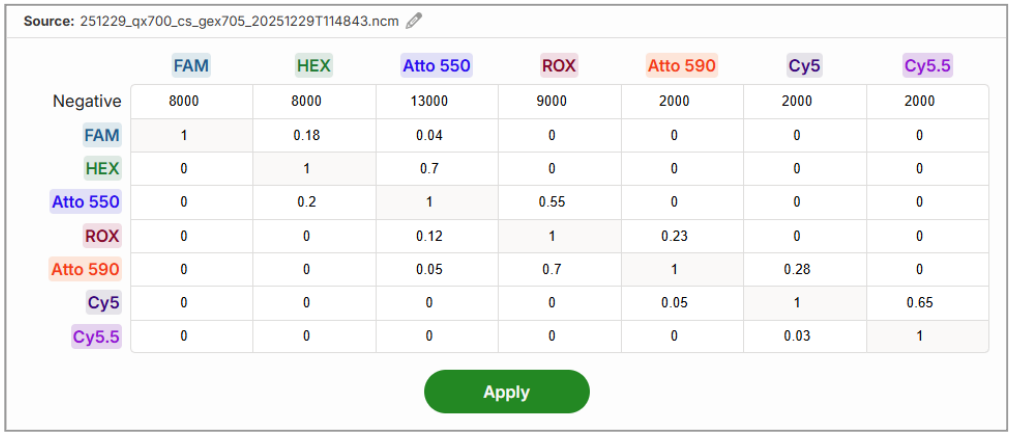
	FAM	HEX	Atto 550	ROX	Atto 590	Cy5	Cy5.5
Negative	0	0	0	0	0	0	0
FAM	1	0	0	0	0	0	0
HEX	0	1	0	0	0	0	0
Atto 550	0	0	1	0	0	0	0
ROX	0	0	0	1	0	0	0
Atto 590	0	0	0	0	1	0	0
Cy5	0	0	0	0	0	1	0
Cy5.5	0	0	0	0	0	0	1

Apply

- In a *compensated* experiment, the fields where the fluorophore intersects with itself are populated with a 1 and the remaining fluorophores contain the compensation values. Uncompensated fluorophores in a compensated experiment contain a 0.

  Note: If you imported the values from a saved compensation matrix, the existing file name appears next to Source in the upper-left corner.

Fig. 34: Compensated experiment



For information on the spillover compensation options, see Analysis Options for Spillover Compensation on page 139.

Analysis Options

QX Insight Software provides a variety of options that compliment data analysis:

■ General Analysis Options on page 107 ■ Dashboard Options on page 121

■ Analysis Options for Amplitude Displays on page 127

■ Analysis Options for Data Point Displays on page 135

■ Analysis Options for Spillover Compensation on page 139 ■ Data Table Options on page 147

General Analysis Options

Using main menu and toolbar options in the QX Insight displays, you can ■ Show or hide the well selector or data table

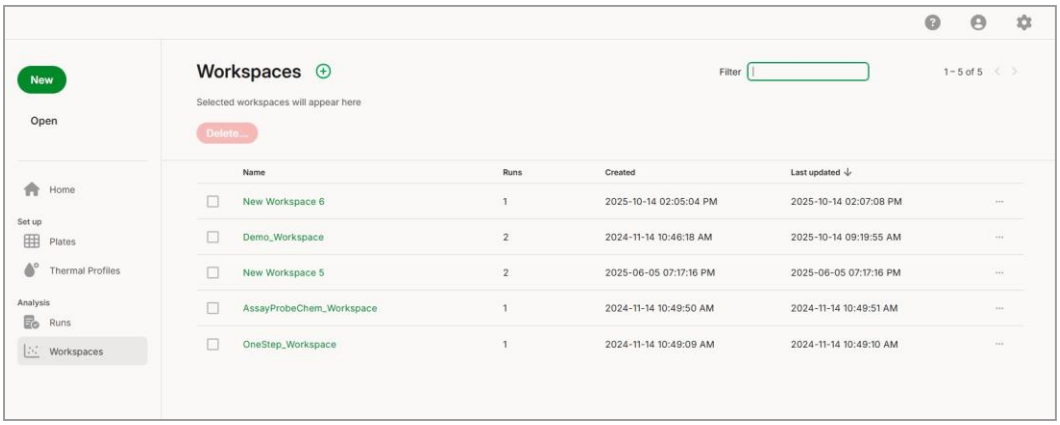
■ Select contiguous or non-contiguous wells in the well selector to display the corresponding data in the chart and data table

■ Select a different display from the dropdown list ■ Merge and unmerge wells ■ Export data and images

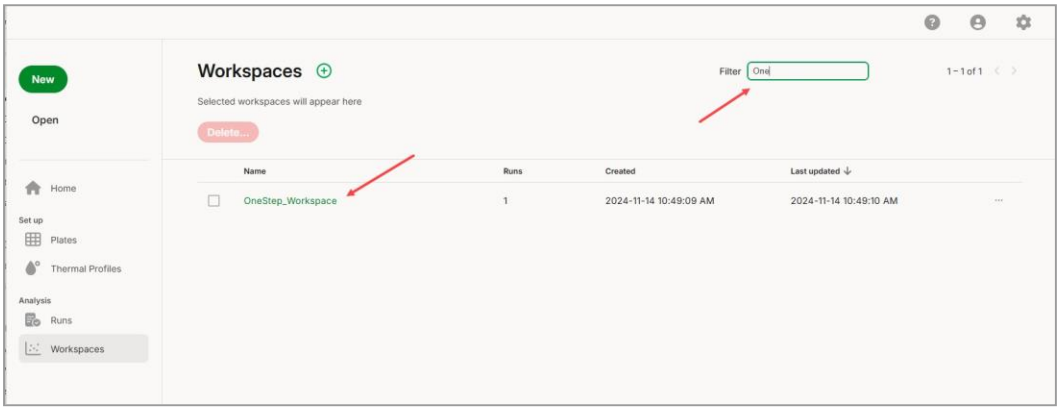
■ Adjust axis values to apply fixed boundary scales or restore automatic boundary scales ■ Pause on text or calculated values to display tool tips for additional information ■ Right-click for additional selection menus

Filtering for Workspaces

1. Open QX Insight Software and click Workspaces to display the Workspaces view.



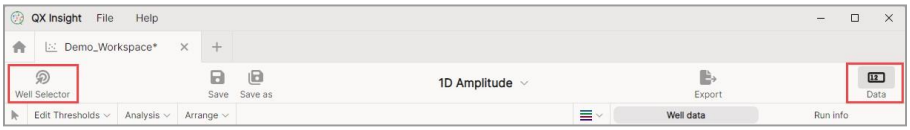
2. In the Filter field, enter all or part of the workspace name. As you enter alphanumeric characters, the list of workspaces narrows to those matching your entry.



Show or Hide Panes

The Well Selector and Data panes appear by default in the workspace layout but you can hide them on demand to create more space to accommodate multi-well layouts.

Note: Both panes are automatically hidden In the Plate Editor and Spillover Compensation (QX700 only) views.



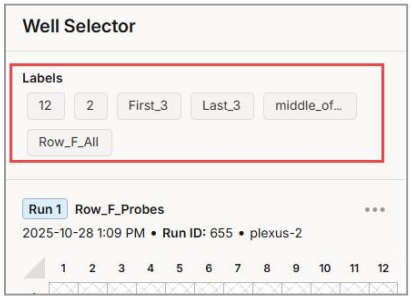
▶ Click the following toggle buttons:

■ Well Selector to show or hide the plate or cartridge layout ■ Data to show or hide the content in the well data and run information tabs

Selecting Wells

To display different well arrangements, you can select wells using the methods described in the following bullet points:

■ If labels are assigned in the plate, click a label (displayed above the Well Selector) to automatically select the corresponding wells.



- To select non-contiguous wells, press the CTRL key and select the wells.

Fig. 35: QX Continuum

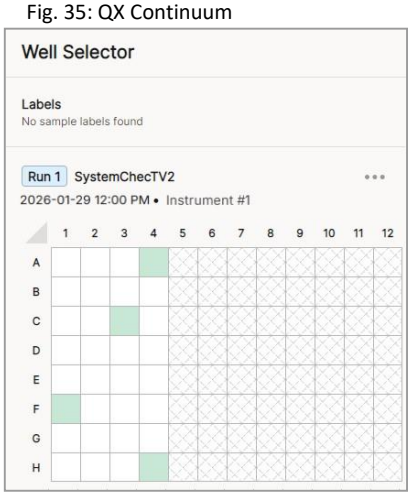
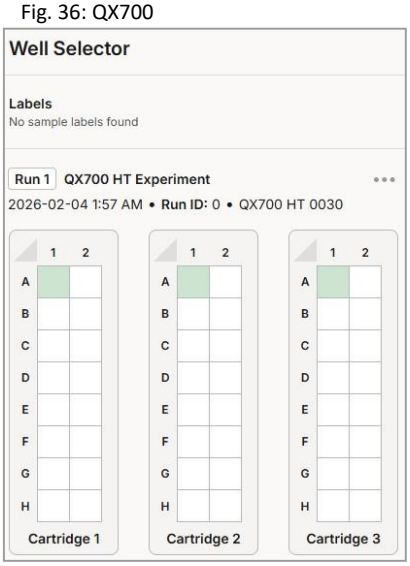
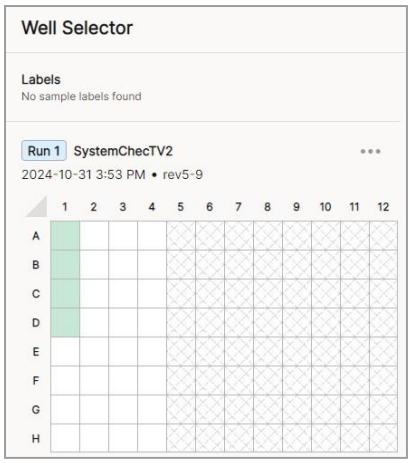
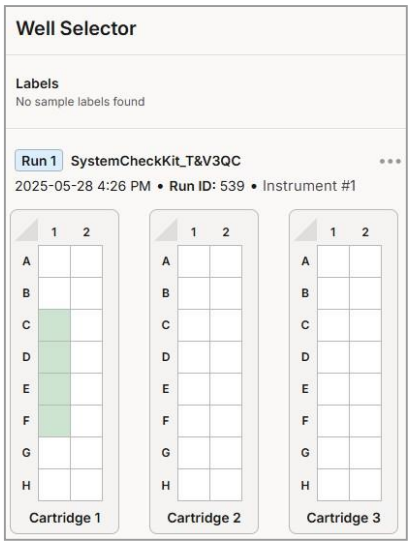


Fig. 36: QX700

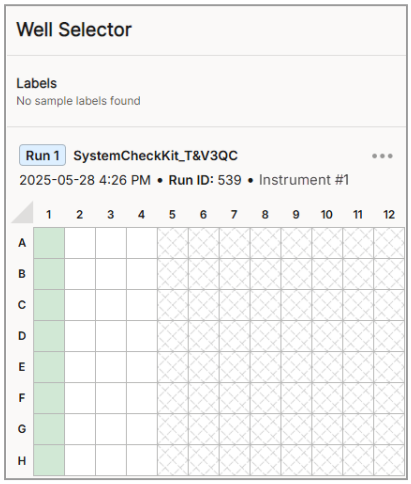


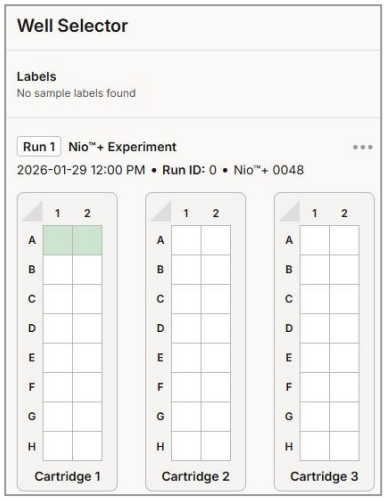
- To select contiguous wells, click a well and drag up, down, left, or right to extend the selection.



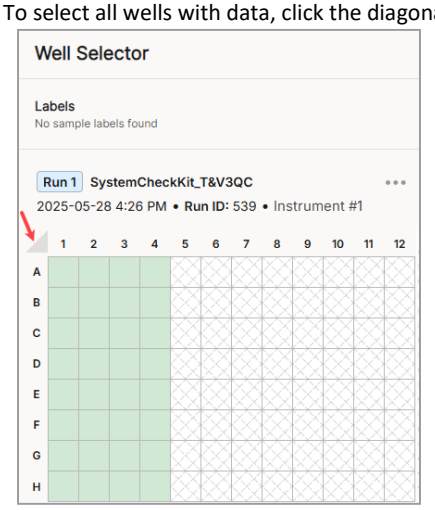


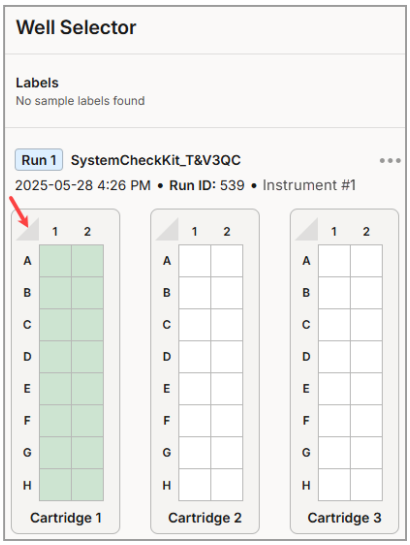
- To select a row or column, click the letter to select the row or the number to select the column. QX Insight selects only the wells in which data was transferred. An X appearing in the well selector indicates no data.





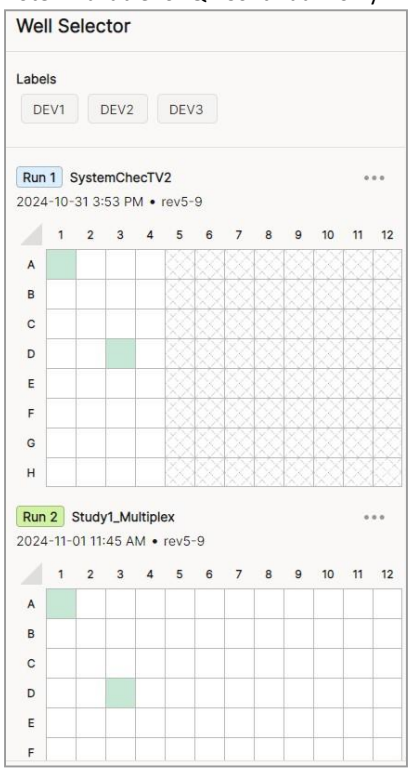
- To select all wells with data, click the diagonal in the upper-left corner.





- To select wells from different runs, press the CTRL key and select each applicable well.

Note: Available for QX Continuum only.

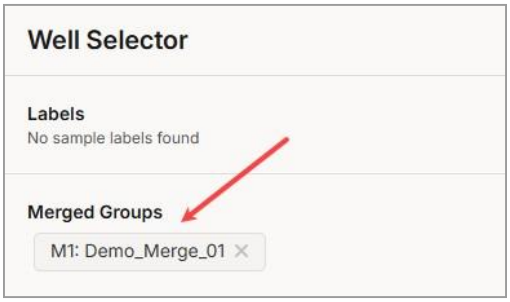


Merging and Unmerging Wells

For additional sensitivity and greater accuracy in run data analysis calculations, you can merge wells in the Well Selector to define separate well groups in the plate or cartridge layout. Each well group is calculated for the average value by aggregating each value for the selected wells and then calculating the average.

Note the following:

- Merged well data appears in all analysis views and in the exported data files.
- QX Insight Software assigns a merged well group identifier (M1, M2, and so forth), followed by the merged well name.



- If you merge a well into one well group, you cannot merge the well into another well group. ■ You cannot merge wells from different plates. ■ Merged well data is included in well tool tips.
- You can select each well in a merged group to display the individual data values, but you must unmerge the wells to modify the well configuration.
- If you try to merge wells with different targets, reference targets, reference copy number, or samples, an error message appears.
- If you manually delete all wells in a well group, the group itself is automatically deleted.
- Merged well groups defined in the QX700 software appear in QX Insight Software when you open the .niodata file and are saved as part of the workspace (.qxi) file.

The following graphic shows the Well data table for the merged well group (Demo_Merge_01), which displays the averaged values, and thetables containing the original data calculated for each individual well (A1, A2, and A3).

Well data | Run info

Run 1 M1 Demo_Merge_01 (73444 Droplets)

Target	Dye	c/µl	(+)	(-)
Target-1	FAM	991.79	27479	45965
Target-2	HEX	978.57	27191	46253
Target-3	ROX	979.08	27202	46242
Target-4	Cy5	961.85	26824	46620

Run 1 A1 (24167 Droplets)

Target	Dye	c/µl	(+)	(-)
Target-1	FAM	982.42	9005	15162
Target-2	HEX	968.15	8902	15265
Target-3	ROX	986.03	9031	15136
Target-4	Cy5	938.27	8684	15483

Run 1 A2 (24771 Droplets)

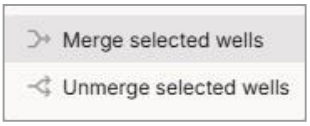
Target	Dye	c/µl	(+)	(-)
Target-1	FAM	994.16	9267	15504
Target-2	HEX	970.07	9090	15681
Target-3	ROX	971.83	9103	15668
Target-4	Cy5	974.81	9125	15646

Run 1 A3 (24506 Droplets)

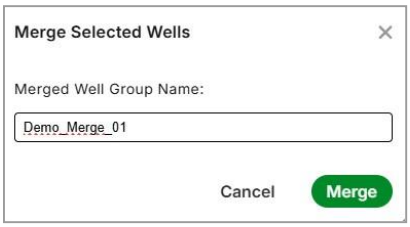
Target	Dye	c/µl	(+)	(-)
Target-1	FAM	998.71	9207	15299
Target-2	HEX	997.61	9199	15307
Target-3	ROX	979.54	9068	15438
Target-4	Cy5	972.28	9015	15491

To merge two or more wells

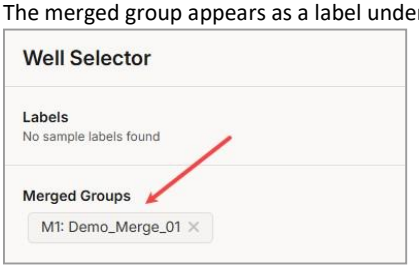
1. Select the wells in the Well Selector plate or cartridge layout and right-click to open the popup menu.
2. Select Merge selected wells.



3. In the Merge Selected Wells dialog, keep the default name or enter a new one (64-character limit) and then click Merge.



The merged group appears as a label under Merged Groups in the Well Selector.

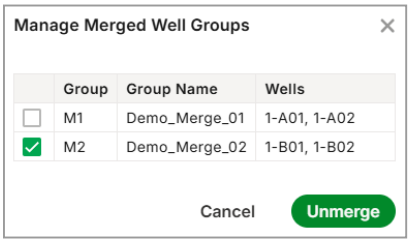


To unmerge a well group

1. Select a Merged Group label to highlight the wells.
2. Do one of the following:

   ■ Pause on the merged well group and right-click to display the pop-up menu; click Unmerge selected wells; continue to Step 3 ■ Click the X on the right side of the label to delete the group, thereby unmerging the wells

3. In the Managed Merged Well Groups dialog, select the groups to delete and click Unmerge.



The wells are returned to their original state and the well group no longer appears under Merged Groups.

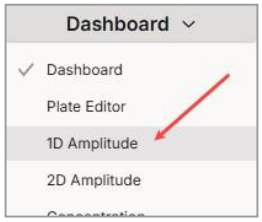
Changing the Chart Scale

For each chart, you can modify the chart scale by manually setting boundary values from a minimum of –10,000 to a maximum of 80,000 in the boundary fields.

Note: To change the axis scale from linear or logarithmic, see Changing the Axis Scale Type on page 138.

To manually adjust the boundary range

1. In a new or saved workspace, select the desired chart from the dropdown menu.

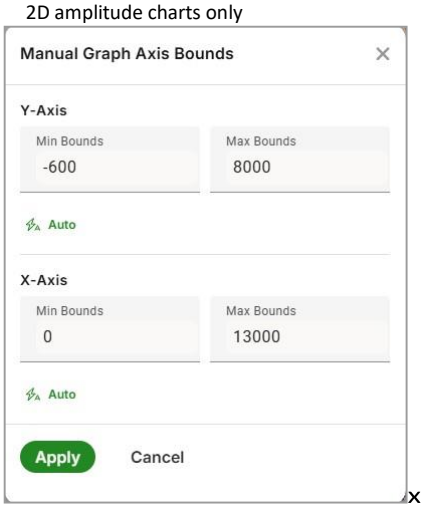


2. Click the Settings icon in the upper right corner.

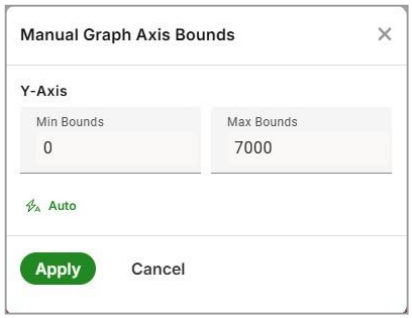
Note: The Settings icon appears in the same location in all charts. *You might need to pause the cursor on the area to display the icon*.

Important: In the dialog for 2D amplitude (next page), you can adjust values on the y-axis and x-axis. For all other charts in the dropdown, you can adjust values on the y-axis only.

2D amplitude charts only



All other charts



3. Enter new minimum and maximum boundary values:

- If the field is empty, or if you enter an invalid value (higher than the maximum or lower than the minimum), an error message appears.
- In data point charts, you must enter linear values for minimum and maximum boundaries even if you have selected a logarithmic axis scale.

4. Click Apply to display the new values and adjust the chart scale accordingly.
5. To reset the boundaries to the default values, click Auto, and then click Apply.

Copying Chart Images

In each of the chart displays, you can select an image to copy to the clipboard and then paste it into a document or graphics program image template.

1. In the graphical display portion of the workspace, right-click on a chart.

   Note: In the 1D and 2D amplitude charts, you must copy the chart for each fluorophore or fluorophore combination separately.

2. From the pop-up menu, select Copy Chart Image.

   The graphic is copied immediately.

3. Paste the graphic into your graphics program or document file.

Exporting Analysis Data and Images

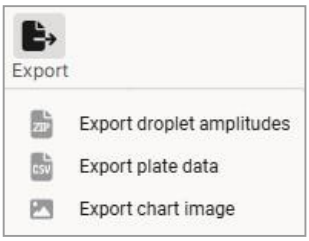
Note: For information on exporting spillover compensation data, see

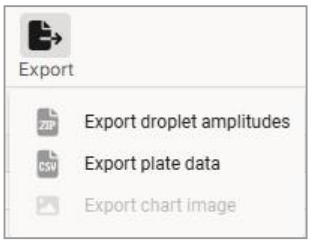
In any workspace view, you can use the workspace Export option to export the following: ■ Droplet amplitudes for each target in each well ■ Plate data, which exports Data Table ■ Chart images for each fluorophore

Note: The export of chart images is automatically excluded when you are viewing the Data Table and Analysis Plate Editor.

Fig. 37: Export options for amplitude, data droplet count views

Fig. 38: Export options for the analysis plate editor, point, and data table, and spillover views





Important: QX Insight exports the data from all runs that were added to the workspace.

1. Click Export to open the list.

   Tip: You can also right-click the image to display the pop-up menu.

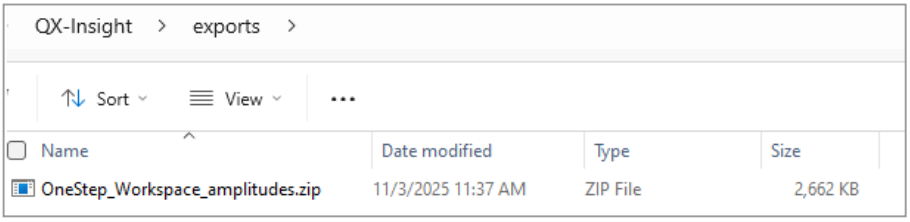
2. Select an option.

   - If you select Export droplet amplitudes or Export plate data, the Export dialog opens to the export library.
   - If you select Export chart image, the Export Chart Image dialog opens, where you can adjust the image size. When you click Export, the Export dialog opens to the export library.

3. Keep the default file name or enter a new file name for the item.

4. Click Save.

   Data is saved in a zip file and stored in the QX Insight > exports folder.



   The zip file contains an individual CSV file for the amplitudes in each well that was selected for the export.

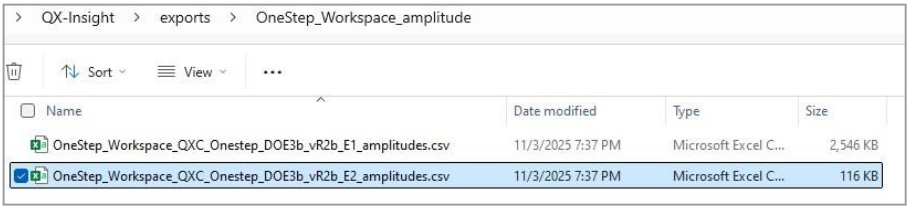


   Plate data is saved in CSV format and charts are saved in PNG format.

Viewing Tool Tips

When you pause on an area in the analysis Well Selector, analysis Plate Editor, or a data point chart, additional values appear in a tool tip. Note: Tool tips do not appear in the amplitude charts. ■ In the Analysis Well Selector and Analysis Plate Editor, pause on a well to display the tool tip.

Fig. 39: Well Selector Tool Tip

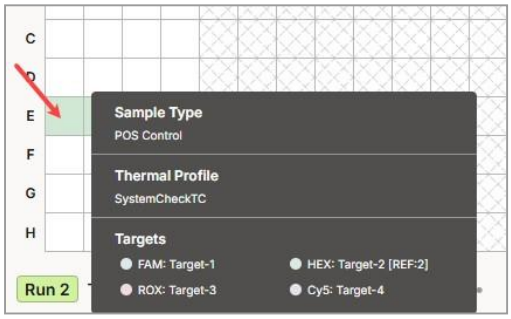
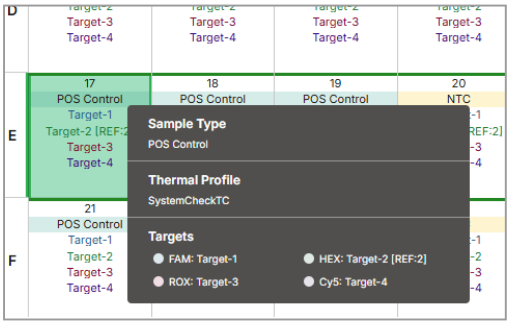
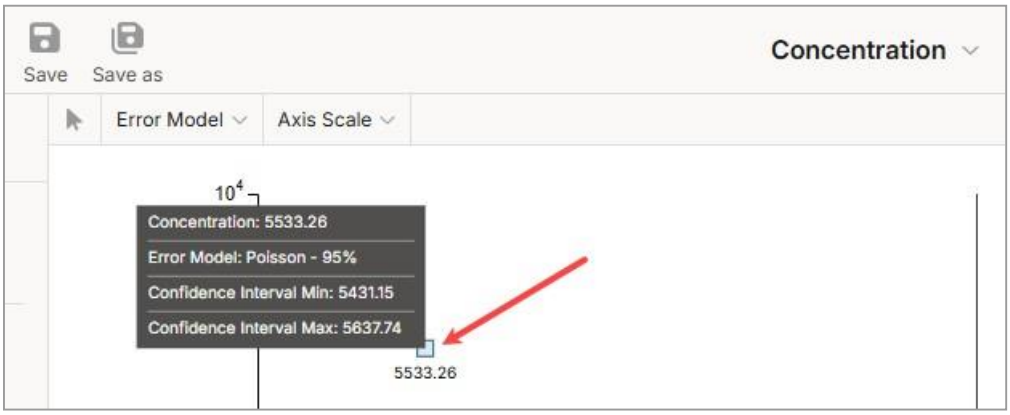


Fig. 40: Plate Editor Tool Tip



■ In the Concentration, Copy Number, Ratio, and Fractional Abundance charts, pause on a data point to display the tool tip.



Dashboard Options

When you select a chart in the Dashboard, a green border appears and options that are available in the standalone view are also available in the Dashboard view. For information, see Analysis Options for Amplitude Displays on page 127 and Analysis Options for Data Point Displays on page 135.

In the Dashboard view, you can ■ View multiple charts simultaneously ■ Insert, remove, or replace charts ■ Save a

reconfigured Dashboard layout as the default ■ Before saving,

restore the original default display

For information , see Changing, Inserting, and Removing Charts in the Dashboard on page 124 and Saving or Restoring a Dashboard Layout on page 126.

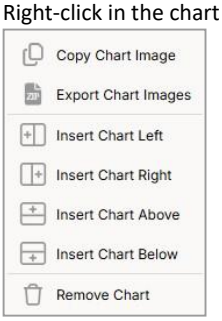
Note: You can select one chart at a time, and the selected chart appears with a green border.

Copying and Exporting Chart Images from the Dashboard

You can copy a chart image from the Dashboard and then paste it into a document and you can export chart images to a specified folder.

To select the wells and display the options

1. In the Well Selector, select the desired wells in the plate or cartridge layout.
2. Right-click in the chart to display the dropdown list of options.



Follow the procedures below.

To copy the chart image

1. Select Copy Chart Image.
2. Open the document where the image should be included, and then right-click and select Paste.

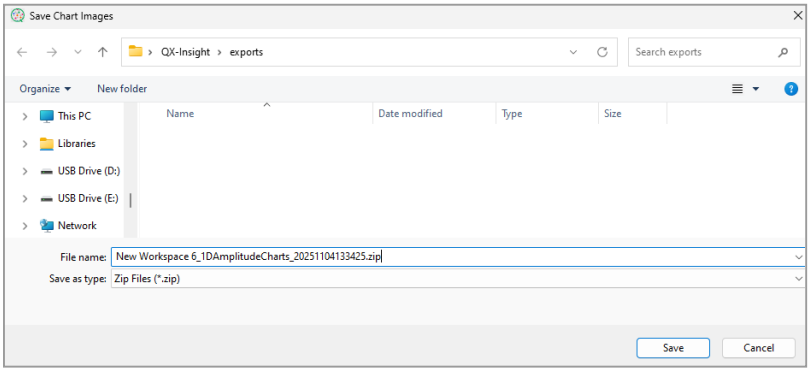
Important: In the 1D Amplitude chart, only one image is captured at a time. For example, if you right-click in the FAM dot plot, only the FAM dot plot is copied, even though the HEX dot plot is also visible in the 1D Amplitude block.

To export the chart image

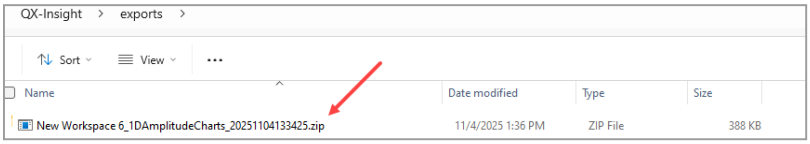
1. Select Export Chart Images.
2. (Optional) In the Export Chart Image dialog, change the image dimensions.

3. Click Export.

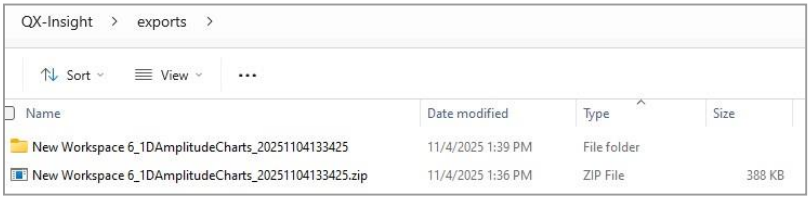
The QX-Insight > exports folder opens.



4. Click Save to save the zip file in the exports folder.

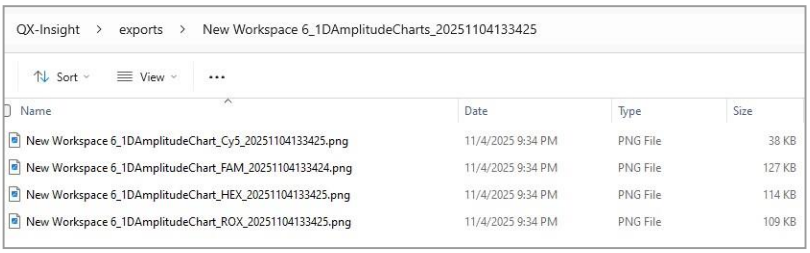


5. To unzip the file, navigate to the folder and right-click on the zip file, and then select Extract all.



6. Double-click the folder to display its contents.

The amplitude charts for each fluorophore appear for the selected wells.

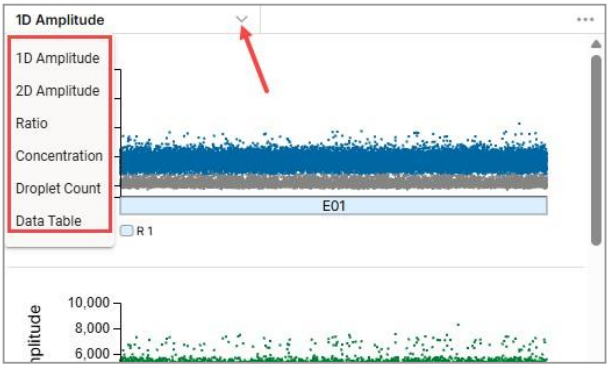


Changing, Inserting, and Removing Charts in the Dashboard

In the chart display below the Dashboard toolbar, each individual Dashboard window provides a dropdown menu to select a different chart type and another dropdown menu to insert or remove charts.

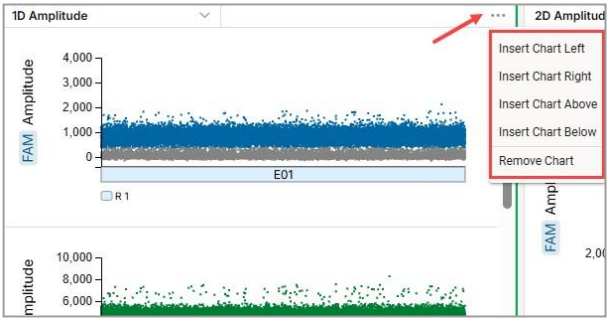
Note: You can display up to eight charts in the Dashboard. A minimum of one chart is required.

To change the chart type ▶ Click the chart type dropdown arrow and select a different chart.

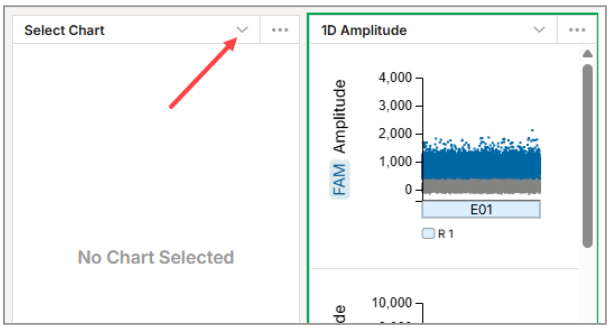


To insert a chart

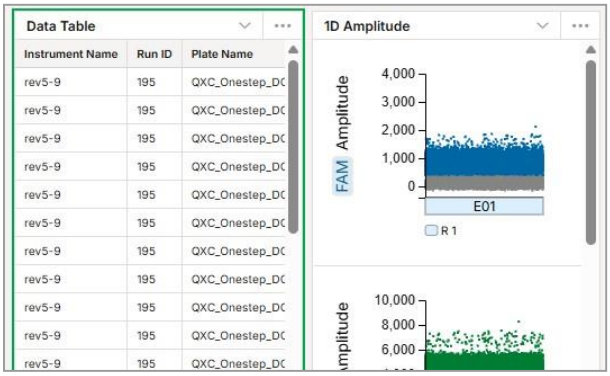
1. Click the ellipsis on the right and select an Insert option from the dropdown menu.



2. Click the Select Chart dropdown arrow and select a chart.



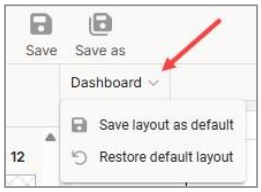
The selection appears in the space.



To remove a chart ▶ Click the ellipsis in the upper-right corner of the chart and select Remove Chart.

Saving or Restoring a Dashboard Layout

After you finish reconfiguring your Dashboard display, you can save it as the new default layout. The Dashboard for all future workspaces will display the run data in the specified layout. You can also restore the original layout before you save the changes.



To save the modified layout as the new default

▶ After you make your changes, click the Dashboard dropdown arrow and select Save layout as Default.

To discard changes and return to the most recently saved default display

- If you prefer to cancel your changes, click the Dashboard dropdown arrow and select Restore default layout.

Analysis Options for Amplitude Displays

If the well data meets certain quality metrics (for example, a minimum of 17,500 droplets), QX Insight automatically determines which droplets are considered positive and negative for each fluorophore and creates default thresholds. You can rely on these automated calculations or you can use the analysis tools to manually adjust the dot plot thresholding. Using the thresholding tools and settings, you can ■ Manually adjust line thresholds in 1D amplitude dot plots ■ Manually adjust line and cluster thresholds in 2D amplitude dot plots ■ Restore the default thresholds that the software set automatically

- When multiple wells are selected, arrange the 1D amplitude data by collection time, row processing order, or column processing order
- Manually adjust and restore the axis boundary values

Changing Fluorophores in 1D and 2D Amplitude Plots

Plot data for all fluorophores appears by default (Show all) but you can select one fluorophore at a time to display the corresponding plot data.

Note: In QX700 data files, fluorophores that were not enabled during the run are grayed out.

Fig. 41: QX Continuum

Fig. 42: QX700

To display a different fluorophore in a 1D Amplitude plot

1. Click the dropdown arrow to display the list offluorophores, and then select a fluorophore to display the corresponding plot data.
2. To restore all fluorophores to the display, click the icon in the upper-right corner and select Show all.

To display different dye combinations in a 2D Amplitude plot

1. Click the dropdown arrow next to the fluorophore (FAM or HEX in this example) to display the list of other dyes, and then select an alternative dye.

2. Repeat for the second fluorophore.

Fig. 43: QX Continuum

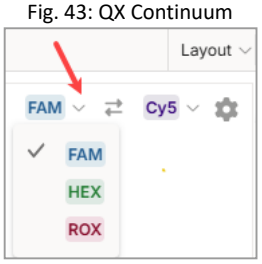
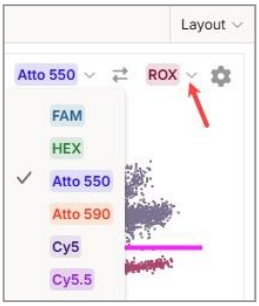


Fig. 44: QX700



Automatic Thresholding

For wells in which enough droplets are recognized (17,500 droplet minimum per well), QX Insight automatically distinguishes between positive and negative droplets in the well and calculates an automatic amplitude threshold for each dye, where all droplets above the threshold are considered positive.

If you manually adjust the automatically calculated thresholds, you can click Analysis > Auto to restore the default automatic thresholds.

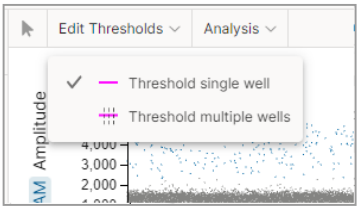
Manual Thresholding for 1D Amplitude Plots

To change the automatic thresholds, experienced users can employ the manual thresholding options and draw thresholds for a single well or multiple wells to ensure correct quantification of positive and negative droplets. Manual thresholding is often used in the following circumstances: ■ Certain sample types are used that are difficult to automatically threshold (such as FFPE) ■ There is variability in target sequences

■ There is failure to distinguish between positive and negative droplets, which can result in No Call wells

To manually adjust the thresholds

1. From the Edit Thresholds dropdown list, select a thresholding option.

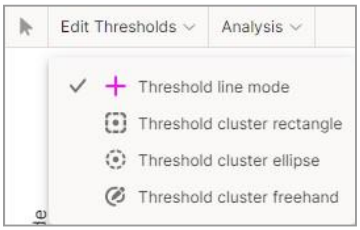


2. Click in the plot and drag the threshold line to a new location.

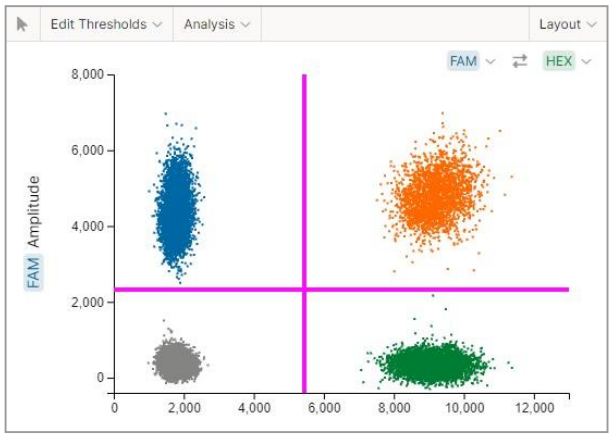
QX Insight displays the manual threshold and changes the color of the droplets that are now above and below the threshold.

Manual Thresholding in 2D Amplitude Plots

When you select 2D Amplitude, you can manually change thresholds and cluster droplets. ▶ Click Edit Thresholds and select a thresholding mode.



To use the line mode ▶ Select threshold line mode, and then click in the plot to draw the corresponding quadrants.

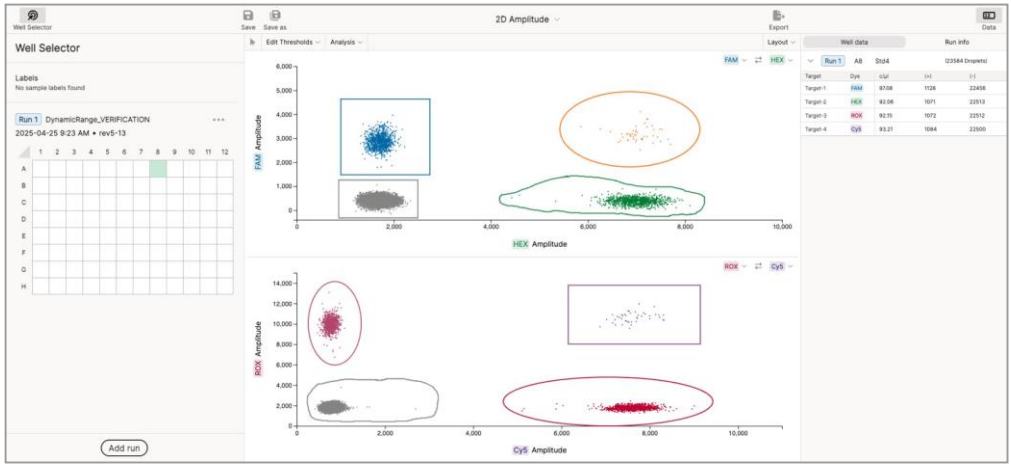


To use a cluster mode

1. Select Threshold cluster rectangle, ellipse, or freehand, and then click in the plot to draw the corresponding shape.

   When the Assign Cluster pop-up appears, select a color to classify the cluster.

   The shape and dots appear in the selected color.



2. To make changes, click Reset and click a different line or cluster option. Restoring the Original Amplitude

If you adjusted the thresholds using the manual thresholding options, you can use the Auto option to restore the original thresholds. For information on setting manual thresholds, see 1D Amplitude on page 88 and 2D Amplitude on page 90.

1. To restore the automatic thresholds, click Analysis > Auto.

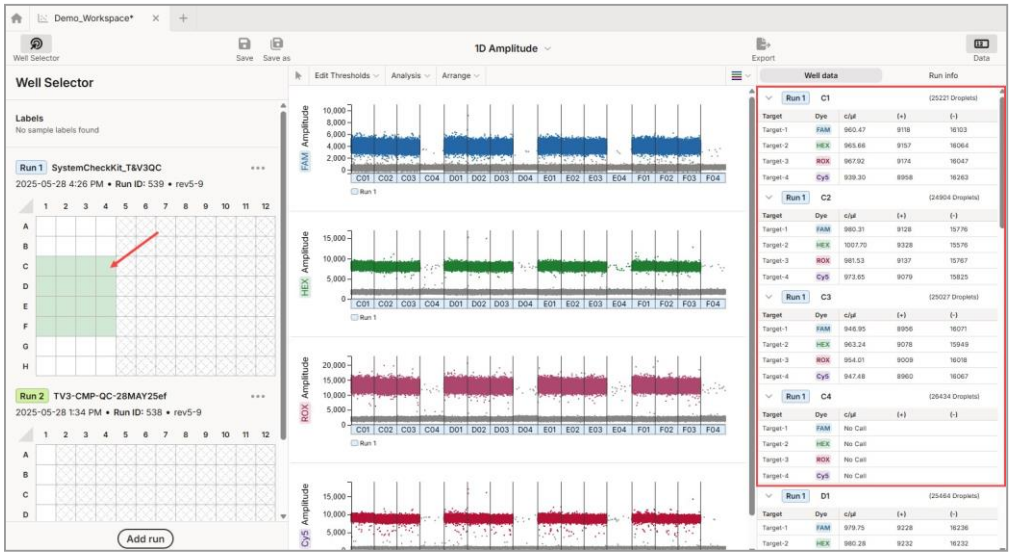
   For each dye in each well, QX Insight Software recalculates and displays the positive and negative droplet counts and concentration per microliter on the Well data tab, and adjusts the coloring of droplets in the dot plot to identify the positive droplets by fluorophore color and the negative droplets in gray.

2. To exit the Edit Threshold mode, press ESC.

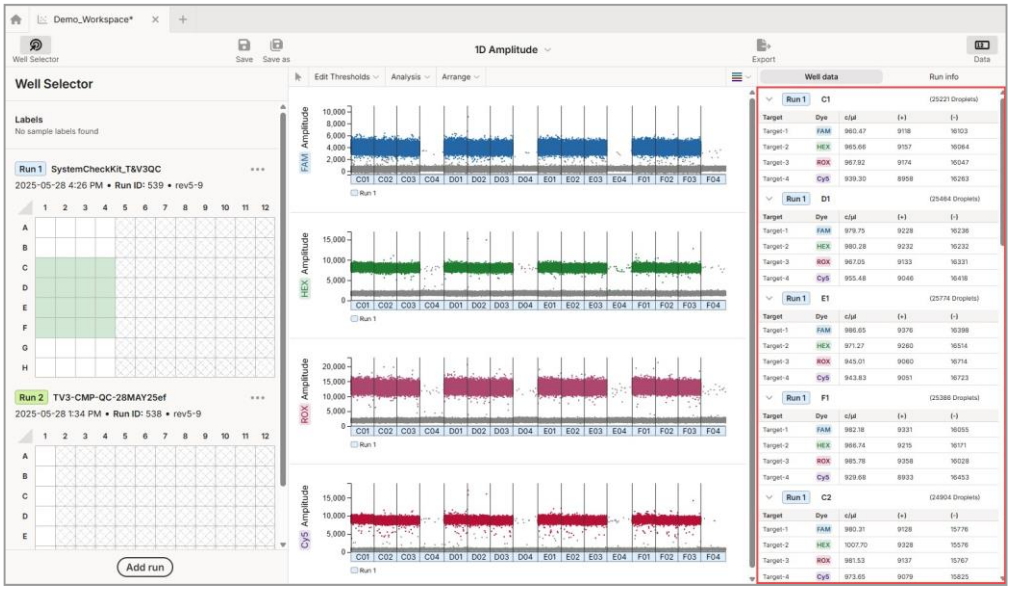
Changing the Droplet Collection Type

For 1D amplitude analysis, you can use the Arrange options to plot the droplet data, as follows:

- By Collection Time — chronological collection of sample, based on the order defined during plate setup
- By Row — processing order of the wells in the selected rows ■ By Column — processing order of the wells in the selected columns By default, wells are arranged by row, as shown in the following graphic.



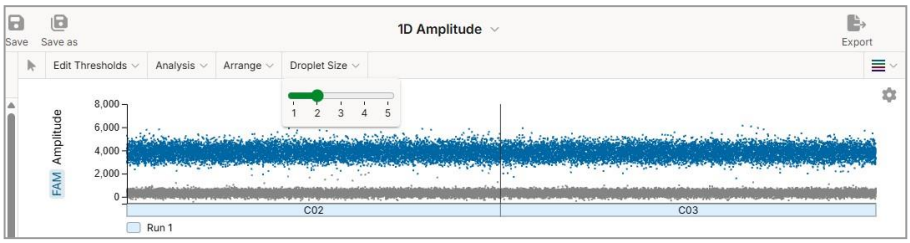
If you switch to a By column view, the wells are rearranged as shown in the following graphic.



Changing the Droplet Size in Amplitude Plots

You can increase or decrease the size of the droplets represented in the amplitude dot plots. The default value is 2, as shown in Fig. 45.

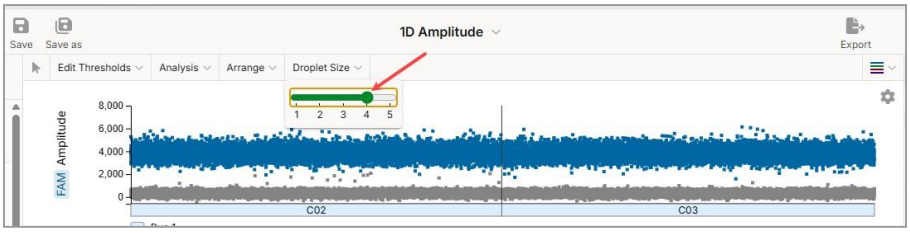
Fig. 45: Droplet size default



To increase or decrease the droplet size

▶ Click and drag the control backward or forward to immediately change the droplet size, as shown in Fig. 46.

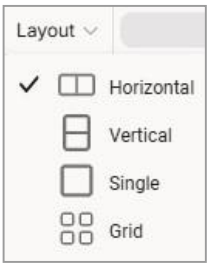
Fig. 46: Droplet size increased



Changing the Layout in 2D Amplitude Plots

▶ To change how the plots appear in the 2D display, click Layout, and then select Horizontal, Vertical, Single or Grid.

Note: Grid is the default display. However, changes to the layout are persistent and appear when you next open the chart in the workspace.



■ Horizontal — displays the 2D plots side-by-side ■ Vertical — displays the 2D plots above and below ■ Single — displays one 2D plot only ■ Grid — displays all 2D plot combinations

Analysis Options for Data Point Displays

If the well data meets certain quality metrics (for example, a minimum of 17,500 droplets), QX Insight automatically determines which droplets are considered positive and negative for each fluorophore and creates default thresholds. You can rely on these automated calculations or you can use the analysis tools to manually adjust the data point value ranges for the selected Poisson confidence interval.

In the data point charts (Concentration and Ratio), QX Insight Software provides the error model and axis scale options described in Table 11.

Note: Values for Ratio appear only if you selected one or more reference targets.

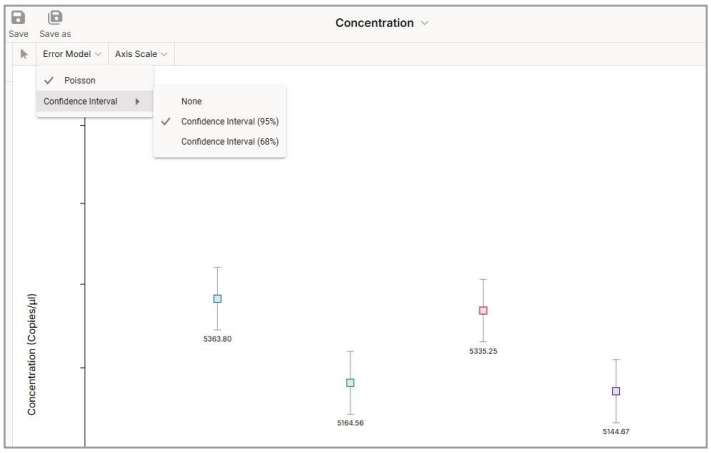
Table 11. Data point chart analysis options

For this option...	You can...
Error models	Specify a Poisson statistical confidence interval You can keep the default calculations that are not modified by the error model, or you can display the data by confidence intervals of 95% or 68%. Brackets are applied above and below to illustrate the range. For more information, see Changing the Confidence Interval on page 136.
Axis scales	■ Display the data points in linear or logarithmic scale. For more information, see Changing the Axis Scale Type on page 138. ■ Apply fixed fluorescence scaling options on the y-axis and restore automatic scaling
Data point tool tips	You can pause on a data point to display an informational tool tip

Confidence Interval

The default Poisson statistical confidence interval in the data point chart displays is 95%, which provides a wider range and a higher degree of confidence that the true target value is within the error bars. However, you can use the Error Model dropdown to recalculate your results based on a different confidence interval. Choosing 68% narrows the range to be more precise, but with less confidence that the true target value is within the error bars. When you change the confidence interval, each data point is updated.

Changing the



1. Click Error Model.
2. Select Confidence Interval, and then select the alternate confidence interval from the dropdown list.

   The error bars widen or narrow immediately.
3. To remove the error bars, repeat steps 1 and 2, and select None.

You can also display more information about a data point. If you pause on a value in the chart, a tool tip opens that contains the original calculated concentration, the selected confidence interval, and the minimum and maximum concentrations based on the confidence interval, as shown in the following graphics.

Fig. 47: 68% confidence interval

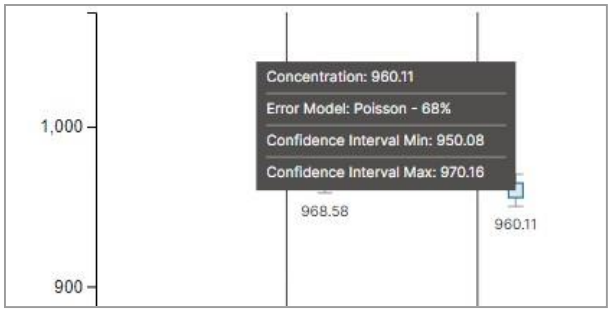
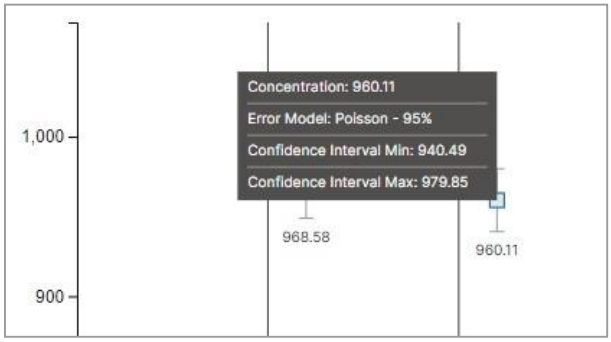


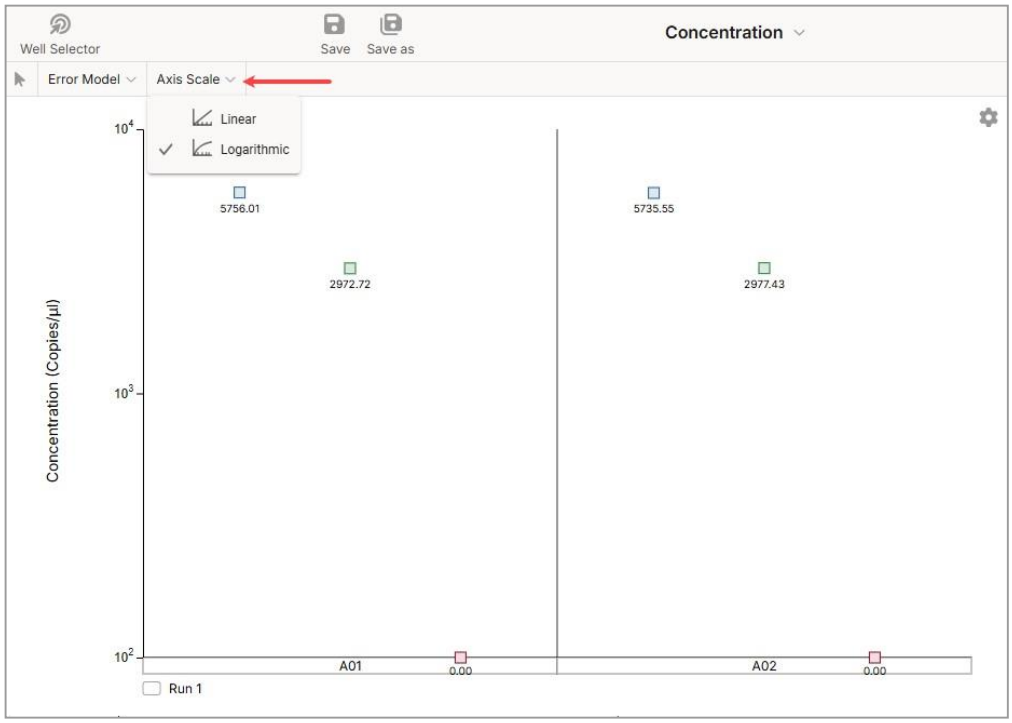
Fig. 48: 95% confidence interval



Axis Scale Type

For data point charts, QX Insight Software provides two axis scales, linear and logarithmic. The following graphic displays the data on a logarithmic scale, which is the default setting for a more efficient display when charts contain a wide range of values. ▶ To change the scale, click Axis Scale and choose the alternate method.

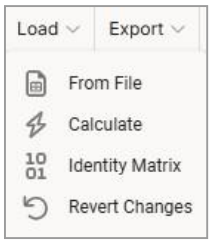
Changing the



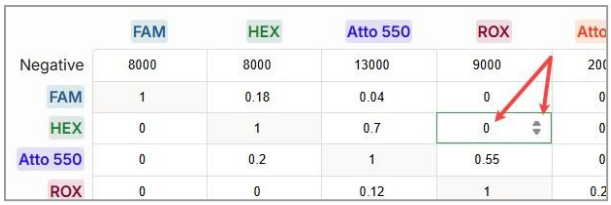
Analysis Options for Spillover Compensation

The Spillover view provides Load (import) options and an Export option.

Load options allow you to load existing compensation matrix files, create a new calculated matrix using monocolor values, and load or reload an identity matrix, which sets the values to the default zeroes and ones. You can also use the Revert Changes option in the Load menu to restore the last applied compensation values.

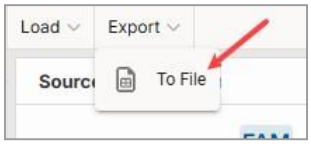


Important: You can manually enter compensation values into the matrix, but this is not recommended unless you are an expert ddPCR user.



	FAM	HEX	Atto 550	ROX	Atto
Negative	8000	8000	13000	9000	200
FAM	1	0.18	0.04	0	0
HEX	0	1	0.7	0	0
Atto 550	0	0.2	1	0.55	0
ROX	0	0	0.12	1	0.2

The Export option allows you to save your compensation matrix in an independent .qx700cm file. The name of the exported file matches the name of the run data file by default.

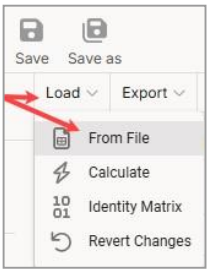


Note: All .ncm files are saved as .qx700cm files in QX Insight.

Loading, Creating, or Updating Compensation Matrix Values

To load a compensation matrix from a file

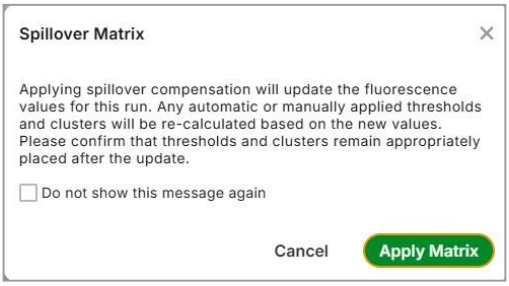
1. Open the run data file and click Load > From File.



2. In the Windows Open File dialog, navigate to the folder containing the compensation matrix files.
3. Select the applicable .ncm or qx700cm file and click Open.

   The matrix grid is populated.
4. If you are satisfied with the values, click Apply.

   The following confirmation message appears.



5. To set the compensation values, click Apply Matrix.

   Important: If a companion .ncm file wasn't created for the QX700 run, then you must select a file with settings that match your run data file. An .ncm file is saved as a qx700cm file in QX Insight.

To calculate a compensation matrix using monocolor controls

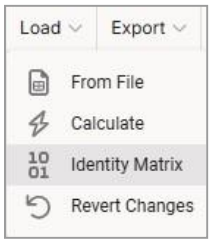
Important: Before you can use the Calculate feature, wells must be set up with monocolor controls in each channel. In the example shown in this section, wells A through G in Cartridge 1 have been set up using monocolor values for each dye.

1. Open the run data file and select Spillover from the chart view dropdown list.

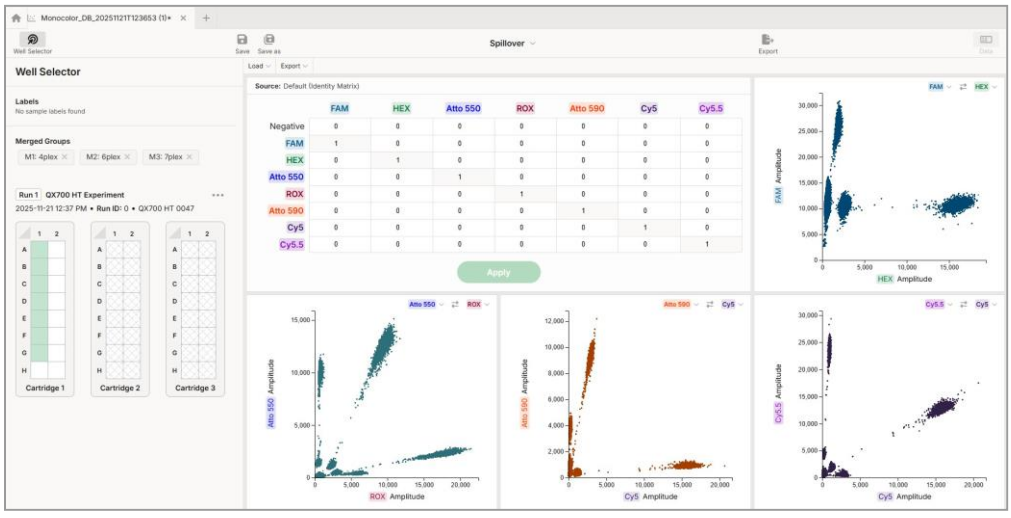
The Spillover view appears.

2. Do one of the following:

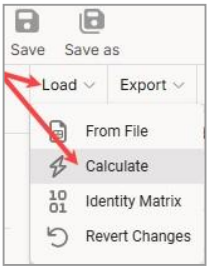
- If a matrix is applied and you want to calculate a new matrix, click Load > Identify Matrix to reset the values to the defaults.



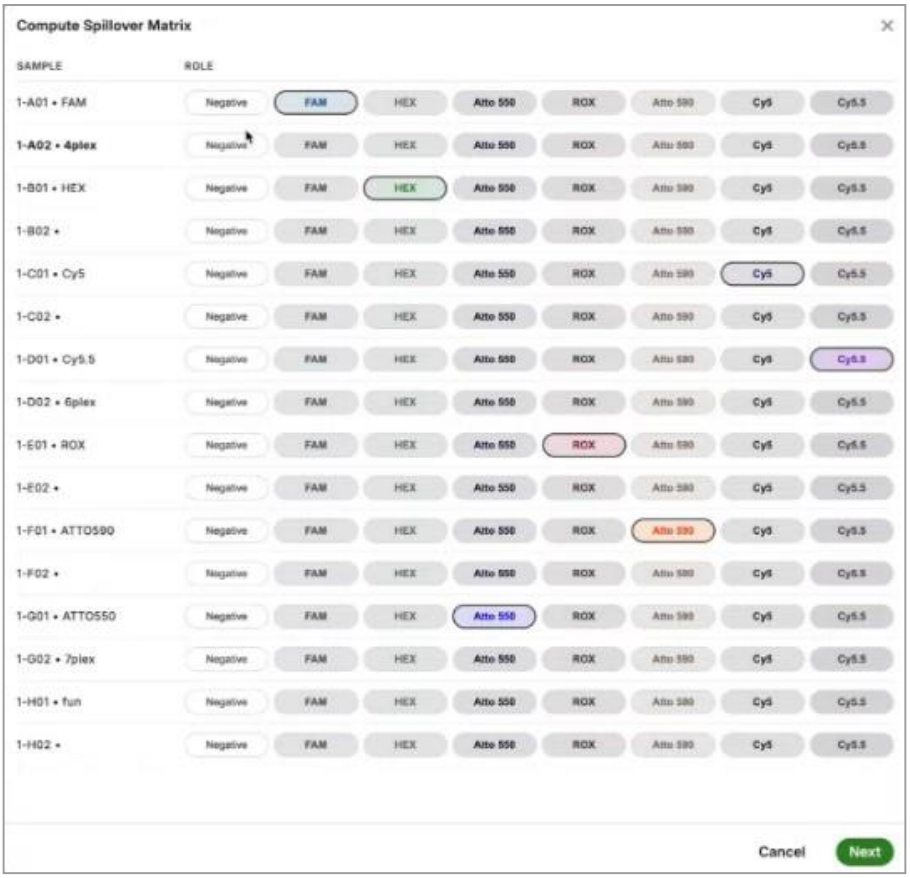
- If compensation has not been applied before, then the matrix appears in default format, with a 1 populating each field where the fluorophore intersects with itself, and zeroes populating the remaining fields.



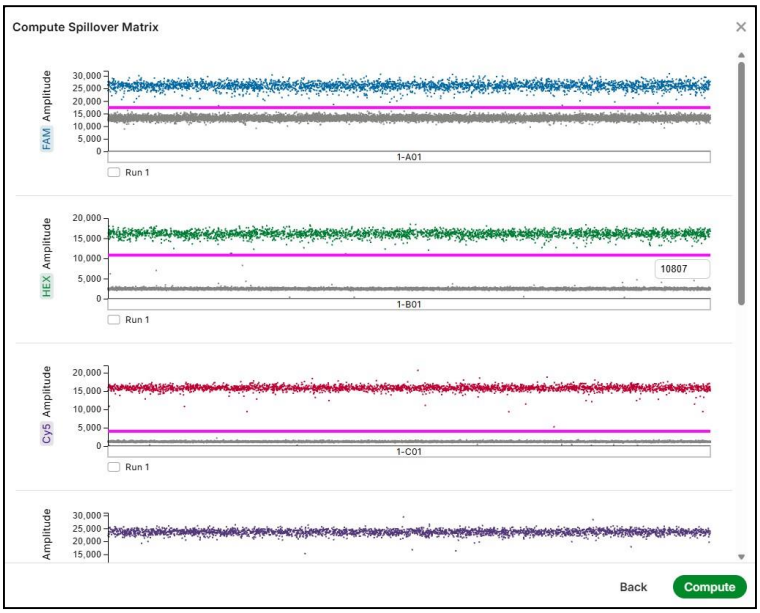
3. Click Load > Calculate.



4. When the Computer Spillover Matrix dialog appears, select the fluorophore for each monocolor well, as shown below.

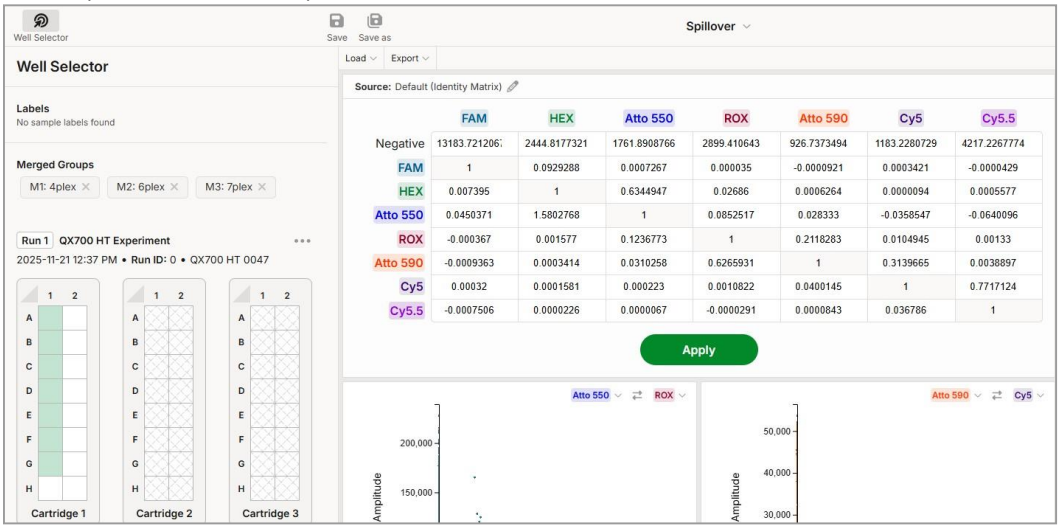


5. Click Next to open the Compute Spillover Matrix 1D amplitude view.



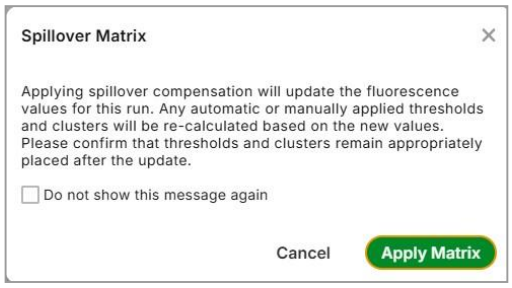
6. Review each chart and, optionally, adjust the thresholds before continuing.
7. Click Compute.

   The compensation values are updated for review.



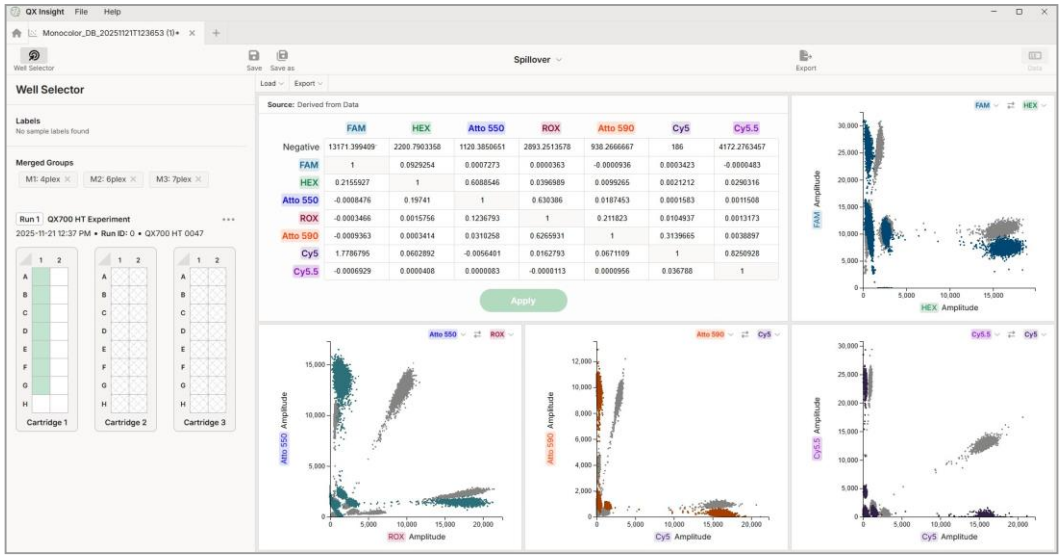
8. If you are satisfied with the values, click Apply.

   The following confirmation message appears.

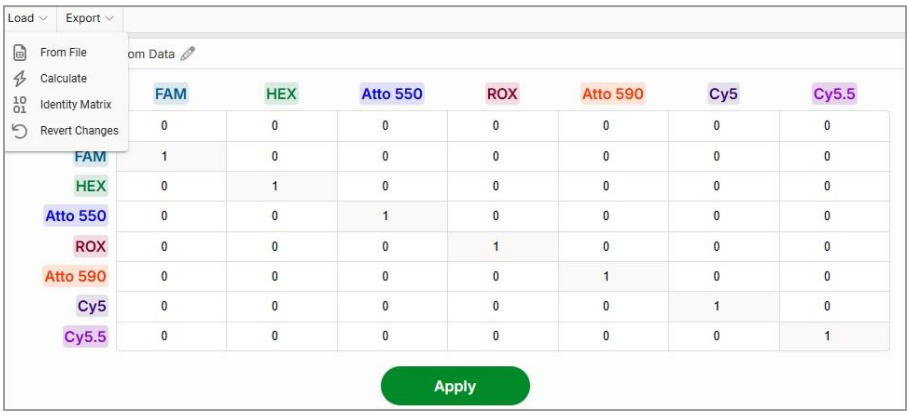


9. To set the compensation values, click Apply Matrix.

   Gray areas indicate droplet placement before compensation was applied, and the droplets identified by fluorophore color indicate compensated data.



To load or reload the Identity Matrix ▶ Click Load > Identify Matrix to restore the 0 and 1 starting values.



To revert changes to the last applied values

▶ Click Load > Revert Changes.

Exporting Spillover Compensation Data

To export your new or modified spillover compensation data to a qx700cm file

1. Click Export > To File.
2. In the Save Compensation Matrix dialog, keep the default file name that matches the run data file name, or change it.
3. Click Save.

   The file is saved to the exports folder. All compensation matrix files saved in QX Insight are save as .qx700cm files.

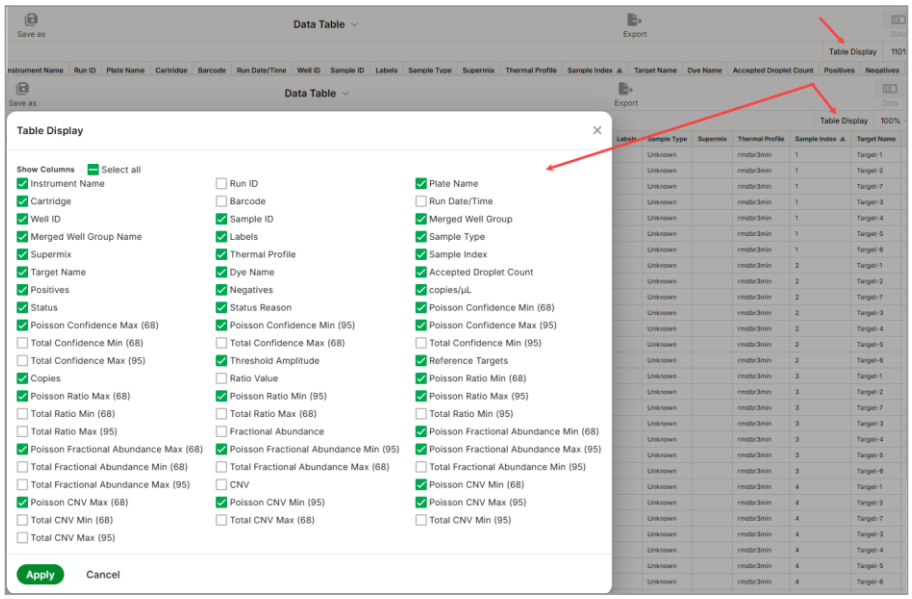
Data Table Options

When you display the Data Table, you can change the array of columns displayed and you can use the Zoom option to enlarge or reduce the display.

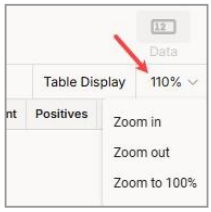
To change the column array

1. Click Table Display to open the Table Display dialog.
2. Select or clear the column checkboxes (or Select all) and click Apply. The Data Table column array expands or shrinks according to your choices.

   Important: If you close the Table Display dialog before you click Apply, your changes are lost.



To zoom in or out ▶ Click the percentage icon to display the menu and select a zoom option.



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Appendix A Installing QX Insight Software

This appendix contains the computer requirements for the QX Insight Software computer, as well as instructions to install and update QX Insight on a Windows PC or a Mac.

Computer and Connection Requirements

Following are the minimum and recommended requirements for the PC or Mac computers on which QX Insight Software will be installed and maintained. Remote transfers are supported between QX Insight and the QX Continuum, but are not supported between QX Insight and the QX700.

Note: WiFi is set up using the customer's vendor.

Table 12. PC requirements

Component	Minimum	Recommended
Operating system	Windows 10 64-bit	Windows 11 64-bit
Hard disk space	500 GB	1 TB SSD
CPU	6th generation Intel 2 core processor	8th generation Intel 4 core processor
RAM	16 GB	32 GB
Display resolution	1920 x 1080	3840 x 2160
Ports [1]	USB Ethernet [2]	USB Ethernet [2]
GPU with WebGPU support	Discrete GPU with 1 GB or more of video memory, or integrated graphics on a system meeting the minimum RAM requirement	

Table 13. Mac requirements

Component	Minimum	Recommended
Operating system	Mac OS Ventura	Mac OS Sonoma
Hard disk space	500 GB	1 TB SSD
CPU	6-core Intel Core i7	Apple M2 chip
System memory (RAM)	8 GB	32 GB

[1] If the computer is not equipped with a required port, you must connect it to a docking station. [2]An Ethernet port is required only if the computers with QX Insight Software installed will be hard-wired through a live network outlet. The computers can also be connected using the company's WiFi network.

Display resolution	1920 x 1080	3840 x 2160
Ports (one each) [1]	USB Ethernet [2]	USB Ethernet [2]
GPU with WebGPU support	Discrete GPU with 1 GB or more of video memory, or integrated graphics on a system meeting the minimum RAM requirement	

To support remote transfers between the connected computers and the QX Continuum, the following requirements must also be met:

- *Ports 7090 and 443 must be open and available* to perform plate uploads and data transfers between QX Continuum and QX Insight.
- *You must use the Ethernet cable supplied by Bio-Rad* and included with your purchase.
- *The QX Continuum is connected to your organization's network*. The supplied Ethernet cable must be connected to the Ethernet port on the instrument and to a live network outlet. The Bio-Rad field service engineer can work with your system administrator to ensure requirements are met and the connection is enabled.

  Note: If your lab doesn't use a network, you can connect the Ethernet cable from the Ethernet port on the instrument to the Ethernet port on the computer or docking station.
- *Computers running QX Insight are connected to the same network using WiFi or an office/cube/ workstation Ethernet port*. Your system administrator can configure the wireless or Ethernet connections on the computers.
- *QX Insight Software is configured to communicate with the QX Continuum using the instrument's IP address*. Any user can create the connections in the software.

For a graphical representation, see Connected Computer Architecture on page 151.

Tip: If the computers and instrument are not connected, you can transfer plates and run data using a USB drive. For information, see Transferring Files Using a USB Drive on page 61.

Connected Computer Architecture

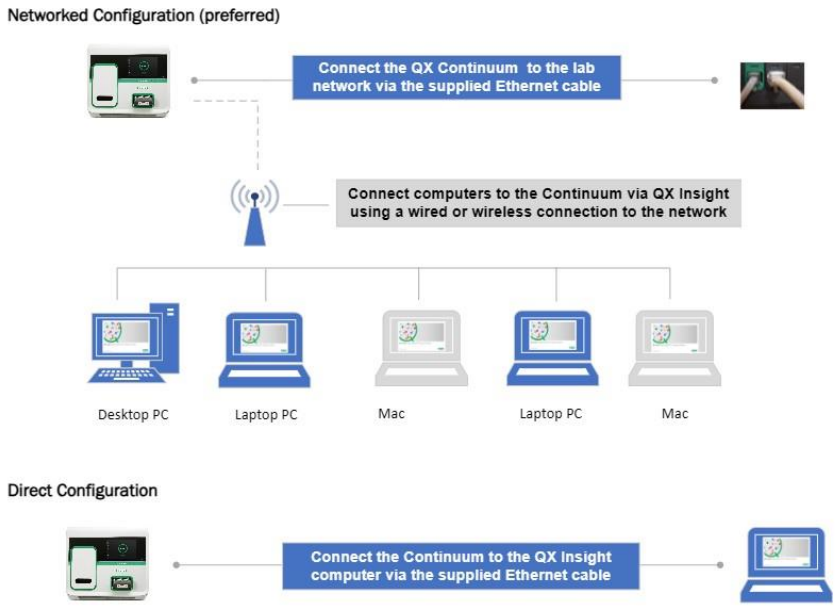
Connected Computer Architecture

This section illustrates how the QX Continuum is connected from the instrument, either directly to a single computer or through the customer network (preferred method) to multiple computers.

Important: The architecture in the graphic shows connectivity options for the QX Continuum Droplet Digital PCR System only. *Connectivity between QX Insight Software and the QX700 ddPCR System is not supported*. Instead, the QX700 can connect to a customer LAN to facilitate access to setup and run data files from a shared location, but the instrument does not have wireless capability.

Each computer can be a PC (desktop or laptop) or a Mac, and must have QX Insight installed. As shown in Fig. 49, the Ethernet cable can be connected between the QX Continuum and a live network outlet or the Ethernet port on the computer with QX Insight Software installed.

Fig. 49: QX Continuum connected computer architecture



If you connect using the network outlet, the computers with QX Insight installed can connect to the QX Continuum using a wired or wireless network connection. This allows multiple computers to be simultaneously connected to the instrument, thereby allowing multiple signed-in users to upload plates and transfer completed runs for data analysis.

Installing on a PC

1. Turn on the computer and log in using your local user or network account.
2. Open a browser and navigate to the Downloads location provided by Bio-Rad, and then download the installation executable.
3. Right-click on the installation file and select Run as administrator.
4. Follow the prompts to install the software.

Installing on a Mac

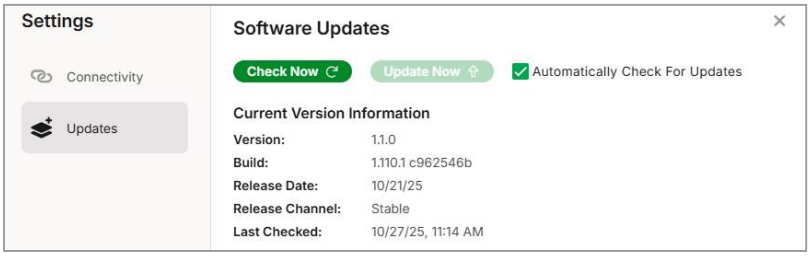
1. Turn on the computer and log in using your local user or network account.
2. Double-click the .dmg file.
3. Click and drag the QX Insight icon to the Applications folder.

Updating QX Insight Software

To allow customers to quickly upgrade their instances of QX Insight Software with new or improved functionality and usability, Bio-Rad provides the software upgrade packages through the application.

Important: Save your work before you start this procedure or the software will interrupt the installation. You must save your work and then initiate a manual installation to continue.

You can choose to automatically check for software updates or you can manually prompt a check when desired. If the automatic feature is enabled, QX Insight checks for a new software upgrade executable each time the software is launched, and hourly if the software is running.



An advisory message notifies you when an update is available and you can download and install the update while you are signed in.

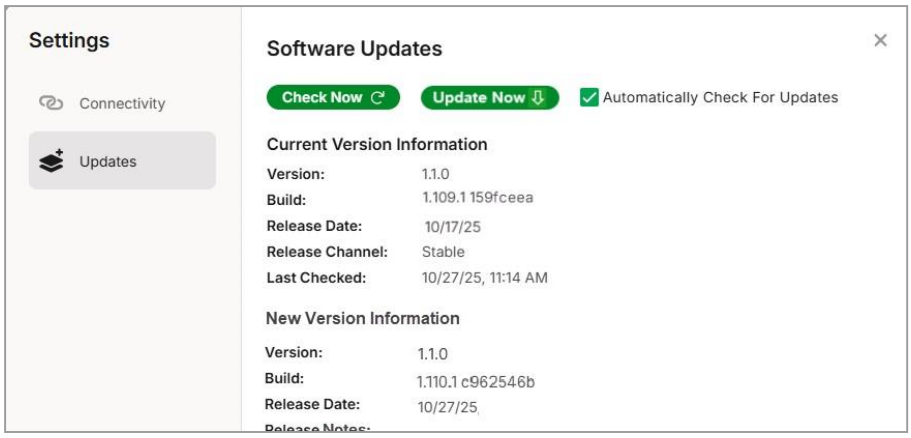
To check for updates

1. Do one of the following:

   - To manually check for an update, click the Settings icon in the upper-right corner to open the Settings dialog and click Updates.
   - If the settings are configured to automatically check for software updates, then the checkbox is selected and you are automatically notified when a software update is available. To open the Settings dialog, tap View Updates in the notification.

     Note: The dialog appears once per QX Insight session until the software is upgraded. To stop the notifications for the specified update, select the checkbox. *This does not stop notifications of future updates.* To close the message, click Cancel.

2. To install the software update, click Update Now in the Software Updates dialog.



The software download begins when you click View updates. When the download is complete a message appears asking you to confirm proceeding with the upgrade.

3. Click Yes.

   The software installation proceeds, and you are notified when the installation is finished.

Bio-Rad
Laboratories, Inc.

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Group

Website bio-rad.com **USA** 1 800 424 6723 **Australia** 61 2 9914 2800 **Austria** 00 800 00 24 67 23 **Belgium** 00 800 00 24 67 23 **Brazil** 55 11 3065 7550
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