
QX ONE Software

Standard Edition

User Guide

Version 1.1



QX ONE Software Standard Edition

User Guide

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BIO-RAD

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Revision History

Document	Date	Description of Change
QX ONE SoftwareUser Guide, Standard Edition and Regulatory Edition	October 2020	Add brief descriptions, bullet points and cross- references to the instrument guide in both software guides

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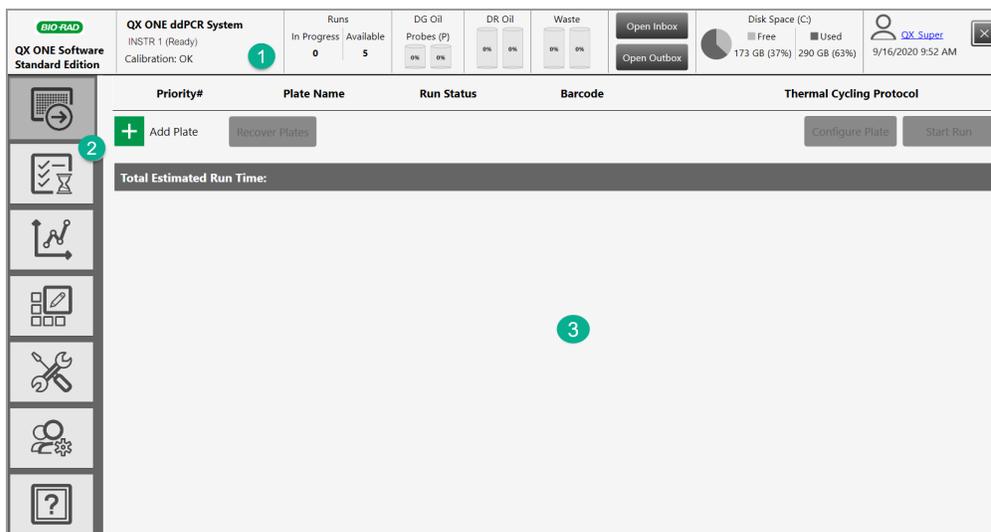
Chapter 1 About QX ONE Software

QX ONE Software, as part of your QX ONE Droplet Digital PCR System, provides all necessary functionality to create, run, and analyze ddPCR experiments on your samples.

Note: In the QX ONE guides, you are instructed to tap options on the touchscreen. If you are using QX ONE Software with a keyboard and mouse, click the options instead. If QX ONE Software goes into standby mode after being idle, tap in the lockout screen to sign into the software again and redisplay the last active window.

For information on using the instrument, refer to the QX ONE Droplet Digital PCR System User Guide, Standard Edition or Regulatory Edition.

The following image highlights functional areas:



LEGEND

- 1 The status bar displays information about the instrument and user.
- 2 Tabs provide access to the main functional windows.
- 3 The main pane displays the details of the selected tab.

A Bio-Rad service engineer sets up your QX ONE Droplet Digital PCR System instrument and installs QX ONE Software onto the touch screen computer. Your installation includes activation of a primary superuser account, to be used by a system administrator in your organization. The superuser is responsible for setting up additional users and assigning user privileges. For information, see [Managing Users on page 149](#).

Note: If you will be installing the software on additional computers to use for ddPCR file analysis, see [Appendix B, Additional Computers](#).

Using the instrument and software, you can

- Set up customized ddPCR experiments for plate runs in the QX ONE Droplet Digital PCR System
- Create and store plate, thermal cycling protocol, and report templates
- Use the live analysis function during the droplet reading phase
- Analyze your data files in a variety of charts and tables in the Analysis module
- Generate reports on your data
- Safeguard user access with session time-out settings
- Restrict template access to ensure the use of approved templates only

Note: When you open QX ONE Software on the touch screen, the Add Plate window appears by default. If you open the software from a standalone computer, the Data Analysis window appears by default and the Add Plate and Run Status tabs are not available.

Chapter 2 Getting Started in QX ONE Software

Use the information in this chapter to

- Sign into the software or change users
- Understand your assigned user privileges and modify your personal preferences
- Become familiar with the functional windows
- Learn the differences between the software installed on the instrument touch screen versus a standalone computer
- Understand the compatible file types for your software edition

Signing Into the Software

Your system administrator sets up users and privileges for the instrument and software, and communicates the information to you.

To sign in

1. Tap the QX ONE Droplet Digital PCR System touch screen to open the Sign in dialog box.

The Sign in dialog box opens.



2. Enter your user name.
3. If the Sign in to: label shows the correct domain name, enter only the user name. If the label is blank or shows a different domain then enter it as **domain name\user name**

Note: The domain and computer names shown in the graphics are examples only. If you are unsure of the domain name, contact your system administrator.

4. Enter the password, and then tap Sign in.

On first use, you must agree to the end user license agreement.



5. Clear the Show EULA on sign in checkbox, and then tap I Agree.

Note: If you tap I Disagree, the application closes immediately. If you leave the checkbox selected, you must agree to the EULA each time you log into the application.

When you agree to the EULA, the dialog box closes and the application opens. Your user name appears in the Instrument Status bar.

If you are opening the software on a standalone computer, the Data Analysis window opens.

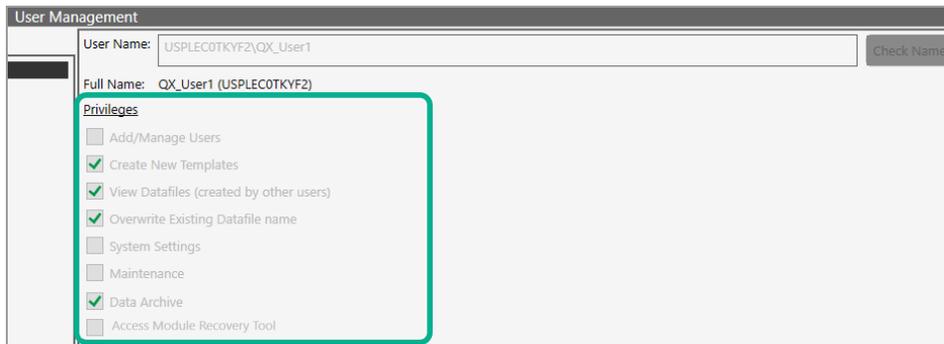
Viewing Your User Privileges

In the User Setup and Preferences window, your privileges are identified by a green check mark in the corresponding checkbox.

- ▶ Select the User Setup and Preferences tab.



You can view your assigned privileges at any time, but the check boxes in the display are not enabled unless you are assigned the Add/Manage Users privilege.



Note: Users who are assigned the Add/Manage Users privilege can also create users and update privileges and preferences for any user. Only the superuser can remove the Add/Manage user privilege from a user account.



Table 1 lists the available privileges, which can be assigned in any combination.

Table 1. User privileges

Privilege	Purpose
Add and manage users	Add or remove users, set privileges, and change preferences. Note: Only the superuser can remove this privilege from other users.
Create new templates	Save a plate, thermal cycling protocol, or analysis report design as a template.
View data files (created by other users)	View files created by other users.
Overwrite existing data file name	Use Save or Save As capability. <ul style="list-style-type: none"> ■ Selecting Save replaces the original file content with any changes made by the user, without changing the file name. ■ Selecting Save As allows the user to save either existing or new content with a new file name. Whether or not changes are made, files are automatically saved as .ddpcrone files. Note: Users without this permission can open files and perform analysis but cannot save their changes.
System settings	View logs, and view and modify the preferred or shared data file and template locations. Note: All users can view the file locations. Important: Your system administrator can set preferred locations in System Settings, which override individual user location preferences.
Maintenance	View the event log. Note: All users can view the maintenance log and maintenance reports.
Data archive	Move raw data from the touch screen computer to free up disk space needed for runs on the instrument.
Access the Module Recovery Tool	For manual plate recovery, users can specify the processes to be rerun.

Managing Your Preferences

Use the User Setup and Preferences window to modify your personal user preferences.

Default folder locations appear in your preferences, but you can change the storage locations for your templates and data files. You can also choose to keep all your data files and templates private. The software prompts you to choose either your personal folder or the shared folder each time you save a file or template.

Important: If your system administrator enables the Preferred Locations in System Settings, data file folder locations in User Preferences are overridden, and all data files are saved to the preferred file paths. Users can still choose where to store templates.

To modify your preferences

1. Tap the User Setup and Preferences tab.
2. Change any of the following preferences:
 - Enter a different file path for your data files and templates.
 - Select or clear the check boxes to change your data file and template privacy settings.

Note: The paths and checkboxes are disabled if your administrator has set preferred locations in System Settings.
 - Enter a different system timeout period.
 - Enter a different total of completed plates to show in the Run Status window, up to a maximum of 100.
3. Tap Save.
4. When the confirmation message appears, tap Yes to save the changes, and then tap OK.

Signing Out or Changing Users

While the software is running, one user can sign out and a different user can sign in.

Note: Where the screen is locked and the initial user left unsaved changes, an advisory prompt appears. The subsequent user has the option to save or discard the changes before completing the sign-out process.

To sign out

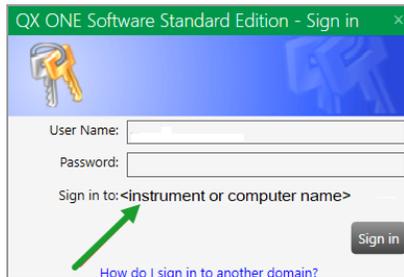
1. Tap the user name link in the upper-right corner and select Sign Out.
2. Tap Yes to confirm.

If there are unsaved changes, QX ONE Software displays a prompt.

- To discard the changes and proceed, tap Yes.
- To cancel the sign out, tap No. Save the changes, and then repeat Steps 1 and 2.

To change users

1. Tap anywhere in the lockout screen to display the Sign in window.
2. Enter a user name.
 - The domain name appears below the user login fields. If you are on the same domain as the previous user, you can enter your user name only.



- If the user is on a different domain, enter the domain name followed by a backslash and the user name.

<domain name>\<user name> *(for example, global\john_smith)

3. Enter the password and tap Sign in.

Functional Windows

This section briefly describes the functional areas in QX ONE Software. [Table 2](#) explains the primary window accessed from each tab.

Table 2. Window Tabs

Tab	Name	Purpose
	Add Plate	<ul style="list-style-type: none"> ■ Add a plate. ■ Access the Plate and Protocol Configuration window, where you can set up your plate for the run. ■ Recover a plate from a failed run
	Run Status	<ul style="list-style-type: none"> ■ In the top pane, view the runs in progress. ■ In the bottom pane, view up to 100 completed runs (successful, failed, recovered).
	Data Analysis	Access the Data Analysis and Gene Study modules.
	Template Setup	<ul style="list-style-type: none"> ■ Access windows where you can set up plate, thermal cycling protocol, and report templates. ■ Search for existing template files.
	System Utilities	<ul style="list-style-type: none"> ■ Access file storage information, event and maintenance logs, maintenance reports. ■ Archive data. <p>Note: Functionality available to you depends on your assigned user privileges.</p> <p>Important: Instrument calibration functionality is available only to the Bio-Rad service engineer.</p>
	Users and Preferences	<ul style="list-style-type: none"> ■ View the user privileges you have been assigned, and view and modify your personal preferences. ■ Create, edit, or remove QX ONE Software users (if you are assigned the Add/Manage Users privilege).
	Help	Access software version information, the End User License Agreement, the Bio-Rad website, open source software license information, and this document in PDF format.

Instrument Status Bar

An instrument status bar appears above all windows except the analysis displays, which open in a separate module.

The status bar on the touch screen displays the information and indicators shown below



LEGEND

- 1** Software name and version

- 2** Instrument name and status information

- 3** Number of runs in progress and runs available

- 4** Oil and waste levels

- 5** Open or close the Inbox or Outbox

- 6** Used and free disk space in gigabytes (GB)
Note: When QX ONE Software detects low disk space, the software displays an advisory prompt to archive data.

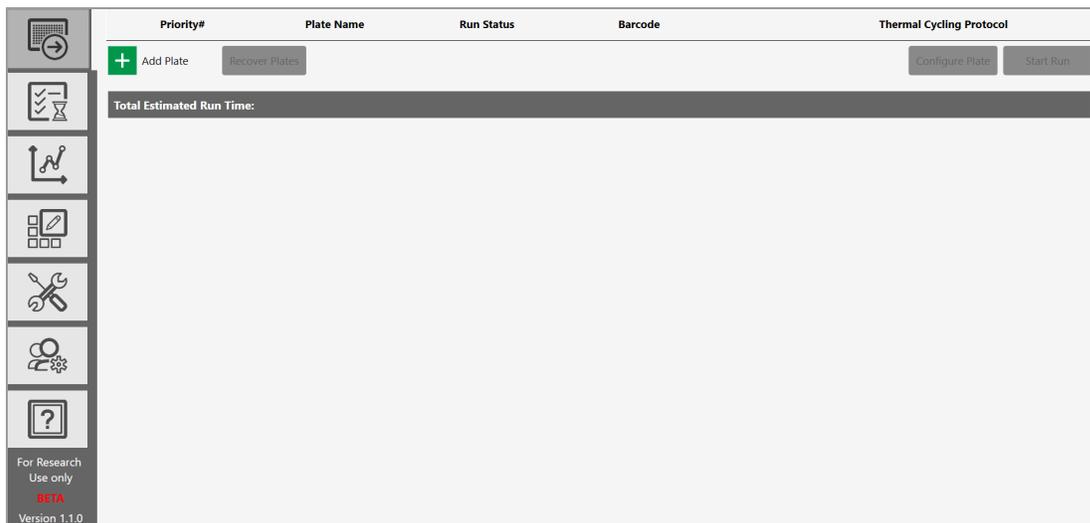
- 7** Name of the current user, along with the current date and time

- 8** Closes the QX ONE Software application

Add Plate Window

On the QX ONE Droplet Digital PCR System touch screen, QX ONE Software opens to the Add Plate window, where you can set up a plate for an experiment and start a ddPCR run. You can also recover a plate that experienced a failure during the run. For information, refer to the QX ONE Droplet Digital PCR System and QX ONE Software User Guide.

Note: If you are using the software on a computer other than the touch screen, the Add Plate window is not accessible and QX ONE Software displays the Data Analysis window by default.



From the Add Plate window you can

- Add plates in the software
- Recover a plate from a failed run
- Access the Plate and Protocol Configuration window to configure your plates and PCR protocols
- Run experiments on your plates and protocols

QX ONE Software enables each button sequentially (Add Plate, Configure Plate, Start Run) when you complete the prerequisite tasks. If the QX ONE Droplet Digital PCR System experienced a failure during the run, the Recover Plate button is enabled. For more information on adding and recovering plates, refer to the QX ONE Droplet Digital PCR System and QX ONE Software Instrument Guide.

Run Status Window

You can view the Run Status window on the QX ONE Droplet Digital PCR System touch screen.

Note: If you are using the software on a computer other than the touch screen, the Run Status window is not available.

The Run Status window displays information on

- The runs in process in the top pane
- Up to 100 completed runs for the current user in the bottom pane, including plate failures and recoveries

Note: You set the number of completed runs to show in your user preferences. For information, see [Managing Your Preferences on page 13](#).

For more information, refer to the QX ONE Droplet Digital PCR System and QX ONE Software Instrument Guide.

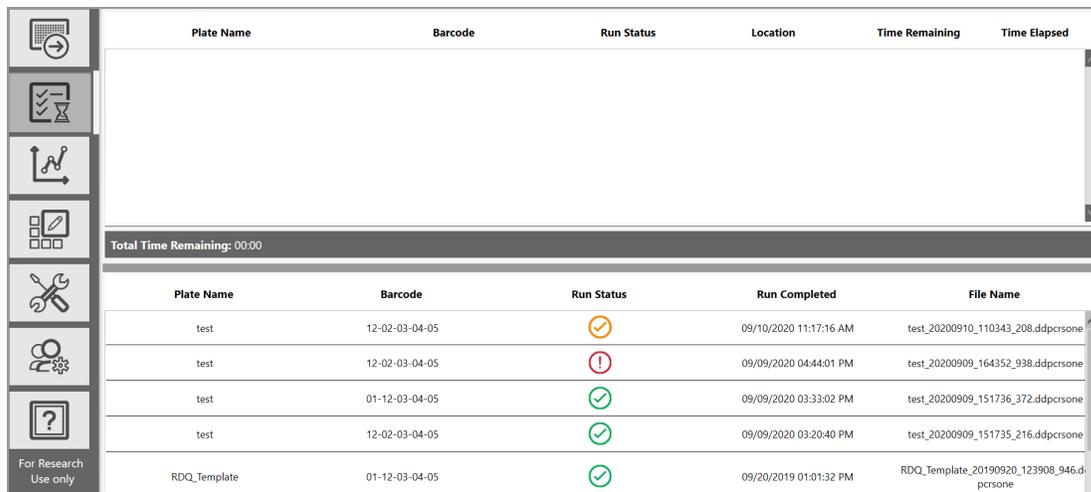
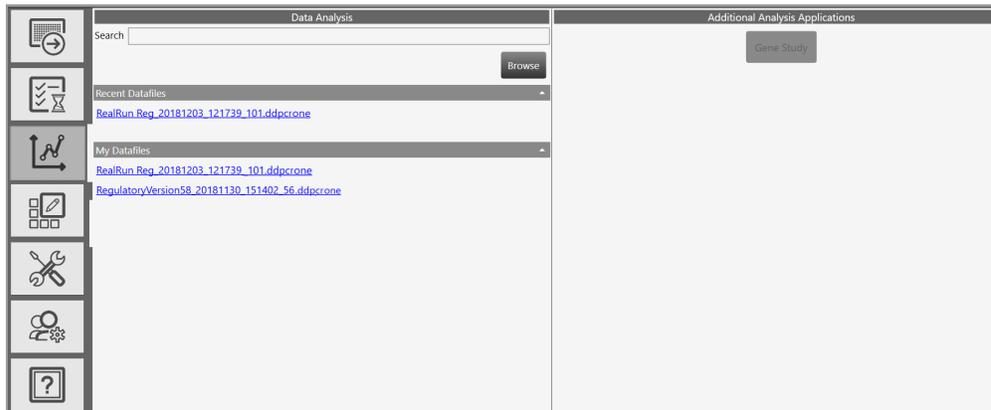


Plate Name	Barcode	Run Status	Location	Time Remaining	Time Elapsed
Total Time Remaining: 00:00					
Plate Name	Barcode	Run Status	Run Completed	File Name	
test	12-02-03-04-05	✓	09/10/2020 11:17:16 AM	test_20200910_110343_208.ddpcrsone	
test	12-02-03-04-05	⚠	09/09/2020 04:44:01 PM	test_20200909_164352_938.ddpcrsone	
test	01-12-03-04-05	✓	09/09/2020 03:33:02 PM	test_20200909_151736_372.ddpcrsone	
test	12-02-03-04-05	✓	09/09/2020 03:20:40 PM	test_20200909_151735_216.ddpcrsone	
RDQ_Template	01-12-03-04-05	✓	09/20/2019 01:01:32 PM	RDQ_Template_20190920_123908_946.dpcrsone	

Data Analysis Window

The Data Analysis window displays a list of analysis files created at the end of each run.



From the left panel, you can select files from the following storage areas to open in the Data Analysis module:

- **Recent Datafiles**

A list of files that you have opened.

- **My Datafiles or System Datafiles**

If the system administrator has

- Not set shared locations in System Settings, all data files are automatically saved to the file path specified in the user's preferences.
- Set shared locations in System Settings, all data files are automatically saved to the path specified for Datafile Storage Location. Preferred locations override individual user preferences.

Important: For either choice, the specified path must be a local folder on the QX ONE Droplet Digital PCR System touch screen computer.

Note: For information on files types you can open and save, see [Compatible File Types on page 25](#).

- From the right panel, you can access the Gene Study module. For information on Gene Studies, see [Gene Study Module on page 123](#).

Template Setup Window

The Template Setup window allows you to

- Create new plate, thermal cycling protocol, and report templates
- Search for existing templates
- Modify existing templates

If you are assigned the Create New Template user privilege, you can save new or modified templates for reuse. You can save templates to a personal or system folder (My Templates or System Templates, respectively), or you can save them to the Shared Templates location.

Note: If your system administrator has specified Preferred Locations in the System Settings window, the System Templates location overrides the folder specified in your user preferences. However, you have the option to save the template to the shared file location instead.

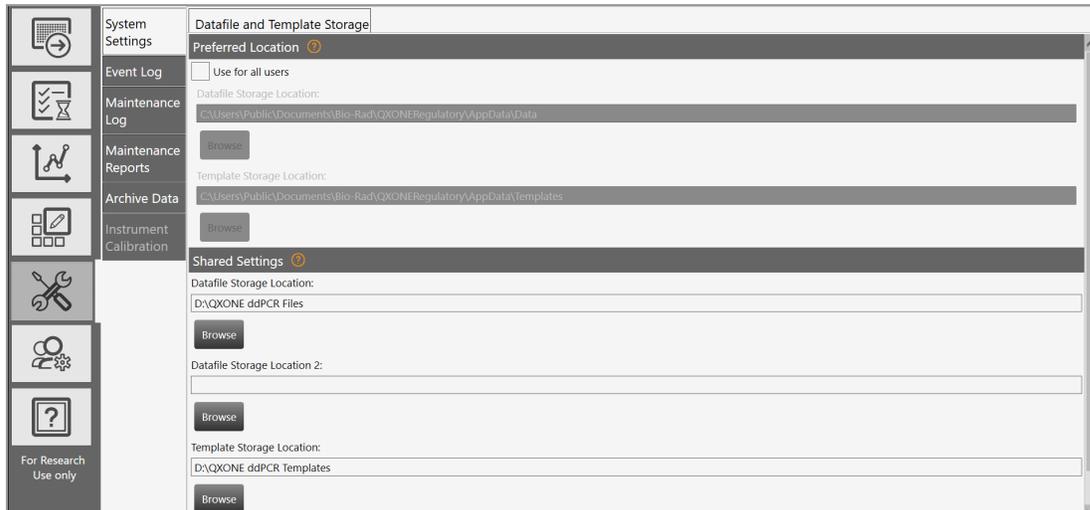


For information on creating or editing templates, see

- [Creating or Editing Plate Templates on page 27](#)
- [Creating or Editing Thermal Cycling Protocol Templates on page 43](#)
- [Creating Report Templates on page 117](#)

System Status Window

The System Status window displays information about your QX ONE Droplet Digital PCR System and QX ONE Software.



Depending on your permission level, you can

- View or change shared storage folder locations
 - Important:** System administrators can override individual storage locations and set preferred locations for data files and templates. Only administrators can establish or change these locations.
- View event and maintenance logs, and maintenance reports
- Archive raw data

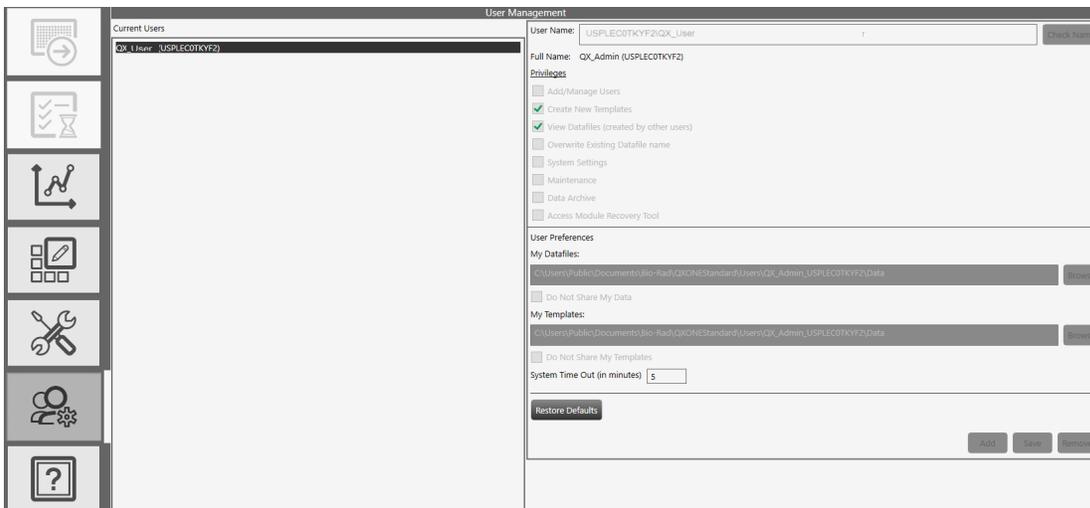
Note: The Instrument Calibration tab is enabled only for the Bio-Rad service engineer user account.

User Setup and Preferences Window

The User Setup and Preferences window displays the privileges that a software administrator, if assigned the Add/Manage Users privilege, can assign to each user, and personal preferences that can be set or modified by users and administrators.

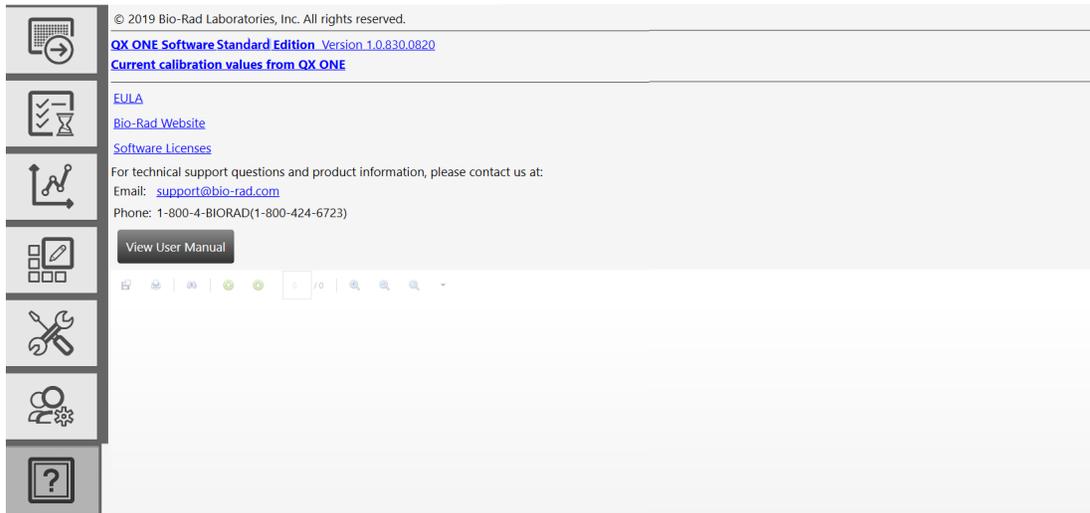
Note: Users who are not assigned any of the privileges listed can still execute runs and use the Analysis module.

For information on user privileges, see [Viewing Your User Privileges on page 11](#) and for information on user preferences, see [Managing Your Preferences on page 13](#).



Help Window

The Help window provides links to important information about QX ONE Software and Bio-Rad Laboratories, Inc.



Select the Help tab to display links to

- Software and firmware version information
- End user license agreement (EULA)
- Bio-Rad website
- Open-source software license information
- QX ONE Software User Guide (this document)

Touch Screen Differences

As shown in [Table 3](#), QX ONE Software functionality varies between the QX ONE Droplet Digital PCR System touch screen and a standalone computer.

Table 3. Touch Screen Differences

Feature	Instrument Touch Screen	Standalone Computer
Gene Study	Not available	Available
Files open	Only one file at a time	Up to 5 files concurrently
Run Setup	Enabled	Disabled
Run Status	Enabled	Disabled
Maintenance Log	Enabled	Disabled
Archive Data	Enabled	Disabled

Compatible File Types

This section describes the file types you can open in QX ONE Software.

The following applies if your organization is using more than one edition of the software:

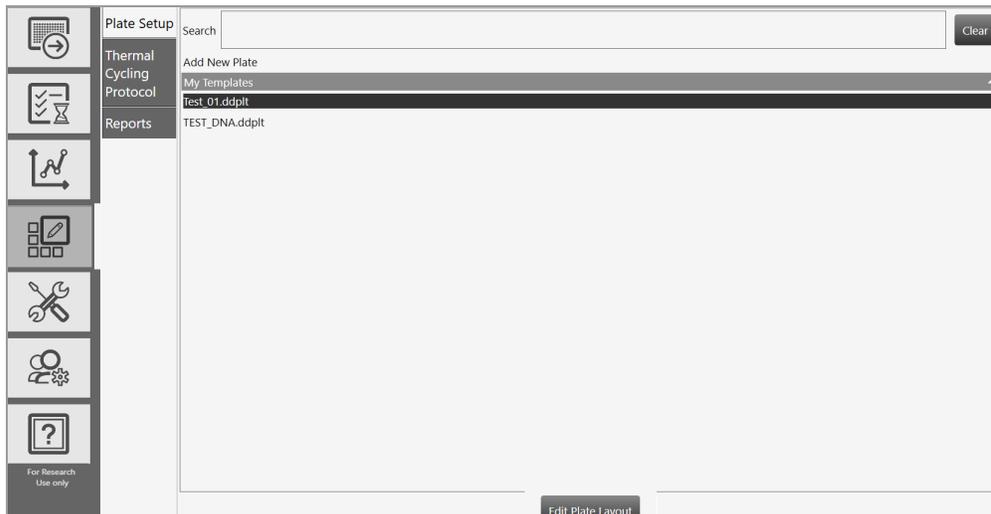
- Analysis files created in QX ONE Software Regulatory Edition open only in Regulatory Edition.
- Analysis files created in QX ONE Software Standard Edition open only in Standard Edition.

Table 4. Compatible software file types

File Type	Extension	Details
Plate	.ddplt	Plate template file containing setup details to perform experiments; this file type opens in the Plate Editor.
Protocol	.ddthp	Protocol template file containing setup details to perform experiments; this file type opens in the Protocol Editor.
Data	.ddpcrone .ddpocr	Contains the results of an experiment run and data analysis performed in QX ONE Software, Standard Edition; this file type opens in the Analysis module. Note: You can open a .ddpocr file in the QX ONE Software Analysis module, but the file is saved as a .ddpcrone file.
Data	.qlp	File type from QuantaSoft versions, for two-channel experiment runs and data analysis; this file type opens in the Analysis module. Note: You can open .qlp files in QX ONE Software, but if you make any changes, the software prompts you to save the file as a .ddpcrone file.

Chapter 3 Creating or Editing Plate Templates

If you are assigned the Create New Templates user privilege, you can save a new or reconfigured plate as a template for reuse from the Add Plate and Template Setup windows.



Templates that are available to you appear in the default view of the Template Setup tab, as follows:

- Templates you created and saved, which are saved to one of the following paths:
 - The path specified in your user preferences; these appear under My Templates
 - The path specified under Preferred Locations in System Settings, if your system administrator has specified the path for all users, these appear under System Templates
- **Important:** If your administrator has specified the Preferred Location for all users, the path overrides the path specified in user preferences. Preferred locations can be enabled only by users with the System Settings user privilege.
- Templates created and saved by any user and designated as shared; these appear under Shared Templates.

Plate Editor Window

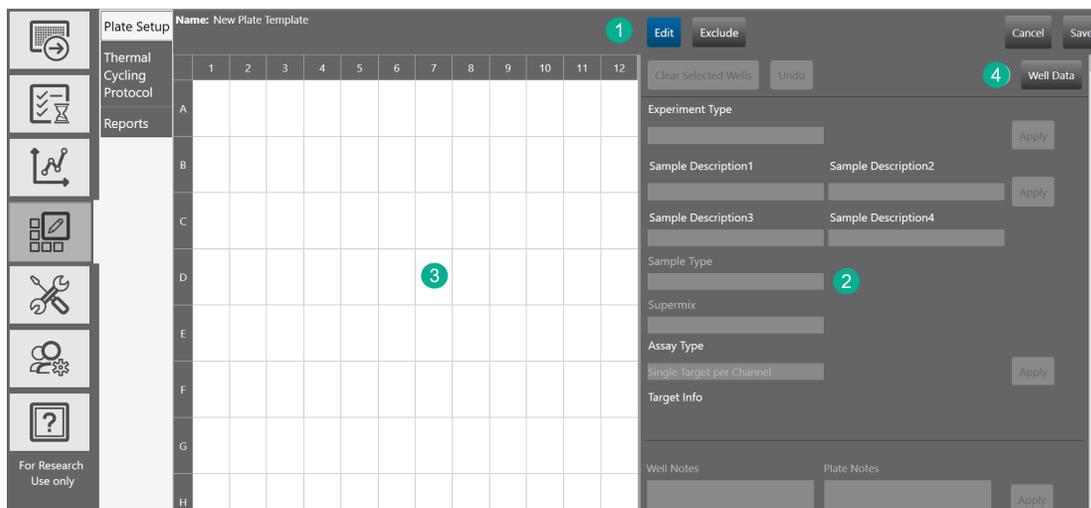
Use the Template Setup Plate Editor to

- Create new or edit existing plate template files
- Save new or modified templates for reuse (with Create New Templates permission only)
- Define or change experiment type, sample type, supermix, and assay type for each well

Tip: Except for the supermix, which you must select pre-run, you can configure the plate experiment parameters before, during, or after a run.

- Set reference targets and control samples
- Exclude wells from droplet generation and droplet reading, or just droplet reading
- Add applicable plate or well notes

The Plate Layout view is the default template view.



LEGEND

1 You can access the Edit and Exclude buttons from the top of the screen.

2 The right pane contains the interface for defining your experiment parameters.

LEGEND , CONTINUED

- 3 The left pane displays the plate grid and configuration information in each well.
- 4 The Well Data button toggles to the Well Data table, where you can see your plate setup in a tabular format, and then back to the Plate Editor. When the Well Data table is displayed, the button name changes to Plate Layout.

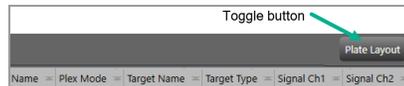


Plate Editor Tools

Use the buttons described in [Table 5](#) to define your experiment in the Plate Template Editor.

Table 5. Plate Editor Buttons

Button	Description
Edit	<p>Allows you to</p> <ul style="list-style-type: none"> ■ Select wells or clear well selections ■ Set the experiment type, sample name and type, supermix, and assay type ■ Set the target name, type, and fluorophore for each channel ■ Add well or plate notes <p>Note: This button is enabled by default when you open the window.</p>
Exclude	Allows you to exclude wells from droplet generation and droplet reading, or from droplet reading only
Cancel	Allows you to cancel and return to the Template Setup window
Save	Opens a dialog box where you can enter a file name and specify a storage location
Clear Selected Wells	Allows you to delete information from selected wells
Undo	<p>Allows you to reverse your last action</p> <p>Note: This button is enabled only after you have applied at least one experiment parameter (for example, the selection for Experiment Type).</p>
Well Data or Plate Layout	Toggle button to access the Well Data table and return to Plate Layout view
Apply	Applies your entries and choices for selected wells

Experiment Types

QX ONE Software offers seven experiment types from which you can choose, each enabling different assay options and analysis tools. [Table 6](#) briefly explains each experiment type.

Tip: You can assign multiple experiment types within the plate, but you can assign only one experiment type to each well.

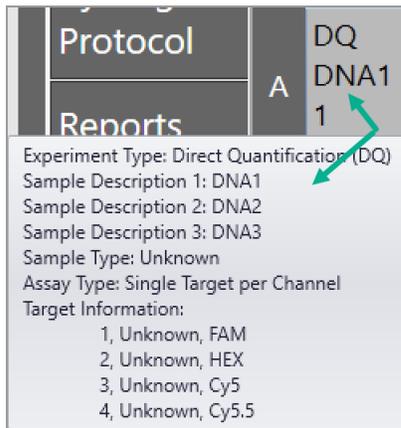
Table 6. Experiment types

Type	Description
Direct quantification (DQ)	DQ uses absolute quantification to determine the concentration (copies/ μ l) of target DNA copies in a sample. DQ measures the number of positive and negative droplets for each target in a well based on their fluorescence amplitude, and uses a Poisson algorithm to calculate the starting concentration of each target DNA molecule. Note: The ABS experiment type created in QuantaSoft Software versions 1.4 through 1.7 is automatically mapped into the software as a DQ experiment type.
Copy number variation (CNV)	CNV determines concentration (as with DQ) and calculates the copy number of an unknown (CNV) target relative to a known reference or references within the same well.
Mutation detection (MUT)	MUT determines concentration (as with DQ) and calculates the fractional abundance of an unknown mutant present at a low frequency in a wild-type background.
Rare event detection (RED)	RED determines the concentration of a known mutant or a rare target species relative to a given reference species within a large pool of background DNA.
Drop-off (DOF)	DOF determines absolute quantification of targets for assays designed to detect non-wild type sequences, such as indels and genome edits; DOF is designed to support an assay strategy where one probe counts all alleles and one “drop-off” probe sits on top of a predicted cut site.
Gene expression (GEX)	GEX determines concentration (as with DQ) and calculates relative expression levels of an unknown target relative to a known reference or references within the same well.
Residual DNA quantification (RDQ)	RDQ provides a precise method for residual host cell DNA quantification. Note: Users can enter a conversion factor to calculate the mass for a well, up to three decimals. The software displays the result in the Molecular Weight column of the Data Table.

Sample Descriptions

QX ONE Software allows you to enter descriptive words or phrases for your sample in up to four fields per well. Descriptors can include information such as research type, dilution factors, and so forth.

Tip: Only the information from the first field appears in the Plate Editor layout. However, you can see entries in the second, third, and fourth fields in the Well Data table, or you can pause on a well to display a tool tip, as shown in the following graphic.



Sample Types

QX ONE Software offers four sample types:

- Unknown
- NTC (no template control)
- Positive control, where one or more targets are expected
- Negative control, where no response is expected

Tip: You can assign multiple sample types within the plate, but you can assign only one sample type to each well.

Supermixes

Important: Use of unapproved supermixes can harm the instrument and void the warranty.

QX ONE Software offers five PCR supermixes. Each supermix is optimized to deliver maximum PCR efficiency and sensitivity for the amplification and detection of DNA and RNA targets.

- **ddPCR Multiplex Supermix** — For use in nucleic acid sample preparations to amplify and detect DNA targets in probe-based experiments involving multiple targets

Note: Bio-Rad recommends using ddPCR Multiplex Supermix for Cy5 and Cy5.5 probes assays.

- **ddPCR Supermix for Probes (No dUTP)** — For use in nucleic acid sample preparations, with sensitivity for the amplification and detection of DNA targets using hydrolysis probe-based assays
- **ddPCR Supermix for Probes** — For use in sample preparation with uracil N-glycosylase (UNG) decontamination protocols to prevent the reamplification of carryover PCR products between experiments
- **ddPCR Supermix for Residual DNA Quantification** — For use in residual DNA detection
- **One-Step RT ddPCR Advanced Kit for Probes** — For use in absolute quantification of target RNA molecules

Assay Types and Fluorophores

QX ONE Software offers a total of five assay methods, which are explained in [Table 7](#).

Table 7. Assay types

Assay type	Description
Single target per channel	<p>Method assuming up to four probe colors (FAM, HEX/VIC, Cy5 and Cy5.5) and up to four targets per well, with a single target detected per channel. This is the default assay type, with the maximum four rows displayed. You can delete up to three rows.</p> <p>This assay type is available for the DQ, CNV, MUT, RDQ, RED, and GEX experiment types.</p>
Amplitude multiplex	<p>Method to increase multiplexing up to eight targets per well, with one or two targets detected per channel. The maximum of eight rows are displayed. You can delete up to seven rows.</p>
Probe mix triplex	<p>Triplex mode that allows two groups of three targets each. Group 1 has 1 target in FAM, 1 target in HEX/VIC, and 1 target in both channels. Group 2 has 1 target in Cy5, 1 target in Cy5.5, and 1 target in both. You can delete a group, but not the individual targets within the group.</p> <p>Note: The channels within each group are not interchangeable therefore FAM is always paired with HEX/VIC and Cy5 with Cy5.5.</p>
Advanced classification	<p>Method to increase multiplexing rate up to 10 targets per well in any combination of method targets detected per channel. This method requires manual classification of droplets.</p> <p>This assay type is available for the DQ and RED experiment types.</p>
Basic drop-off	<p>This mode allows two groups of two targets each. Group 1 is shown by default, with one target in FAM <u>and</u> HEX/VIC and one target in FAM <u>or</u> HEX/VIC.</p> <p>This assay type is available only for the DOF experiment type.</p> <p>You can add one more group of two targets, with one target in Cy5 <u>and</u> Cy5.5, one target in Cy5 <u>or</u> Cy5.5. You can delete a group, but not the individual targets in the group.</p> <p>Note: The channels within each group are not interchangeable therefore FAM is always paired with HEX/VIC and Cy5 with Cy5.5.</p>

The experiment type you select determines the available assay methods. [Table 8](#) displays the sample type associated with each assay method.

Table 8. Assay types by experiment type

Assay type	Experiment type						
	DQ	CNV	MUT	DOF	GEX	RDQ	RED
Single target per channel	✓	✓	✓		✓	✓	✓
Amplitude multiplex	✓	✓	✓		✓	✓	✓
Probe mix triplex	✓	✓	✓		✓	✓	✓
Advanced classification	✓						✓
Basic drop-off				✓			

QX ONE Software automatically populates the fluorophore in each channel for each target but you can change the default selections

Use [Table 9](#) to select from the fluorophore options for each assay method listed.

Table 9. Fluorophore options

Assay method	Signal Ch1	Signal Ch2	Signal Ch3	Signal Ch4
Single target per channel, for 1 to 4 targets	FAM	HEX	Cy5	Cy 5.5
	None	VIC None	None	None
Amplitude multiplex, for 1 to 8 targets	FAM Hi	HEX Hi	Cy5 Hi	Cy5.5 Hi
	FAM Lo	HEX Lo	Cy5 Lo	Cy5.5 Lo
	None	VIC Hi	None	None
		VIC Lo None		
Probe mix triplex, for 6 targets	FAM	HEX	Cy5	Cy5.5
	None	VIC None	None	None
Advanced classification	FAM 1-10	HEX 1-10 VIC 1-10	Cy5 1-10	Cy5.5 1-10

Opening a New Plate Template

You can open a blank plate template from either the Template Setup window or the Add Plate window.

- Use Template Setup if you are creating one or more templates to store for future use.
- Use Add Plate if you are creating a template to correspond to a plate you are about to run.

To open a new plate template from the Template Setup window

1. Tap the Template Setup tab.



2. Tap Plate Setup.
3. Tap Add New Plate.
4. Tap Edit Plate Layout.

The Plate Editor opens, displaying a blank plate template.

5. To set up your plate, see [Template Setup — Setting Up Your Wells on page 37](#).

To open a new plate template from the Add Plate window

1. Tap the Add Plate tab.



2. Tap the + icon next to Add Plate and follow the prompts to insert a plate into the instrument.
3. When the software enables the Configure Plate button, tap Configure Plate.

The Plate Configuration window opens to the Plate Information tab by default.

4. Under Plate Template, tap Create New.

The template Plate Editor opens, displaying a blank plate layout.

5. To set up your plate, continue to [Template Setup — Setting Up Your Wells on page 37](#).

Opening an Existing Plate Template

You can open an existing plate template from either the Template Setup window or the Add Plate window.

- Use Template Setup if you are editing one or more templates to store for future use.
- Use Add Plate if you are editing a template to correspond to a plate you are about to run.

To open an existing plate file from the Template Setup window

1. Tap the Template Setup tab.



The Plate Setup window appears by default and a list of available plate templates appears.

2. Select a template and tap Edit Plate Layout.

Tip: You can also double-click the template file to open it.

The Plate Editor opens, displaying the plate template as it is currently configured.

To make changes to the plate, continue to [Template Setup — Setting Up Your Wells on page 37](#).

To open an existing plate template from the Add Plate window

1. Tap the Add Plate tab.



2. Tap the + icon next to Add Plate and follow the prompts to insert a plate into the instrument.
3. When the software enables the Configure Plate button, tap Configure Plate.

The Plate Configuration window opens to the Plate Information tab by default.

4. Under Plate Template, tap the dropdown arrow and select a template from the list.

The template Plate Editor opens, displaying the configured layout.

You can run the plate without changing the template, or you can modify the configuration. To make changes to the plate, continue to [Template Setup — Setting Up Your Wells on page 37](#).

Template Setup — Setting Up Your Wells

When you open the Plate Editor from the Template Setup tab, the Edit button is selected by default. You can assign different experiment parameters to each well or groups of wells.

The screenshot shows the Plate Setup interface. On the left is a sidebar with icons for Thermal Cycling Protocol, Reports, and a 'For Research Use only' section. The main area is a grid with 12 columns and 7 rows (A-G). The first two columns of each row contain the text 'MUT DNA1 1'. To the right of the grid is a configuration panel with the following sections:

- Experiment Type:** Mutation Detection (MUT) (with an Apply button)
- Sample Descriptions:** Sample Description1 (DNA1), Sample Description2 (DNA2), Sample Description3, and Sample Description4 (with an Apply button)
- Sample Type:** Unknown (dropdown)
- Supermix:** ddPCR Supermix for Probes (dropdown)
- Assay Type:** Single Target per Channel (dropdown with an Apply button)
- Target Info:** A table with columns: Target Name, Target Type, Signal Ch1, Signal Ch2, Signal Ch3, Signal Ch4.

Target Name	Target Type	Signal Ch1	Signal Ch2	Signal Ch3	Signal Ch4
1	Unkn	FAM	None	None	None
2	Unkn	None	HEX	None	None
3	Unkn	None	None	Cy5	None
4	Unkn	None	None	None	Cy5.5

For each well, or group of wells, you must

- Choose an experiment type
- Select a sample type
- Select a supermix
- Select an assay method

Optionally, you can

- Enter up to four sample descriptors for each well
- Modify fluorophore assignments
- Add notes for each well
- Add notes for the plate

If you are creating a new template, the grid is blank. If you are editing an existing template, previously saved information appears when you select one or more configured wells.

To define well parameters

1. Select a well or group of wells.
2. From the Experiment Type dropdown list, select a new or different experiment type. For more information, see [Experiment Types on page 30](#).
3. In the Sample Description fields, enter or change up to four words or phrases that describe the sample. For more information, see [Sample Descriptions on page 31](#).
4. From the Sample Type dropdown list, select a new or different sample type. For more information, see [Sample Types on page 31](#).
5. From the Supermix dropdown list, select or change the supermix. For more information, see [Supermixes on page 32](#).
6. The Assay Type field populates automatically based on your experiment selection. To change it, select a different assay type from the dropdown list. For information, see the fluorophore options in [Assay Types and Fluorophores on page 33](#).

Note: Available assay types vary, depending on the selected experiment type.

7. (Optional) Under Target Info, change the fluorophore assigned to a channel.

The fluorophore information populates automatically for each target, with the maximum number of rows allowed for the assay method, but you can modify the default fluorophore assignments, if applicable.
 - a. Tap the field dropdown arrow and select an alternative.
 - b. To delete a row, tap the minus (-) icon next to the target.
 - c. To re-add a row, tap the plus (+) icon.
8. (Optional) To clear one or more wells at any time, select the wells and tap Clear Selected Wells.
9. Tap Apply.

Note: You must tap Apply for the software to recognize your new or edited information, and before you can save the template. Once applied, you can pause on a well to see the corresponding information in a tool tip.

	DQ	DQ	DQ	DQ	DQ	DQ
	NTC/1cpd	0.125 cpd	0.25 cpd	0.5 cpd	1 cpd Ch1	2 cpd Ch1
E	HSV	Experiment Type: Direct Quantification (DQ)			HSV-1	HSV-1
	B2N	Sample Description 1: 0.5 cpd Ch1/1cpd Ch2			B2M-Hex	B2M-Hex
	Sample Type: Unknown					
	Supermix: ddPCR Supermix for Probes					
	Assay Type: Single Target per Channel					
	Target Information:					
	NTC	HSV-1, Unknown, FAM			cpd Ch1	2 cpd Ch1
F	HSV	B2M-Hex, Unknown, HEX			HSV-1	HSV-1
	B2M-Hex	B2M-Hex	B2M-Hex	B2M-Hex	B2M-Hex	B2M-Hex

10. Exclude wells in the plate from droplet generation and droplet reading, or from droplet reading only. For information, see [Template Setup – Excluding Wells](#).

Important: The QX ONE Droplet Digital PCR System processes all 96 wells in the plate by default. If you have filled fewer than 96 wells with sample or buffer, you should exclude the remainder.

11. If you are finished with your plate configuration, tap Save in the upper-right corner and then in the Save dialog box, enter a new name for the template and tap Save again.

Note: You must save the template with a new name. You cannot overwrite existing templates. If you tap the Save button before the plate layout is complete, the file closes and you must reopen it from the saved template location.

Template Setup – Excluding Wells

Optionally, you can exclude wells from the droplet digital PCR run.

Important: The QX ONE Droplet Digital PCR System processes the entire 96-well plate by default. If you have added your sample to fewer than 96 wells in the plate, you must exclude the remainder.

The GCR96 cartridges that are inserted into the instrument contain six separate sections, each with two rows of eight wells each. The QX ONE Droplet Digital PCR System automatically generates droplets by section rather than by well or by individual column. In the QX ONE Software well exclusion design, the first two columns represent the first section, the second two columns the second section, and so forth.

When you select Droplet Generation and Droplet Reading, the software allows you to exclude each section from both processes, but if you try to exclude an odd number of columns, QX ONE Software automatically selects the remaining column.

When you select Droplet Reading, the software allows you to exclude any well or group of wells from the droplet reading phase.

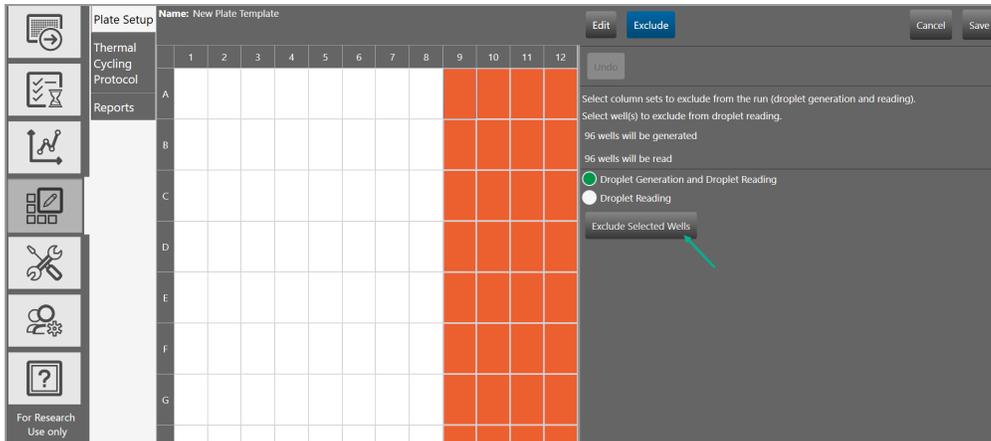
Wells excluded from droplet generation and droplet reading are displayed with a criss-cross pattern.

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												

Wells excluded from droplet reading are identified by a diagonal pattern.

To exclude columns from droplet generation and droplet reading

1. Select one or more column pairs to exclude, and then tap Droplet Generation and Droplet Reading.



Note: If you selected an odd number of columns, the software automatically selects the remaining column to represent a section. You can exclude that column from droplet reading only.

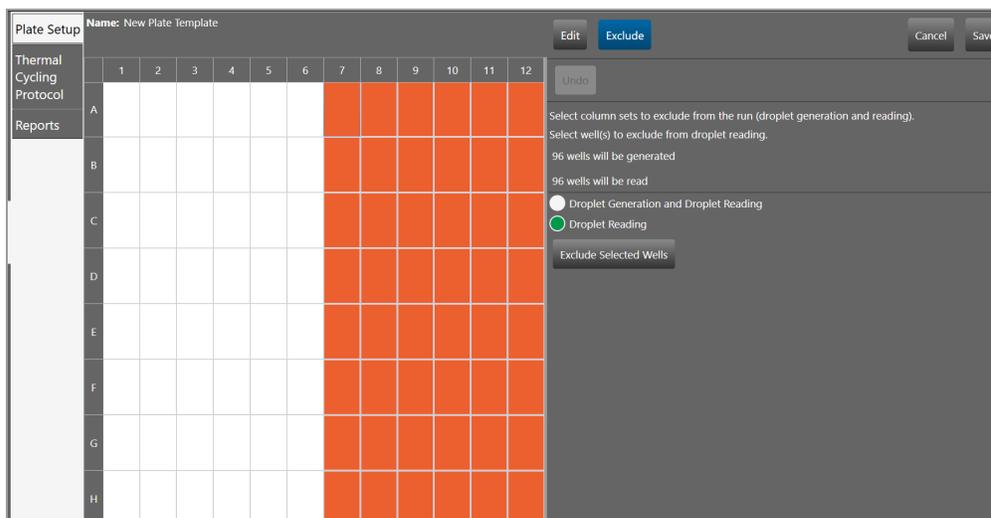
2. Tap Exclude Selected Wells.

To exclude wells from droplet reading

1. Select one or more wells to exclude, either individually or in groups, and then tap Droplet Reading.

Tip: To exclude multiple wells, press the CTRL key, and then select the wells to exclude.

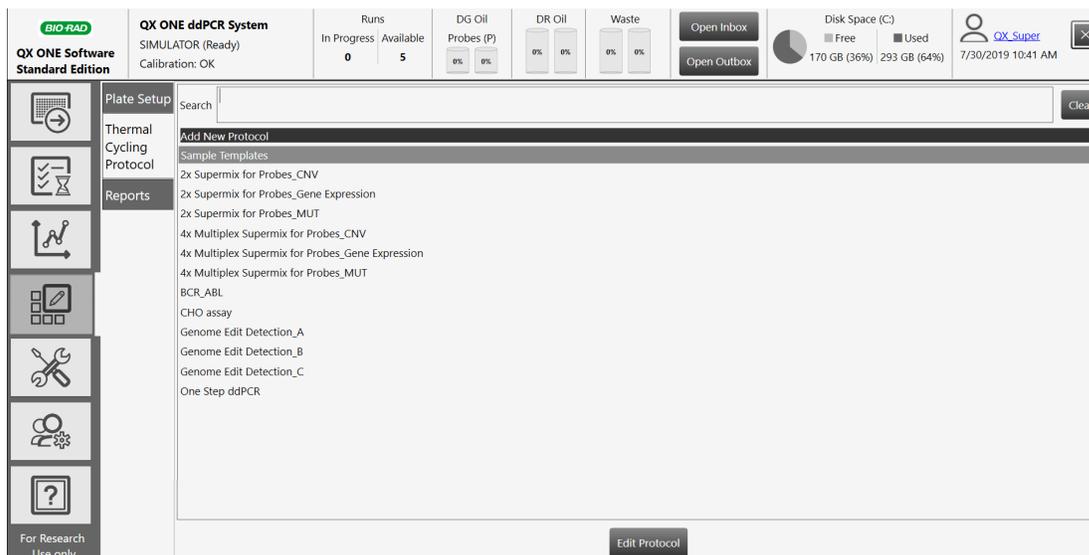
2. Tap Exclude Selected Wells.



Note: The Exclude Selected Wells button is a toggle button. If you select wells that have been excluded, the button name changes to Include Selected Wells. If you excluded one or more wells that should be read, you can select the wells and then tap the button to include them.

Chapter 4 Creating or Editing Thermal Cycling Protocol Templates

From the Templates tab you can access the Protocol Templates window, where you can create a new or open an existing protocol template in the Protocol Editor window. Using the Protocol Editor, you can create custom protocols that are specific to the thermal cycling phase of the QX ONE Droplet Digital PCR System.



Thermal Cycling Protocol Editor Window

The Thermal Cycling Protocol Editor includes

- Standard protocol controls to quickly create or edit protocols
- Ability to quickly calculate a gradient for a specific temperature range
- Ability to quickly calculate run time for the plate type
- Ability to edit protocol steps
- Ability to save protocols for reuse

By default, the window opens to a generic 4-step protocol

The screenshot shows the Thermal Cycling Protocol Editor window. At the top, the 'Protocol Name' is 'New Protocol' (marked with a green circle 1) and the 'Estimated Run Time' is '1:43:42'. There are 'Cancel' and 'Save' buttons on the right. The left sidebar contains icons for 'Plate Setup', 'Thermal Cycling Protocol', 'Reports', and 'For Research Use only'. The main area is divided into two panes. The upper pane (marked with a green circle 2) contains 'Step Options' with fields for Temperature (95.0 °C), Time (10:00), Ramp Rate (2.0 °C/sec), Increment, and Extend. It also has a 'Gradient' section with buttons A-H and a 'Goto' section with 'Goto Step Number' and 'Number of repeats' fields. The main pane (marked with a green circle 3) displays a graphical representation of the protocol with four steps. Step 1 is at 95.0°C for 10:00 (marked with a green circle 4). Step 2 is at 94.0°C for 0:30. Step 3 is at 55.0°C for 1:00. Step 4 is labeled 'GOTO 2' and '39 X'. A red arrow points from the 'GOTO 2' label back to the start of step 1.

LEGEND

- 1 The top section provides quick access to the name of the protocol, estimated protocol run time, and save or cancel functions.
- 2 The upper pane displays the protocol controls that you can use to customize the protocol.
- 3 The main pane displays a graphical representation of the protocol.

- 4 The lower pane displays the following toolbar commands:



- The trashcan command deletes the selected step.
- The plus sign to the left of the trashcan adds a step before the selected step.
- The plus sign to the right of the trashcan adds a step after the selected step.

Opening a New Thermal Cycling Protocol Template

QX ONE Software provides the following two options to open a new protocol template:

- From the Template Setup window
- From the Add Plate window

To open a new protocol template from the Template Setup window

1. In the Template Setup window, select the Thermal Cycling Protocol tab.

The Thermal Cycling Protocol window appears, displaying a list of sample and custom protocol templates.

2. Tap Add New Protocol.

The Protocol Editor window opens, displaying the default 4-step protocol.

To open a new protocol template from the Add Plate window

1. In the Plate Setup window, tap Add Plate.
2. After the plate is loaded, tap Configure Plate.

The Plate Setup window opens, displaying the Required Plate Information window.

3. In the Thermal Cycling Protocol pane, tap Create New.

The Protocol Editor window appears, displaying the default 4-step protocol.

Opening an Existing Thermal Cycling Protocol Template

QX ONE Software provides sample thermal cycling protocol templates that you can edit and save as new templates. You can also edit and save a custom protocol as a new protocol template.

To open an existing protocol from the Template Setup window

1. Select the Thermal Cycling Protocol tab.

The Thermal Cycling Protocol window appears, displaying a list of sample protocol templates.

2. Locate the protocol of choice.
3. Select the protocol and tap Edit Protocol at the bottom of the window.

The protocol opens in the Thermal Cycling Protocol Editor window.

4. (Optional) tap Save to save the protocol with a new name or in a new storage location.

To open an existing protocol from the Add Plate window.

1. In the Plate Setup window, tap Add Plate.
2. After the plate is loaded, tap Configure Plate.

The Plate Setup window opens, displaying the Required Plate Information window.

3. In the Thermal Cycling Protocol pane, tap the Choose Existing dropdown arrow, and then select an existing protocol.
4. Click the “i” button to the right of the dropdown arrow.

The Protocol Editor window opens, displaying the selected protocol in a pop-up window.

5. Click Edit.
6. Continue to [Adding Steps to a Protocol Template on page 47](#).

Adding Steps to a Protocol Template

To add a step to a protocol template

1. In the Protocol Configuration window, determine where to insert the new step. Step 1 is selected by default.



You can add a step before or after an existing step.

2. On the graph, select a step and do one of the following:
 - To insert the new step before the selected step, tap the + icon to the left of the trash can.



- To insert the new step after the selected step, tap the + icon to the right of the trash can.

3. Do one of the following:

- To make the step a gradient step, tap the Gradient checkbox.
- To make the step a goto (repetition) step, tap the Goto checkbox.

Note: If the step type can be converted to Goto, the Goto checkbox is enabled.

4. Do one of the following:

- To adjust the values in the protocol step options before saving, see [Adjusting Step Options on page 48](#).

- To save the protocol with the default step values, tap Save. In the Save dialog box, enter a name for the protocol and tap Save again.

Note: The protocol closes after you save it. You can reopen the protocol from the Protocol Templates window.

Adjusting Step Options

QX ONE Software enables you to change the step options in an existing or added step to customize the protocol. You can set values for the following step types:

- Temperature
- Gradient
- Goto

Important: You can save the changes to the steps as you go, or save them all at once when you are finished with your modifications.

To configure a temperature step

1. From the graph, select the temperature step containing the parameters to be changed.
Temperature steps contain a single temperature line.
2. Under Step Options, change or enter the following values where applicable:
 - Temperature (°C)—target temperature (0 to 100°C) for the step
 - Time (HH:MM:SS)—hold time (0–64800 sec) for the step
The hold time is the duration after the block has reached the target temperature.
 - Ramp Rate (°C/sec)—(Optional) the rate (0.1–5°C per second) at which the instrument heats or cools to achieve the target temperature.
 - Increment (°C/cycle)—(Optional) the degrees to increase or decrease the step's temperature with each successive cycle. The entry must be between -10 and 10°C
 - Extend (sec/cycle)—(Optional) amount of time (-60–60 sec) to increase or decrease the step's hold time with each successive cycle.

To configure a gradient temperature step

1. From the graph, select the gradient step containing the parameters to be changed.
2. Under Step Options, change or enter the following values where applicable:
 - Temperature (°C)—temperature (30.0–99.0°C) of the last row in the plate.
 - Time (HH:MM:SS)—hold time (0–64800 sec.) for the step.

Hold time is the duration after the block has reached the target gradient.

Note: Note: Setting the time to 0 sec instructs the instrument to hold at the current step until resumed manually from the instrument’s user interface.

- Extend (sec/cycle)—(Optional) amount of time (-60–60 sec) to increase or decrease the step’s hold time with each successive cycle.
- Range—(1°C–16°C) range between the lowestr temperature and the highest temperature.

Note: The temperatures for rows A through H automatically populate with updated values when you change the temperature or temperature range. You can also change the temperature for each row, and the temperature and range are automatically updated.

To configure a goto (repetition) step

A Goto step loops back to an earlier step and repeats a sequence.

1. From the graph, select the Goto step containing the parameters to be changed.
2. Under Step Options, change the following value, if applicable:
 - Goto step number—step to which to return to repeat a sequence
 - Number of repeats—number of times to repeat the sequence

Deleting Steps from a Protocol Template

Important: You cannot undo this function. Take care when deleting steps.

To delete a step in the protocol

1. Select the step in the graph.
2. Tap Delete Step.
3. Save the protocol.

Chapter 5 Data Analysis Module Overview

As the instrument reads droplets in each well, the QX ONE Droplet Digital PCR System collects raw data. At the end of each run, QX ONE Software uses the raw data to produce a data file, which contains analysis information based on the number of positive and negative droplets identified for each target in each sample. The fraction of positive droplets is fit to a Poisson algorithm to determine the starting concentration of the target DNA molecule in units of copies/ μ l input.

When you open the data file in the Analysis module, you can view the data with the automatic analysis thresholds and calculations, or you can choose different options to recalculate, replot, or recolor the results.

When you select the Data Analysis tab from the touch screen, you can access the Data Analysis module. When you select the tab from the software installed on a standalone computer, you can also access the Gene Study module.



The Data Analysis module functionality allows you to

- View your droplet data in 1D, 2D, and 3D plots
- Analyze multiple targets within a well using amplitude multiplexing and probe mixing strategies
- Detect genomic editing events and non-wild type events with a “drop-off assay” analysis option
- Visualize and export data with improved flexibility
- Generate reports for different analysis types

The Gene Study module allows you to

- Collect data by adding one or more data files
- View gene expression calculations across multiple experiments

Note: The maximum number of samples you can analyze in a gene study is limited by the size of the computer's RAM and virtual memory.

This section summarizes the analysis functionality, and also describes the general options governing your chart displays. More detailed information, including specific information and uses for each analysis window, as well as information on data calculations, is contained in [Chapter 6, Data Analysis Methodology](#).

For information on analysis reporting, see [Report Elements on page 113](#). For information on gene studies, see [Gene Study Module on page 123](#).

Opening a Datafile for Analysis

You can open the following file types in the Data Analysis module:

- .qlp

When you save this file type in QX ONE Software, the software automatically saves the file as a .ddpcrone file. This creates a new file and does not overwrite the original.

- .ddpcr

When you save this file, the software automatically saves it as a .ddpcrone file. This creates a new file and does not overwrite the original.

- .ddpcrone

Analysis data files are typically stored in one of the following defined storage locations:

- Personal folder for the individual user on the touch screen computer or the system folder specified for Preferred locations in the System Settings window

Note: Your personal folder location is displayed in your user preferences. You can open the System Settings window to see the preferred or shared storage locations.

Important: If your system administrator has specified preferred location file paths for all users, the file paths specified in user preferences are overridden.

- Shared folder in a different location, such as a network path

Note: The software also saves data files to a shared folder if it is accessible.

To open data files, you must be on a computer on which QX ONE Software is installed and have access to the storage locations.

To open a data file for analysis

1. Open QX ONE Software and log into the application.
2. Select the Data Analysis tab.



Available data files are displayed under the following headings.

■ Recent Datafiles

Displays a list of files you have recently opened.

■ System Datafiles or My Datafiles

Only one of the following locations is available:

- If your system administrator has enabled Preferred Locations for all users, all data files are stored in that folder, and files appear under System Datafiles.
- If preferred locations are not enabled for all users, your data files are saved to your personal folder and appear under My Datafiles.

■ Shared Datafiles

Displays a list of data files saved to the shared location, if one has been specified in the System Settings window.

Important: Data files are always saved to either My Datafiles or System Datafiles, data files are also saved to the Shared location (such as a network directory) if it is accessible when the software saves the data file.

3. Tap a file to open it in the Analysis module.

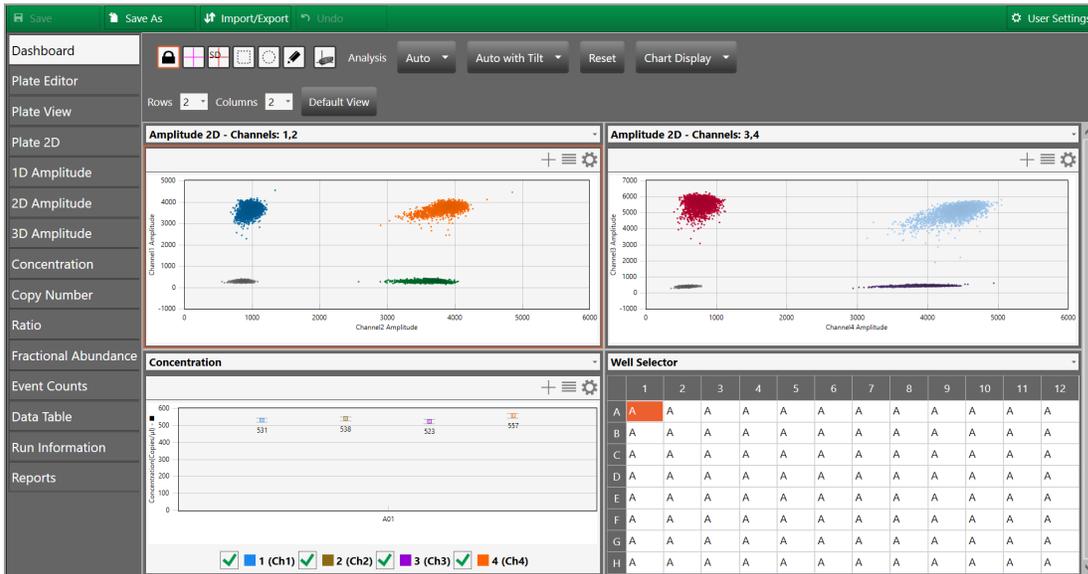
Tabs for each analysis and data window appear on the left of the Analysis window..

Tip: From the touch screen, you can also open the data file from the Run Status window. In the completed files section, tap the completed run icon (✔) for the file, and then tap Launch Analysis.

Analysis Dashboard

When you open a data file, the Analysis module opens to a Dashboard window containing default analysis and data panes in a summary view. You can customize the view to increase or decrease the number of panes that appear in the Dashboard, and you can also change the selection of panes that appear.

For information on the available options in the Dashboard view, see [Dashboard Options on page 55](#).



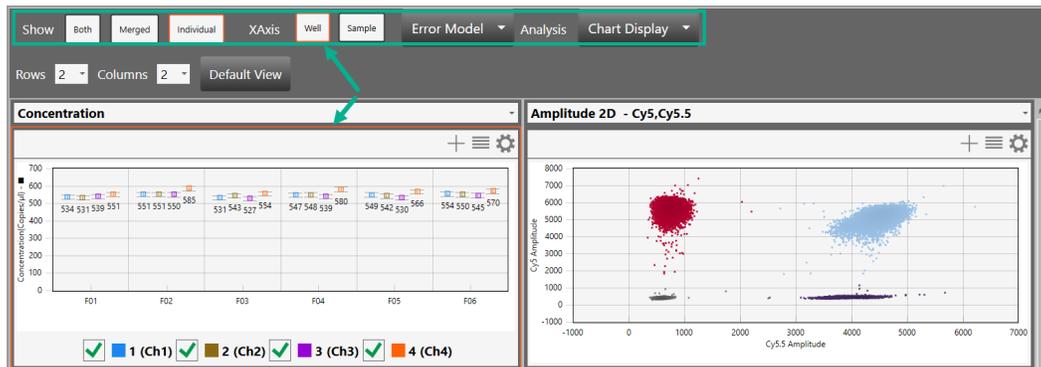
Note: If you open a .qlp file, the Plate Editor opens as the default view and you must select the Dashboard tab to open it.

From the tabs on the left, you can access plate views, amplitude charts, statistical probability distribution charts, droplet counts, and a complete data table. You can also view data specific to the run in the Run Information window, and run reports on any of the analysis charts from the Reports window.

Dashboard Options

From the Dashboard window, depending on the individual dashboard window you have selected, you can use a range of options as described below:

- When you select a window, the options that appear in the menu bar correspond to the options you see if you open the same window from the side menu. For example, if you select the Dashboard Concentration chart, the Concentration menu options appear.



- You can reset the entire dashboard display by adding or removing rows or columns to display more or fewer individual windows.



Tap Default View to return to the default display of two rows and two columns.

- From any window you can
 - Expand or collapse the window
 - Copy, save, or print the image
 - Select display, scale, axis, and logarithmic options
 - Use the dropdown list to replace the current display with a different display
 - Use the menu bar options for the particular chart type

Table 10 on page 56 describes the option sets identified in the following graphic.

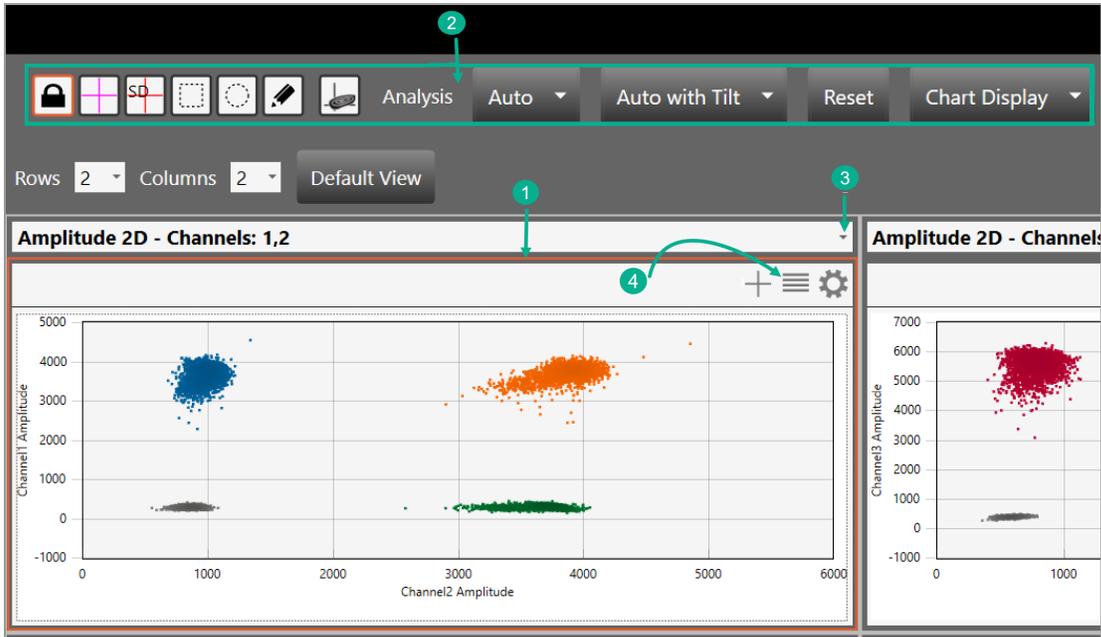


Table 10. Dashboard options

Item	Description
1	Border to identify selected window
2	Toolbar corresponding to the selected window type in the Dashboard
3	Dropdown arrow to replace the current display with a new display
4	Chart options

Analysis Display and Output Options

QX ONE Software provides the following miscellaneous option types in the Analysis module:

- **Universal Options** — available from the primary toolbar to save, import, or export data files
- **Chart Display Preferences** — to change how your chart appears in the analysis window
- **Chart Scale Options** — to change the scale represented on an axis
- **Chart Menu Options** — available in chart views (from the  icon) to copy, save, and print chart images. In Amplitude charts showing data points, you can show or hide
- **Table Menu Options** — available in table views (from the  icon) to copy data, export data to Excel or CSV files, and show or hide columns

You can also expand the chart display to full-screen size and then return to the original display.

Universal Options



Universal options are explained in [Table 11](#), and are available in all Analysis module windows.

Table 11. Universal options

Option	Description
Save	Saves changes to the analysis file under its current file name or file extension
Save as	Saves the file as a different file extension (for example, .qlp or ddpcr to .ddpcrone, or saves changes to the file under a different file name)
Import/export	<ul style="list-style-type: none"> ■ Imports or exports plate setup data ■ Exports amplitude, cluster and linkage data ■ Exports and prints the visible charts ■ Saves the charts as image files
Undo	Enabled in the Plate Editor only
User Settings	Allows you to restore your user preferences to the default settings

To save the data file

1. Tap Save or Save As.
 - If you tap Save, the software immediately saves any changes you made under the original file name.
 - If you tap Save As, continue to Step 2.
2. In the Save As dialog box, enter a new file name.
3. (Optional) enter a file location.
4. Tap Save.

The file is saved with the new file name.

To import or export setup or analysis data

1. Tap Import/Export.

[Table 12](#) describes the available import/export options.

Note: If there is unsaved data, the software prompts you to save the file before its contents can be imported or exported.

Table 12. Import/export options

Import/export option	Description
Import plate setup CSV	Import plate layout data from a CSV file to the Plate Editor Note: This option is only available in the Plate Editor. The new plate layout overwrites the existing layout.
Export plate setup CSV	Export a configured plate layout to a CSV file Note: This option is only available in the Plate Editor.
Import plate setup	Import a plate layout from a plate template (ddplt) file Note: This option is only available in the Plate Editor.
Export plate setup	Export the plate layout to a plate template file. Note: This option is only available in the Plate Editor.
Export visible data to CSV	Export only the data from the wells you selected in the Well Selector to a CSV file

Table 12. Import/export options, continued

Import/export option	Description
Export amplitude data	Exports the data to an Excel spreadsheet, and indicates whether or not the target was found (1 = yes, 0 = no)
Export cluster data	Exports the data to an Excel spreadsheet Note: Includes (for each well) whether or not the target was found (1 = yes, 0 = no), the droplet count, and the mean and standard deviation for each dye, and the cluster ID
Export linkage data	Exports to a CSV file the number of copies of a target that are present in overabundance, versus. the expected value in copies per μl
Export visible charts	Exports all charts that are displayed
Print visible charts	Prints all charts that are displayed

Table 13 shows where the options are available.

Table 13. Option availability

Import/export option	Plate editor	Plate view	Plate 2D	Amplitude plots	Statistical probability charts	Event counts	Data table
Import plate setup from CSV	✓						
Export plate setup to CSV	✓						
Import plate setup	✓						
Export plate setup	✓						
Export visible data to CSV				✓	✓	✓	✓
Export amplitude data	✓	✓	✓	✓	✓	✓	✓
Export cluster data	✓	✓	✓	✓	✓	✓	✓

Table 13. Option availability, continued

Import/export option	Plate editor	Plate view	Plate 2D	Amplitude plots	Statistical probability charts	Event counts	Data table
Export linkage data	✓	✓	✓	✓	✓	✓	✓
Export visible charts			✓	✓	✓	✓	
Print visible charts			✓	✓	✓	✓	

Note: Amplitude plots are available in 1D, 2D and 3D, and statistical probability charts are Concentration, Copy Number, Ratio, and Fractional Abundance. In the Dashboard view corresponding options are available for each plot or chart displayed.

Expanding or Reducing the Chart

You can expand or reduce the chart size in the following windows:

- Dashboard
- 1D Amplitude
- 2D Amplitude
- 3D Amplitude
- Concentration
- Copy Number
- Ratio
- Fractional Abundance
- Event Counts

If you expand an amplitude or statistical probability chart to full size, the Well Selector and the Well Data table are hidden from view until you collapse the chart again.

To expand or reduce the chart size

1. To increase the chart size to the full window, tap the  icon in the upper-right corner of the chart.
2. To reduce the window to its original size, tap the  icon.

Selecting Chart Display Preferences

The Chart Display preferences primarily control font sizes and positioning. [Table 14](#) explains each preference, as well as the windows in which it is available.

Table 14. Chart display preferences

Preference	Window	Description
Channel or Dye	1D Amplitude 2D Amplitude 3D Amplitude	Select Channel or Dye to identify your chart accordingly. If you select Channel, the channel number appears as the chart title. If you select Dye, FAM, HEX, Cy5, or Cy5.5 appears as the chart title.
Axis Data Label Size	1D Amplitude 2D Amplitude 3D Amplitude Concentration Copy Number Ratio Fractional Abundance Event Counts	Select a different font size.
Chart Data Label Size	Concentration Copy Number Ratio Fractional Abundance Event Counts	Select a different font size.
Chart Marker Size	Concentration Copy Number Ratio Fractional Abundance	Select a different marker size.
Chart Marker Label Position	Concentration Copy Number Ratio Fractional Abundance	Reposition the marker label.

To select chart display preferences

1. Tap the Chart Display button.
2. Select your options.

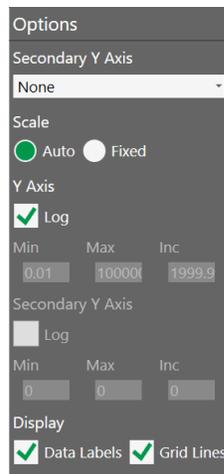
The display changes immediately to reflect your choices.

3. Do one of the following:

- To save your choices as your default settings, tap Save as my default. When the success message appears, tap OK to close the dialog.
- To close the menu without saving the settings, tap anywhere in the chart display.

Selecting Chart Scale Options

Chart scale options primarily control chart scale displays.



Each preference, as well as the windows in which it is available, is explained in [Table 15](#).

Table 15. Chart scale options

Option	Description
Scale	<p>Appears in options for all charts</p> <p>The Scale option determines the numerical range for your chart data:</p> <p>Auto — Selected by default; QX ONE Software automatically displays a scale to match the data distribution.</p> <p>Fixed — Selecting Fixed enables the Axis fields, and allows you to manually adjust the range and increment.</p>
Axis	<p>Appears in options for all charts</p> <p>When the Axis fields are enabled, you can manually adjust the minimum (Min) and maximum (Max) points for the data display on each axis, as well as the increments (Incr).</p> <p>X — Horizontal axis Y — Vertical axis</p> <p>Note: You can modify the x axis scale in the 2D Amplitude chart only.</p>
Secondary Y Axis	<p>Appears in options for Concentration only</p> <p>From the dropdown list, you can select from Channel 2 concentration, ratio, copy number, or fractional abundance to display the corresponding data in a secondary row on the y axis.</p>
Log	<p>Appears in options for all statistical probability charts (Concentration, Copy Number, Ratio, and Fractional Abundance)</p> <p>You can select the Log checkbox to display the y axis in base 10 logarithmic format.</p> <p>Note: In the Concentration chart, you can also select Log for the secondary y axis.</p>
Inverse	<p>Appears in options for Ratio and Fractional Abundance only</p> <p>The Inverse option equals the opposing number of the Ratio data point to a total of 2, and the opposing number of the Fractional Abundance data point to a total of 100.</p>
Data labels	<p>Appears in options for all statistical probability charts</p> <p>A data label appears by default for each point in the chart, but you can clear the checkbox to remove the labels.</p>
Grid Lines	<p>Appears in options for all charts</p> <p>Gridlines appear by default. You can clear the check box to remove them.</p>

To select chart scale options

1. Tap the  icon in the upper-left corner of the chart to display the corresponding chart options.
2. To manually define the chart scale, select Fixed.

Note: Auto is the default setting.
3. In the Min, Max, and Inc fields, enter your lower and upper limits, and the increment, for the y axis. For 2D Amplitude, you can also change the scale on the x axis.
4. For Concentration, Copy Number, Ratio, Fractional Abundance, and Event Counts, select an option for a secondary y axis display. To change to a base 10 logarithmic scale on the y axis, select the Log checkbox.
5. For Ratio and Fractional Abundance, to view an inverse calculation, select the Inverse checkbox.
6. To remove the data labels or grid lines from the display, clear the Data Labels or Grid Lines checkbox.

Using Chart Menu Options

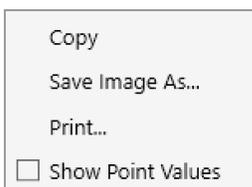


Chart menu options are explained in [Table 16](#).

Table 16. Chart menu options

Option	Description
Copy	Copies the chart to the clipboard, which you can paste into a separate document, such as an MS Word file
Save Image as	Saves the chart as an image file
Print	Prints the image
Show Point Values	Shows corresponding point values when you pause on a data point

Tip: Select the Show Point Values checkbox to see the data. Data is available for 1D and 2D amplitude only.

Chart menu options are available in the following windows:

- 1D Amplitude
- 2D Amplitude
- 3D Amplitude
- Concentration
- Copy Number
- Ratio
- Fractional Abundance
- Event Counts

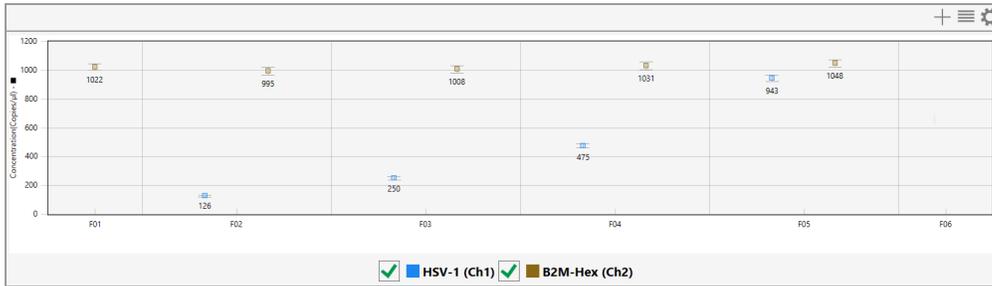
To use the chart menu options

1. Select wells in the Well Selector.

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E	A	A	A	A	A	A						
F	A	A	A	A	A	A						
G	A	A	A	A	A	A						
H	A	A	A	A	A	A						

Corresponding data appears in the Well Data table and the associated chart.

Well Data						
Well	SampleDesc1	Target	Conc(copies/μL)	Status	Experiment	
F01	NTC/1cpd Ch2	HSV-1	No Call	CHECK	DQ	
F01	NTC/1cpd Ch2	B2M-...	1022	OK	DQ	
F02	0.125 cpd Ch...	HSV-1	126	OK	DQ	
F02	0.125 cpd Ch...	B2M-...	995	OK	DQ	
F03	0.25 cpd Ch1...	HSV-1	250	OK	DQ	
F03	0.25 cpd Ch1...	B2M-...	1008	OK	DQ	
F04	0.5 cpd Ch1/...	HSV-1	475	OK	DQ	



2. In the upper-right corner of the chart, tap the menu icon (☰) and select from the dropdown list.
 - Select Copy to copy the chart to the Clipboard.

Note: To paste the data, navigate to the location (for example, a Word file), then right-click and select Paste.
 - Select Save Image As and follow the prompts to save the file.
 - Select Print and follow the prompts to print the image.
3. Tap anywhere in the display to close the dialog box.

Using Table Menu Options

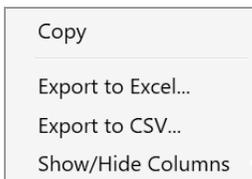


Table menu options are explained in [Table 17](#).

Table 17. Table options

Option	Description
Copy	Copies the information from the selected wells to the clipboard
Export to Excel	Exports the data to an .xlsx file
Export to CSV	Exports the data to a .csv file

Table 17. Table options, continued

Option	Description
Show/Hide Columns	<p>Add or remove columns from a table display</p> <p>When you select Show/Hide Columns, the following dialog box opens. Select or clear check boxes to show or hide columns in the data table.</p> <div data-bbox="565 558 786 1033" style="border: 1px solid black; padding: 5px;"> <p><input checked="" type="checkbox"/> Select All</p> <hr/> <p><input checked="" type="checkbox"/> Well</p> <p><input checked="" type="checkbox"/> Sample description 1</p> <p><input checked="" type="checkbox"/> Sample description 2</p> <p><input type="checkbox"/> Sample description 3</p> <p><input type="checkbox"/> Sample description 4</p> <p><input checked="" type="checkbox"/> Target</p> <p><input checked="" type="checkbox"/> Conc(copies/μL)</p> <p><input checked="" type="checkbox"/> Status</p> <p><input checked="" type="checkbox"/> Experiment</p> <p><input checked="" type="checkbox"/> SampleType</p> <p><input checked="" type="checkbox"/> TargetType</p> <p><input checked="" type="checkbox"/> Supermix</p> </div>

Table menu options are available in the following windows:

- 1D Amplitude
- 2D Amplitude
- 3D Amplitude
- Concentration
- Copy Number
- Ratio
- Fractional Abundance
- Event Counts

Tip: You can access the menu icon from the individual windows, or from the windows in the Dashboard display.

To use the table menu options

If you are using these options in the Data Table, skip to Step 3.

1. Select wells in the Well Selector.

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E	A	A	A	A	A	A						
F	A	A	A	A	A	A						
G	A	A	A	A	A	A						
H	A	A	A	A	A	A						

Corresponding data appears in the Well Data table.

Well Data							+	☰
Well	Target	Conc(copies/μL)	Status	Experiment	SampleType			
F01	1	534	OK	DQ	Unknown			
F01	2	531	OK	DQ	Unknown			
F01	3	539	OK	DQ	Unknown			
F01	4	551	OK	DQ	Unknown			
F02	1	551	OK	DQ	Unknown			
F02	2	551	OK	DQ	Unknown			
F02	2	550	OK	DQ	Unknown			

2. In the upper-right corner of the table, tap the menu icon (☰) and select from the dropdown list.
 - To copy the data from the selected wells to the Clipboard, select Copy.

Note: To paste the data, navigate to the location (for example, an Excel file) and then right-click and select Paste.
 - To export your data, select Export to Excel or Export to CSV, and then follow the prompts to save the file.

Note: Exporting to Excel exports the data in spreadsheet format, while Exporting to CSV exports the data in comma-delimited format.
 - To show or hide the corresponding column in the table, select Show/Hide Columns, and then select or clear checkboxes.
3. Tap anywhere in the display to close the dialog box.

Plate View Windows

In the QX ONE Software Analysis module you can open the following windows that contain data on all wells in the run:

- **Plate Editor (analysis view)** — Displays the experiment type, sample names, sample types, supermixes, and assay type for each well in the run (similar to the Template Setup Plate Editor)
- **Plate View** — Displays concentration results in a text format for each processed well
- **2D Plate View** — Displays a two-dimensional amplitude chart for each processed well

Plate Editor — Analysis View

You can use the Plate Editor tab in the Analysis module to select wells, and then view or change the experiment parameters. Similar to other Plate Editor displays, the Analysis Plate Editor displays the experiment information for each well in the run.

Target Name	Target Type	Signal CH1	Signal CH2	Signal CH3	Signal CH4
1	Unkn	FAM	None	None	None
2	Unkn	None	HEX	None	None
3	Unkn	None	None	Cys	None
4	Unkn	None	None	None	Cys.S

Important: You can change all fields except the supermix.

Wells you select in the Analysis Plate Editor – except for 3D amplitude where you select from a dropdown menu – determine the data initially displayed in other analysis windows.

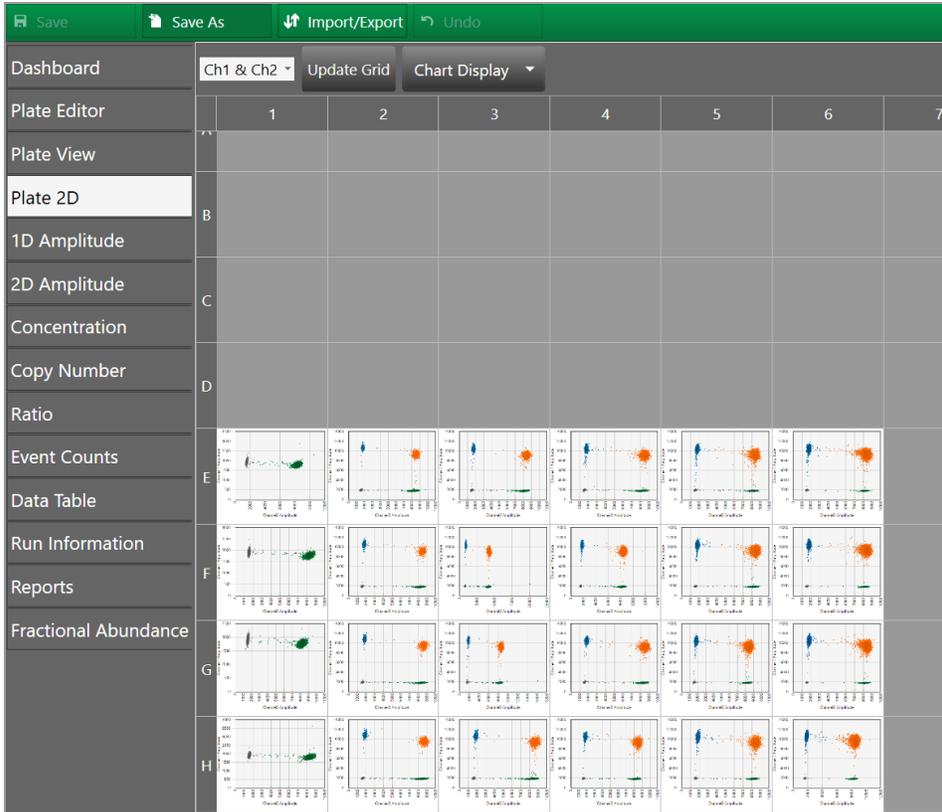
Plate View

The Plate View tab provides, within the plate layout grid, a text view of the calculated concentration for each target in each well processed in the run. For information on the concentration results in chart format, see [Concentration on page 104](#).

Save Save As Import/Export Undo User Settings																								
Dashboard	1	2	3	4	5	6	7	8	9	10	11	12												
Plate Editor	DQ																							
	4 Color																							
	U1	30.87	U1	21.19	U1	520.3	U1	28.69	U1	11.26	U1	14.46	U1	524.5	U1	18.98	U1	33.27	U1	17.65	U1	33.55	U1	38.79
	U2	37.85	U2	17.22	U2	19.06	U2	20.34	U2	29.28	U2	28.56	U2	34.47	U2	26.17	U2	34.02	U2	33.17	U2	25.72	U2	32.45
Plate 2D	U3	22.54	U3	04.86	U3	23.93	U3	25.76	U3	21.27	U3	06.94	U3	27.47	U3	17.79	U3	29.71	U3	24.66	U3	32.83	U3	24.75
	U4	56.75	U4	31.73	U4	54.32	U4	48.68	U4	41.32	U4	55.57	U4	39.33	U4	48.25	U4	46.42	U4	47.64	U4	35.93	U4	57.27
	DQ																							
	4 Color																							
1D Amplitude	U1	33.29	U1	27.73	U1	29.04	U1	14.55	U1	26.06	U1	40.19	U1	529.5	U1	27.23	U1	34.84	U1	28.21	U1	33.14	U1	34.17
	U2	28.05	U2	27.94	U2	35.54	U2	40.89	U2	25.59	U2	28.57	U2	30.89	U2	41.35	U2	39.24	U2	32.46	U2	41.21	U2	540.8
	U3	21.24	U3	26.07	U3	15.81	U3	22.42	U3	21.05	U3	22.81	U3	35.26	U3	30.31	U3	47.65	U3	49.25	U3	23.38	U3	35.92
	U4	59.42	U4	41.18	U4	54.26	U4	52.67	U4	60.95	U4	57.86	U4	60.48	U4	57.84	U4	50.73	U4	60.02	U4	56.77	U4	65.42
2D Amplitude	DQ																							
	4 Color																							
	U1	42.42	U1	24.96	U1	32.11	U1	30.58	U1	27.62	U1	27.34	U1	37.44	U1	26.62	U1	33.85	U1	36.25	U1	536.7	U1	38.18
	U2	543.4	U2	41.81	U2	40.33	U2	39.55	U2	527.8	U2	36.33	U2	29.13	U2	40.87	U2	42.85	U2	32.16	U2	46.32	U2	39.92
3D Amplitude	U3	33.99	U3	36.95	U3	22.79	U3	31.11	U3	35.35	U3	30.65	U3	30.77	U3	22.05	U3	42.49	U3	536.9	U3	31.39	U3	34.69
	U4	59.11	U4	51.01	U4	51.26	U4	42.94	U4	46.17	U4	57.62	U4	50.19	U4	49.51	U4	56.72	U4	50.69	U4	51.15	U4	52.82
	DQ																							
	4 Color																							
Concentration	U1	55.49	U1	44.89	U1	47.35	U1	521.4	U1	34.06	U1	26.72	U1	24.23	U1	37.67	U1	27.09	U1	34.07	U1	25.71	U1	42.92
	U2	49.61	U2	543.4	U2	44.99	U2	48.42	U2	46.77	U2	31.86	U2	541.4	U2	38.47	U2	50.98	U2	45.85	U2	55.72	U2	44.33
	U3	36.08	U3	20.99	U3	38.07	U3	32.61	U3	27.27	U3	25.64	U3	37.72	U3	533.6	U3	26.99	U3	38.53	U3	34.28	U3	36.48
	U4	69.72	U4	54.95	U4	64.65	U4	60.79	U4	63.16	U4	47.74	U4	52.02	U4	56.76	U4	47.85	U4	58.12	U4	556.8	U4	60.51
Copy Number	DQ																							
	4 Color																							
	U1	56.53	U1	34.43	U1	48.25	U1	39.42	U1	56.78	U1	34.63	U1	56.47	U1	37.76	U1	48.05	U1	39.71	U1	54.65	U1	15.56
	U2	56.84	U2	29.27	U2	63.48	U2	42.32	U2	56.88	U2	45.35	U2	50.45	U2	35.03	U2	54.07	U2	29.39	U2	49.82	U2	46.34
Ratio	U3	562.5	U3	30.19	U3	53.83	U3	41.78	U3	43.66	U3	40.77	U3	34.51	U3	30.74	U3	46.53	U3	31.94	U3	45.88	U3	43.58
	DQ																							
	4 Color																							
	U1	56.53	U1	34.43	U1	48.25	U1	39.42	U1	56.78	U1	34.63	U1	56.47	U1	37.76	U1	48.05	U1	39.71	U1	54.65	U1	15.56
Fractional Abundance	U2	56.84	U2	29.27	U2	63.48	U2	42.32	U2	56.88	U2	45.35	U2	50.45	U2	35.03	U2	54.07	U2	29.39	U2	49.82	U2	46.34
	U3	562.5	U3	30.19	U3	53.83	U3	41.78	U3	43.66	U3	40.77	U3	34.51	U3	30.74	U3	46.53	U3	31.94	U3	45.88	U3	43.58
	DQ																							
	4 Color																							
Event Counts	U1	56.53	U1	34.43	U1	48.25	U1	39.42	U1	56.78	U1	34.63	U1	56.47	U1	37.76	U1	48.05	U1	39.71	U1	54.65	U1	15.56
	U2	56.84	U2	29.27	U2	63.48	U2	42.32	U2	56.88	U2	45.35	U2	50.45	U2	35.03	U2	54.07	U2	29.39	U2	49.82	U2	46.34
	U3	562.5	U3	30.19	U3	53.83	U3	41.78	U3	43.66	U3	40.77	U3	34.51	U3	30.74	U3	46.53	U3	31.94	U3	45.88	U3	43.58
	U4	69.72	U4	54.95	U4	64.65	U4	60.79	U4	63.16	U4	47.74	U4	52.02	U4	56.76	U4	47.85	U4	58.12	U4	556.8	U4	60.51
Data Table	DQ																							
	4 Color																							
	U1	56.53	U1	34.43	U1	48.25	U1	39.42	U1	56.78	U1	34.63	U1	56.47	U1	37.76	U1	48.05	U1	39.71	U1	54.65	U1	15.56
	U2	56.84	U2	29.27	U2	63.48	U2	42.32	U2	56.88	U2	45.35	U2	50.45	U2	35.03	U2	54.07	U2	29.39	U2	49.82	U2	46.34
Run Information	U3	562.5	U3	30.19	U3	53.83	U3	41.78	U3	43.66	U3	40.77	U3	34.51	U3	30.74	U3	46.53	U3	31.94	U3	45.88	U3	43.58
	DQ																							
	4 Color																							
	U1	56.53	U1	34.43	U1	48.25	U1	39.42	U1	56.78	U1	34.63	U1	56.47	U1	37.76	U1	48.05	U1	39.71	U1	54.65	U1	15.56
Reports	U2	56.84	U2	29.27	U2	63.48	U2	42.32	U2	56.88	U2	45.35	U2	50.45	U2	35.03	U2	54.07	U2	29.39	U2	49.82	U2	46.34
	U3	562.5	U3	30.19	U3	53.83	U3	41.78	U3	43.66	U3	40.77	U3	34.51	U3	30.74	U3	46.53	U3	31.94	U3	45.88	U3	43.58
	DQ																							
	4 Color																							

2D Plate View

To facilitate comparisons of amplitude in the processed wells, the Plate 2D tab provides a two-dimensional amplitude view in a separate chart for each well. To see a larger representation of a well, select the well in the 2D Amplitude view.



Analysis Windows

In its Analysis module, QX ONE Software provides amplitude displays and statistical probability charts in the Analysis module.

Amplitude Charts

Amplitude charts illustrate the droplets by degree of dye fluorescence. QX ONE Software displays fluorescence for positive and negative targets identified with FAM, HEX/VIC, Cy5, and Cy5.5 fluorophores in the following analysis windows:

- 1D Amplitude
- 2D Amplitude
- 3D Amplitude

Statistical Probability Charts

Statistical probability charts show calculations based on positive droplet concentrations. QX ONE Software counts the number of positive and negative droplets for each fluorophore in each sample, and then fits the fraction of positive droplets to a Poisson algorithm to determine the starting concentration of the target DNA molecule in units of copies/ μ l input in the following analysis windows:

- Concentration
- Copy Number
- Ratio
- Fractional Abundance

When you open the analysis data file in QX ONE Software and select one or more wells in the grid, the software plots each calculated Poisson probability distribution average as a data point in the grid. You can pause on the data point to see the high, median, and low numbers.

Note: You can display the data using a confidence level of either 68% or 95% (default setting).

Event Counts

Event counts record the number of droplets in which the target was found (positive) and was not found (negative). The Event Counts tab shows a bar chart view of the number of positive, negative, and total droplets for each of the targets in each of the selected wells.

For more detailed information, see [Chapter 6, Data Analysis Methodology](#).

Data Windows

QX ONE Software provides the following table views for your well and run data:

- **Data Table** — available from the main tabs on the left, shows your configured experiment parameters, plus all calculations from the run
Note: You can display the data in individual well format, merged format, or both.
- **Plate Well Data Table** — available in plate setup windows (including the Analysis module Plate Editor), shows only your configuration information for each individual well
- **Analysis Well Data Table** — available in the Analysis module, shows a subset of configuration and run data for the wells selected in the Well Selector

Data Table Tab

The Data Table window contains columns of data generated for each target in each well, as droplet clusters are identified through thresholding or manual clustering tools.

Well	Sample description 1	Target	Conc(copies/μL)	Experiment	SampleType	TargetType	Supermix	DyeName(s)	Copies/20μLWell	To
A06	4 Color	1	514	DQ	Unknown	Unknown	ddPCR Su...	FAM	10289	
A05	4 Color	4	541	DQ	Unknown	Unknown	ddPCR Su...	Cy5.5	10826	
A05	4 Color	3	521	DQ	Unknown	Unknown	ddPCR Su...	Cy5	10425	
A05	4 Color	2	529	DQ	Unknown	Unknown	ddPCR Su...	HEX	10586	
A05	4 Color	1	511	DQ	Unknown	Unknown	ddPCR Su...	FAM	10225	
A04	4 Color	4	549	DQ	Unknown	Unknown	ddPCR Su...	Cy5.5	10974	
A04	4 Color	3	526	DQ	Unknown	Unknown	ddPCR Su...	Cy5	10515	
A04	4 Color	2	520	DQ	Unknown	Unknown	ddPCR Su...	HEX	10407	
A04	4 Color	1	529	DQ	Unknown	Unknown	ddPCR Su...	FAM	10574	
A03	4 Color	4	554	DQ	Unknown	Unknown	ddPCR Su...	Cy5.5	11086	
A03	4 Color	3	524	DQ	Unknown	Unknown	ddPCR Su...	Cy5	10479	
A03	4 Color	2	519	DQ	Unknown	Unknown	ddPCR Su...	HEX	10381	
A03	4 Color	1	520	DQ	Unknown	Unknown	ddPCR Su...	FAM	10406	
A02	4 Color	4	532	DQ	Unknown	Unknown	ddPCR Su...	Cy5.5	10635	
A02	4 Color	3	505	DQ	Unknown	Unknown	ddPCR Su...	Cy5	10097	
A02	4 Color	2	517	DQ	Unknown	Unknown	ddPCR Su...	HEX	10344	
A02	4 Color	1	521	DQ	Unknown	Unknown	ddPCR Su...	FAM	10424	
A01	4 Color	4	557	DQ	Unknown	Unknown	ddPCR Su...	Cy5.5	11135	
A01	4 Color	3	523	DQ	Unknown	Unknown	ddPCR Su...	Cy5	10451	
A01	4 Color	2	538	DQ	Unknown	Unknown	ddPCR Su...	HEX	10757	
A01	4 Color	1	531	DQ	Unknown	Unknown	ddPCR Su...	FAM	10617	

Data Table Column Definitions

Table 18 defines each field in the Data Table.

Table 18. Data Table columns

Column name	Content description
Well	The well location in the plate Note: The instrument accepts 96-well plates only.
Sample description 1 Sample description 2 Sample description 3 Sample description 4	Words or phrases to describe the sample in each well Note: Only the sample description 1 data appears in plate layout views but you can pause on a well in the Plate Editor to display a tool tip containing the entries in all completed Sample description fields.
Target	Target name from Plate Editor tab
Conc(copies/ μ l)	Concentration of the target molecules recorded as copies per microliter
Molecular Weight (pg/ μ l)	Mass concentration of DNA in the well in pg/ μ l
Status	Contains either Check, OK, Multi, or Manual <ul style="list-style-type: none"> ■ If Check, either the automatic analysis for the well failed or fewer than 10,000 droplets were counted in the well ■ If OK, enough droplets were counted to trigger automatic analysis ■ If Multi, the data was automatically analyzed as part of a multi-well selection ■ If Manual, the droplets were analyzed manually
Status Reason	Identifies the reason for the status (unsuccessful automatic analysis, not enough droplets counted, or droplets analyzed manually)
Experiment	Experiment type selected for the well in the Plate Editor
SampleType	Sample type selected for the well in the Plate Editor
TargetType	Target type selected for each dye (FAM, HEX or VIC, Cy5 and Cy5.5) in the Plate Editor

Table 18. Data Table columns, continued

Column name	Content description
Supermix	Supermix selected for the well in Plate Editor
DyeName(s)	Dye assigned to channels 1, 2, 3, and 4, respectively, for the well in the Plate Editor
Copies/20µlWell	Concentration of the target normalized to a volume of 20 µl
TotalConfMax	For merged wells the high error bar for the target concentration of the combined wells at a 95% confidence interval
TotalConfMin	For merged wells the low error bar for the target concentration of the combined wells at a 95% confidence interval
PoissonConfMax	Maximum target concentration normalized for the high error bar of the droplet Poisson distribution for the 95% confidence interval
PoissonConfMin	Minimum target concentration normalized for the low error bar of the droplet Poisson distribution for the 95% confidence interval
Accepted Droplets	Total number of droplets accepted by quality algorithm
Positives	Number of droplets that contain the target
Negatives	Number of droplets that do not contain the target
Ch1+Ch2+	Number of droplets that contain both channel 1 and channel 2 targets
Ch1+Ch2-	Number of droplets that contain just the channel 1 target
Ch1-Ch2+	Number of droplets that contain just the channel 2 target
Ch1-Ch2-	Number of droplets that contain neither channel 1 nor channel 2 targets
Linkage	Shows number of copies of a target that are present in overabundance versus the expected value in copies per µl
CNV	Copy number calculated for the target relative to the reference
TotalCNVMax	For merged wells, the high error bar for the copy number of the combined wells at a 95% confidence interval

Table 18. Data Table columns, continued

Column name	Content description
TotalCNVMin	For merged wells, the low error bar for the copy number of the combined wells at a 95% confidence interval
PoissonCNVMax	Maximum copy number normalized for the high error bar of the droplet Poisson distribution for the 95% confidence interval
PoissonCNVMin	Minimum copy number normalized for the low error bar of the droplet Poisson distribution for the 95% confidence interval
ReferenceCopies	Copy number identified for the reference target in the Plate Editor
UnknownCopies	This field is currently unused in QX ONE Software
Threshold1	Threshold value of first threshold line from left to right and bottom to top
Threshold2	Threshold value of second threshold line from left to right and bottom to top
Threshold3	Threshold value of third threshold line from left to right and bottom to top
ThresholdSigmaAbove	Distance of the threshold from the mean of the negative cluster, as a multiple of the negative cluster standard deviation
ThresholdSigmaBelow	Distance of the threshold from the mean of the positive cluster, as a multiple of the positive cluster standard deviation
ReferenceUsed	Identifies which target was used as a reference
Ratio	The ratio of the target against the identified reference
TotalRatioMax	For merged wells the high error bar for the ratio of the unknown against the reference of the combined wells at a 95% confidence interval
TotalRatioMin	For merged wells the low error bar for the ratio of the unknown against the reference of the combined wells at a 95% confidence interval
PoissonRatioMax	Maximum ratio of the unknown against the reference normalized for the high error bar of the droplet Poisson distribution for the 95% confidence interval

Table 18. Data Table columns, continued

Column name	Content description
PoissonRatioMin	Minimum ratio of the unknown against the reference normalized for the low error bar of the droplet Poisson distribution for the 95% confidence interval
FractionalAbundance	Calculation of fractional abundance of this unknown target vs. the reference target
TotalFractionalAbundanceMax	For merged wells the high error bar for the fractional abundance of the combined wells at a 95% confidence interval
TotalFractionalAbundanceMin	For merged wells the low error bar for the fractional abundance of the combined wells at a 95% confidence interval
PoissonFractionalAbundanceMax	Maximum fractional abundance normalized for the high error bar of the droplet Poisson distribution for the 95% confidence interval
PoissonFractionalAbundanceMin	Minimum fractional abundance normalized for the low error bar of the droplet Poisson distribution for the 95% confidence interval
MeanAmplitudeOfPositives	Mean amplitude value of all droplets that contain the target
MeanAmplitudeOfNegatives	Mean amplitude value of all droplets that contain no target
MeanAmplitudeTotal	Mean amplitude value of all droplets
ExperimentComments	Data populates this field only from files created in QuantaSoft versions 1.4 – 1.7, if comments were added.
MergedWells	Identifies which wells were merged together
TotalConfidenceMax68	For merged wells the high error bar for the target concentration of the combined wells at a 68% confidence interval
TotalConfidenceMin68	For merged wells the low error bar for the target concentration of the combined wells at a 68% confidence interval
PoissonConfidenceMax68	Maximum target concentration normalized for the high error bar of the droplet Poisson distribution for the 68% confidence interval
PoissonConfidenceMin68	Minimum target concentration normalized for the low error bar of the droplet Poisson distribution for the 68% confidence interval

Table 18. Data Table columns, continued

Column name	Content description
TotalCNVMax68	For merged wells the high error bar for the copy number of the combined wells at a 68% confidence interval
TotalCNVMin68	For merged wells the low error bar for the copy number of the combined wells at a 68% confidence interval
PoissonCNVMax68	Maximum target concentration normalized for the high error bar of the droplet Poisson distribution for the 68% confidence interval
PoissonCNVMin68	Minimum target concentration normalized for the low error bar of the droplet Poisson distribution for the 68% confidence interval
TotalRatioMax68	For merged wells the high error bar for the ratio of the unknown against the reference of the combined wells at a 68% confidence interval
TotalRatioMin68	For merged wells the low error bar for the ratio of the unknown against the reference of the combined wells at a 68% confidence interval
PoissonRatioMax68	Maximum ratio of the unknown against the reference normalized for the high error bar of the droplet Poisson distribution for the 68% confidence interval
PoissonRatioMin68	Minimum ratio of the unknown against the reference normalized for the low error bar of the droplet Poisson distribution for the 68% confidence interval
TotalFractionalAbundanceMax68	For merged wells the high error bar for the fractional abundance of the combined wells at a 68% confidence interval
TotalFractionalAbundanceMin68	For merged wells the low error bar for the fractional abundance of the combined wells at a 68% confidence interval
PoissonFractionalAbundanceMax68	Maximum fractional abundance normalized for the high error bar of the droplet Poisson distribution for the 68% confidence interval
PoissonFractionalAbundanceMin68	Minimum fractional abundance normalized for the low error bar of the droplet Poisson distribution for the 68% confidence interval plate list after correcting the issue

Table 18. Data Table columns, continued

Column name	Content description
TiltCorrected	Indicates if tilt correction was applied to the well Note: Tilt correction rotates the angles from the double-negative cluster centroid to each droplet, such that the two single positive cluster centroids are orthogonal with the double-negative cluster centroid.

Plate Well Data Table

From the Plate Editor you can access a window containing the Well Data table, which displays configuration and run information on all wells containing sample.

Note: You cannot edit this table.

Table 19. Plate Well Data Table columns

Column name	Content description
Well	Well number (A01 through H12)
Perform Droplet Generation	Yes, if droplets were generated in the well No, if they are not
Perform Droplet Reading	Yes, if wells are read No, if they are not
Experiment Type	Experiment Type selected in the Plate Editor: <ul style="list-style-type: none"> ■ Direct Quantification (DQ) ■ Copy Number Variation (CNV) ■ Mutation Detection (MUT) ■ Rare Event Detection (RED) ■ Drop-off (DOF) ■ Gene Expression (GEX) ■ Residual DNA Quantification (RDQ)
Sample description 1 Sample description 2 Sample description 3 Sample description 4	Words or phrases to identify each sample, as entered in the Plate Editor Note: You can enter up to four sample descriptions for each well.

Table 19. Plate Well Data Table columns, continued

Column name	Content description
Sample Type	Sample type selected in the Plate Editor: <ul style="list-style-type: none"> ■ Unknown ■ No template control (NTC) ■ Positive control (Pos-Ctrl) ■ Negative control (Neg-Ctrl)
Supermix Name	Supermix selected in the Plate Editor: <ul style="list-style-type: none"> ■ ddPCR Multiplex Supermix ■ ddPCR Supermix for Probes (no dUTP) ■ ddPCR Supermix for Probes ■ ddPCR Supermix for Residual DNA Quantification ■ One-Step RT-ddPCR Advanced Kit for Probes
Plex Mode	Assay method selected in the Plate Editor: <ul style="list-style-type: none"> ■ Single Target per Channel ■ Amplitude Multiplex ■ Probe Mix Triplex ■ Advanced Classification Method
Target Name	Target name entered in the Plate Editor tab
Target Type	Target type selected in the Plate Editor tab
Signal Channel 1	FAM, EvaGreen®, or None
Signal Channel 2	HEX, VIC, or None
Signal Channel 3	Cy5 fluorophore or None
Signal Channel 4	Cy5.5 fluorophore or None
Reference Copies	Copy number identified for the reference target in the Plate Editor tab
Well Notes	Entries by the user
Plate Notes	Entries by the user
Plot?	If selected, target will be used to calculate the copy number for the well

Table 19. Plate Well Data Table columns, continued

Column name	Content description
RDQ Conversion Factor	The Residual DNA Quantification conversion factor for the well
	Note: RDQ is only available in Residual DNA Quantification experiments.

Analysis Well Data Table

A well data table containing both configuration and run data appears in the analysis windows, next to the Well Selector. This table displays a subset of data columns for only the wells selected in the Plate Editor or Well Selector.

Well	Sample description 1	Target	Conc(copies/µL)	Status	Experi
A01		1	1135	OK	DQ
A01		2	1007	OK	DQ
A02		1	1118	OK	DQ
A02		2	991	OK	DQ
B01		1	1094	OK	DQ
B01		2	996	OK	DQ
B02		1	1134	OK	DO

Table 20 defines each column in the consolidated table.

Table 20. Fields in the Analysis Well Data table

Column name	Content description
Well	The well location in the plate the sample is from
Sample description 1 Sample description 2 Sample description 3 Sample description 4	The sample name used to identify the well in the Plate Editor tab
Target	Target Name from Plate Editor tab
Conc (copies/µl)	Concentration identified using the Poisson calculation

Table 20. Fields in the Analysis Well Data table, continued

Column name	Content description
Status	<p>Contains either Check, OK, Multi, or Manual</p> <ul style="list-style-type: none"> ■ If Check, either the automatic analysis for the well failed or fewer than 10,000 droplets were counted in the well ■ If OK, enough droplets were counted to trigger automatic analysis ■ If Multi, the data was automatically analyzed as part of a multi-well selection ■ If Manual, the droplets were analyzed manually
Experiment	Experiment type from the Plate Editor
SampleType	Sample type from the Plate Editor
TargetType	Target type assigned to the target in the Plate Editor
Supermix	Supermix from the Plate Editor
DyeName(s)	Fluorophore assigned to Channel 1, Channel 2, Channel 3, and Channel 4 to a target in the Plate Editor
Accepted Droplets	Total number of droplets accepted by quality algorithms
Positives	Number of droplets that contain the target
Negatives	Number of droplets that do not contain the target

Chapter 6 Data Analysis Methodology

As summarized in [Chapter 5, Data Analysis Module Overview](#), the software presents analysis data in several different formats. When you open the data file in the Analysis module, you can view the data with the automatic analysis thresholds and calculations or you can choose different options to recalculate, replot, or recolor the results.

Data is shown in the following formats:

- Amplitude, which shows each droplet in each well in a scatter plot, in one-dimensional, two-dimensional, or three-dimensional views
- Statistical models, which show estimated target molecule concentration, ratio, and fractional abundance of one target against another target based on selected error model and confidence interval

Note: Copy number variation is also calculated if the user ran a CNV experiment.

- Event counts, which are precise counts of positive and negative droplets for each target in each well

Amplitude Plot Analysis Options

QX ONE Software provides three amplitude plot displays in which you can view the data with automatic thresholding where applicable, or choose different options to replot or recolor the results.

In the 1D amplitude and 2D amplitude plots, you can

- Show well calculations
 - Note:** The 1D amplitude plot shows the data for each selected well in its own section, while the 2D amplitude plot shows all selected wells together.
- Modify thresholds
- View data in a heat map
- View histograms (1D amplitude tab only)
- Re-apply automatic analysis
- Apply tilt correction to automatic analysis

In the 3D amplitude plot, you can

- Rotate the plot to gain a better view of the droplets in 3D orientation
- View thresholds previously set in 1D or 2D amplitude display
- View 2D data behind 3D droplets in clusters or heat maps

Table 21 explains the analysis options that are available in both 1D amplitude and 2D amplitude plots.

Table 21. 1D and 2D amplitude tool options

Icon	Mode	Description
	View mode	Data appears with the thresholds or clusters assigned by QX ONE Software. When this icon is selected, the view is locked and you cannot modify the thresholds.
	Heat map	Shows the distribution of droplets, with higher density of droplets shown as hot.
	Automatic analysis	Disables tilt correction and automatically reanalyzes the data.
	Automatic analysis with tilt correction	Enables tilt correction and automatically reanalyzes the data. Note: Tilt correction rotates the angles from the double-negative cluster centroid to each droplet, such that the two single positive cluster centroids are orthogonal with the double-negative cluster centroid.
	Reset	Resets the data to the original automatically analyzed data.
	Chart display options	Choose whether to display axis with channel number or fluorophore, and change the size of axis markers, data labels, and axis labels.

Table 22 explains the analysis options available only for 1D amplitude plots.

Table 22. 1D amplitude tool options

Icon	Mode	Description
	Threshold, single well	For a single well or multiple wells, the user can manually input thresholding values in the 1D amplitude plot.
	Threshold, multiple wells	You can tap anywhere in the plot to apply threshold lines to the data.
<p>To adjust the threshold values</p> <ul style="list-style-type: none"> ■ Drag the lines horizontally in the plot area ■ Enter a new threshold value in the boxes at the end of each threshold line ■ Tap the up and down arrows to incrementally adjust the numbers 		
	Threshold, single well SD	For a single well or multiple wells, QX ONE Software automatically calculates and populates thresholding values with standard deviations (SD).
	Threshold, multiple wells SD	<p>Important: SD thresholds are not recommended for RMD, since these clusters are not typically located at right angles to each other.</p> <p>Note: The SD thresholds are set at a limit that is less than the mean number of positive targets, and more than the mean number of negative targets. Starting from where the user clicks in the plot, SD locates the threshold between the positive and negative clusters such that the classification error is minimized.</p>
	Show histogram	Hides or displays histogram plots for 1D amplitude

Table 23 explains the analysis options available only for 2D amplitude plots.

Table 23. 2D amplitude tool options

Icon	Mode	Description
	Threshold line	<p>Use the Threshold line icons to establish thresholds across a pair of channels, dividing droplets into distinct clusters separated by different colors.</p> <p>You can tap anywhere in the plot to apply threshold lines to the data.</p> <p>To adjust the threshold values, you can</p> <ul style="list-style-type: none"> ■ Drag the lines horizontally or vertically in the plot area ■ Enter a new threshold value in the boxes at the end of each threshold line ■ Tap the up and down arrows to incrementally adjust the numbers
	Threshold line SD	<p>QX ONE Software automatically calculates and populates threshold line values with standard deviations (SD).</p> <p>Important: SD thresholds are not recommended for RMD, since these clusters are not typically located at right angles to each other.</p> <p>Note: The SD thresholds are set at a limit that is less than the mean number of positive targets, and more than the mean number of negative targets. Starting from where the user clicks in the plot, SD locates the threshold between the positive and negative clusters such that the classification error is minimized.</p>

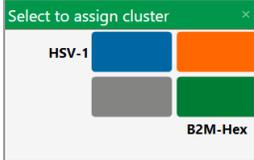
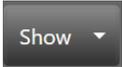
Icon	Mode	Description
	Threshold cluster square	<p>Use the Threshold Cluster icons to establish thresholds across a pair of channels, dividing droplets into distinct clusters separated by different colors.</p> <p>To classify your droplets, you can</p> <ul style="list-style-type: none"> ■ Tap any of the three Threshold Cluster Mode buttons (square, circle, or free form) ■ Draw the chosen shape around the cluster of droplets <p>Note: When using the cluster mode tools to draw a shape around a cluster of droplets, a dialog box with color selections by target appears in the window to help you select the correct target combination.</p>
	Threshold cluster circle	
	Threshold cluster freeform	
		 <p>The screenshot shows a dialog box titled "Select to assign cluster" with a close button (X). It features a 2x2 grid of colored boxes. The top-left box is blue and labeled "HSV-1". The top-right box is orange. The bottom-left box is grey. The bottom-right box is green and labeled "B2M-Hex".</p>
		<p>Target names are shown along the two axes based on the signal values assigned during plate setup. Tap the colored box that best represents the location of the cluster of droplets selected to automatically classify the droplets as positive or negative for each given target.</p> <p>Note: The Cluster dialog box display varies according to the plex mode.</p>

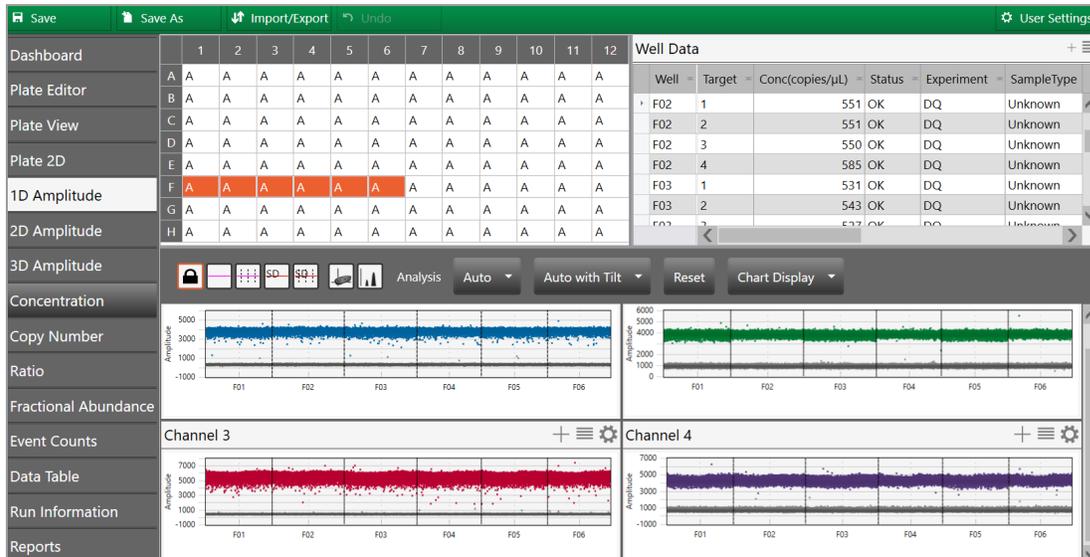
Table 24 explains the analysis options available only for 3D amplitude plots.

Table 24. 3D amplitude tool options

Icon	Mode	Description
	<p>Drop-down menu containing options for data visualization</p> 	<p>Choose to show 3D droplets, 2D amplitude data behind the 3D clusters, thresholds, and 2D color options.</p>
	<p>Axis titles</p>	<p>Choose whether to display axis with channel number or fluorophore name.</p>
	<p>Reset</p>	<p>Reset the display to the default orientation.</p>

1D Amplitude

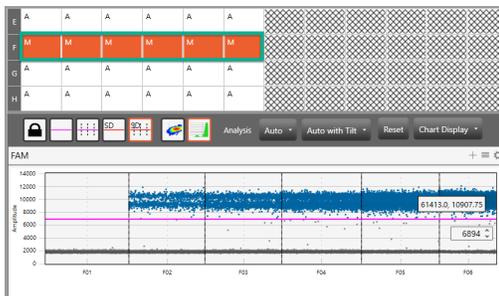
Use the 1D Amplitude analysis window to view droplet amplitudes for each channel, in selection order, for each well selected in the Well Selector.



The 1D Amplitude plots illustrate

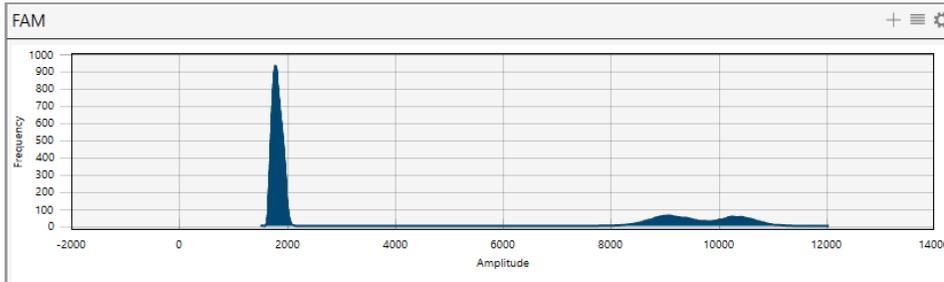
- All accepted droplets, with a visual representation of the positive and negative droplets in each well

Positive droplets appear in color, while negative droplets appear in grayscale. The Well Selector displays an A in each well where QX ONE Software automatically thresholded the droplet data. If you create a manual threshold, the Well Selector displays an M in the wells where the threshold changed.



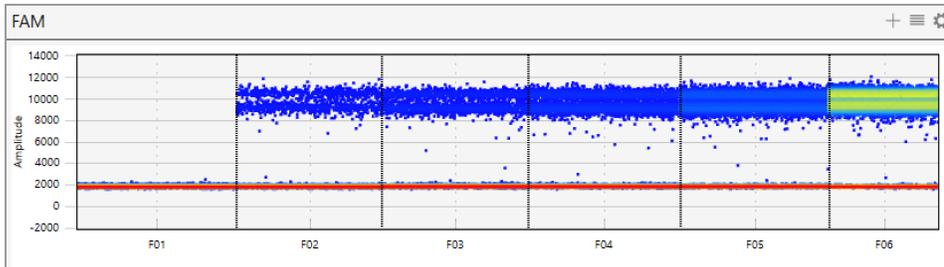
- Amplitude in a histogram plot

The histogram displays frequency of droplets at specific fluorescence amplitudes for each well or group of wells selected. The Channel 1 (FAM) histogram appears in blue and Channel 2 (HEX/VIC) histogram in green.



Note: The Histogram icon is enabled by default so a Histogram plot appears automatically for each fluorophore (channel).

■ Amplitude in a Heat Map view



When you select the 1D Amplitude tab, the toolbar options that allow you to change thresholds and show or hide the heat map and histogram appear above the plots.



Note: For detailed information on each toolbar option, see [Amplitude Plot Analysis Options on page 85](#).

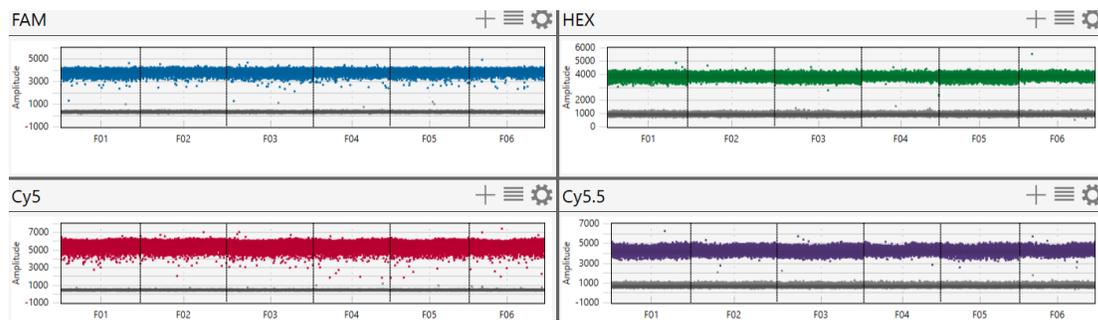
Default color assignments for 1D amplitude plots are

- Positive FAM = Blue
- Positive HEX = Green
- Positive Cy5 = Red
- Positive Cy5.5 = Violet
- Negative = Gray

The images in this section display data from a direct quantification experiment involving four targets:

- The FAM plot (FAM dye in channel 1) shows the positive droplets for the first target in blue.
- The HEX plot (HEX dye in channel 2) shows the positive droplets for the second target in green.
- The Cy5 plot (Cy5 dye in channel 3) shows the positive droplets for the third target in red.
- The Cy5.5 plot (Cy5.5 dye in channel 4) shows the positive droplets for the fourth target in violet.

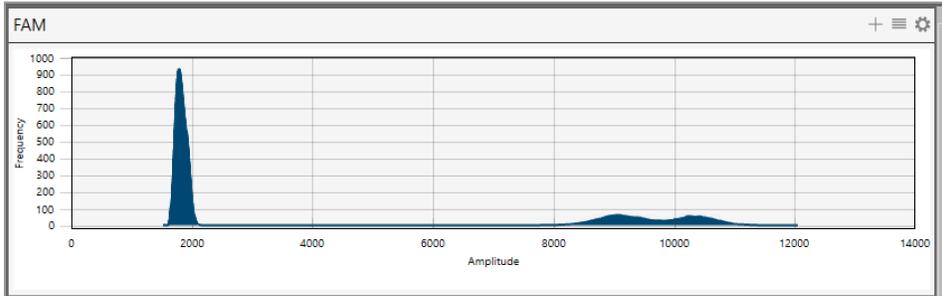
Negative droplets appear in grayscale in each plot.



For experiments where enough droplets are recognized, QX ONE Software automatically configures an amplitude threshold, which distinguishes between positive and negative droplets.

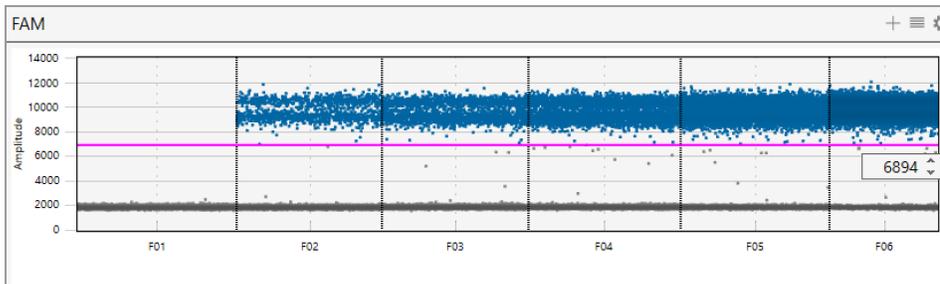
Note: Well data must meet certain quality metrics (minimum of 10,000 droplets) before the software will automatically calculate a threshold above which droplets are considered positive. You can use the manual thresholding tools to ensure correct quantification of positive and negative droplets.

By default, the Histogram icon () is enabled so the Histogram plot automatically appears with the amplitude plot.



To manually adjust the thresholds

1. Select one or more wells.
2. Tap any of the threshold icons () to set manual thresholds:
 - Regular threshold for a single well; when multiple wells are selected, you can set a different threshold for each well
 - Regular threshold for multiple wells, where you can set one threshold for all selected wells
 - Standard deviation threshold for a single well; when multiple wells are selected, you can set a different threshold for each well
 - Standard deviation for multiple wells, where you can set one threshold for all selected wells
3. Tap on the location in the plot where you want to draw the new threshold.
4. When the line appears, use the up or down arrows, or click and drag the threshold, to adjust the location.



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

Note: You can also manually enter a threshold number.

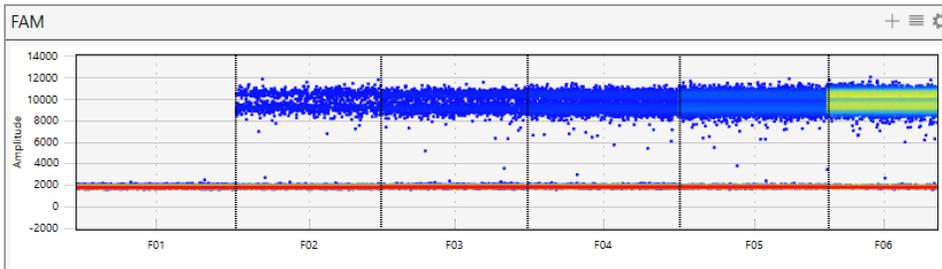
When you manually modify a threshold, the well selector displays an M for manual instead of the A for Automatic.

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	A	A	A	A	A	A	A	A	A	A
B	M	M	M	M	M	M	M	M	M	M	M	M
C	A	A	A	A	A	A	A	A	A	A	A	A
D	A	A	A	A	A	A	A	A	A	A	A	A
E												
F												
G												
H												

- (Optional) To reset to the automatic threshold, tap Auto.
- (Optional) To adjust the automatic threshold to consider tilt correction, tap Auto with Tilt.
- (Optional) To reset everything to the original display, tap Reset.

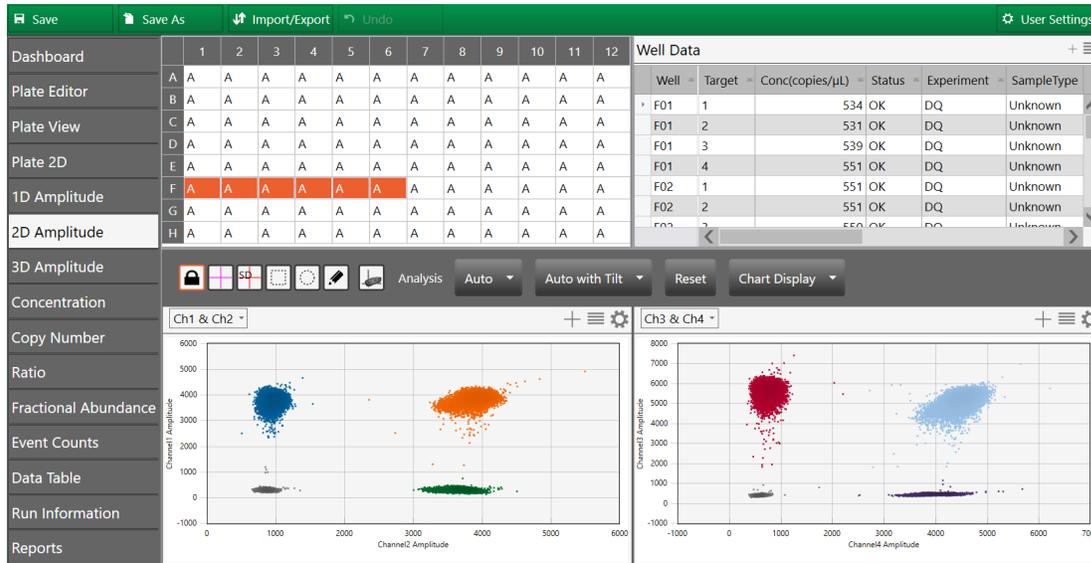
To view a heat map representation of the plot data

▶ Tap the  icon.



2D Amplitude

For the wells you select in the analysis view of the Plate Editor, the 2D Amplitude window illustrates fluorescence for four targets.



When you select the 2D Amplitude tab, the toolbar options allow you to change thresholds, manually cluster wells, and show or hide the heat map above the plots. By default, the left plot displays channel 1 and channel 2 data, and the right displays channels 3 and 4, but these combinations can be changed at the top left of the plot. For detailed information on each toolbar option, see [Amplitude Plot Analysis Options on page 85](#).



Note: For detailed information on each toolbar option, see [Amplitude Plot Analysis Options on page 85](#).

The images in this section display data from a direct quantification experiment involving four targets.

Default color assignments for 2D amplitude plots are

- Positive FAM = Blue
- Positive HEX/VIC = Green
- Positive FAM and HEX/VIC = Orange (may appear brown on some monitors)

Positive Cy5	=	Red
Positive Cy5.5	=	Violet
Positive Cy5 and Cy5.5	=	Light Blue
Double negative	=	Gray

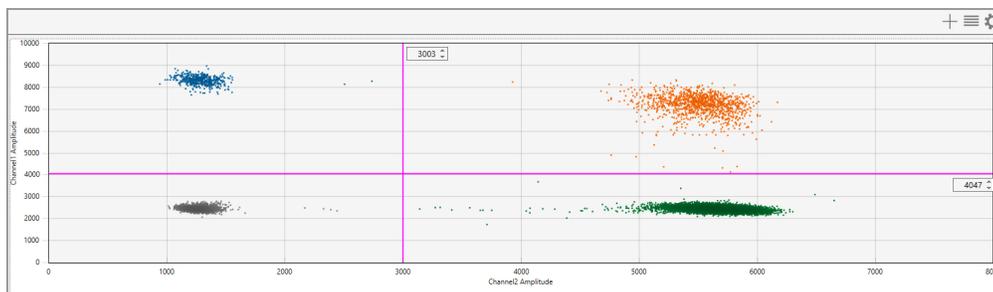
Note: Well data must meet certain quality metrics (minimum of 10,000 droplets) before QX ONE Software will automatically calculate a threshold above which droplets are considered positive. You can use the manual thresholding tools to ensure correct quantification of positive and negative droplets.

To use the 2D amplitude tools

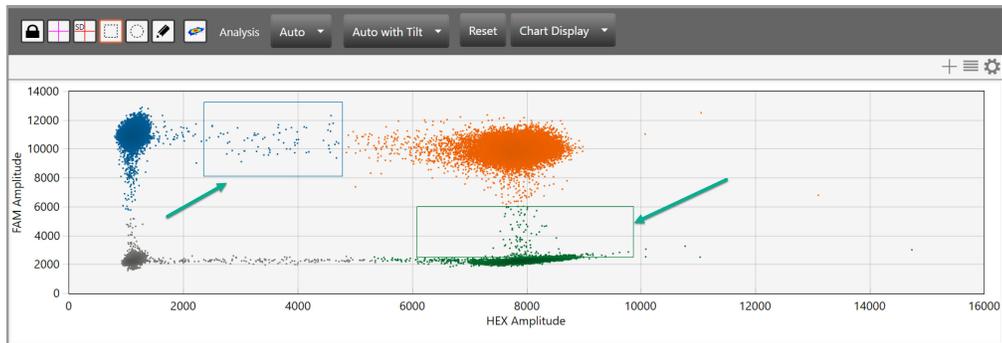
1. Open your data file in the Analysis module and select the 2D Amplitude tab.
2. Choose a threshold line or cluster option.

Note: Use the cluster option when the data is not orthogonal and the threshold lines do not adequately divide the populations of droplets.

- To adjust threshold lines, select a threshold line type () and then tap in the plot to apply crosshair lines. Use any of the following methods:
 - Drag the lines horizontally or vertically in the plot area.
 - Enter a new threshold value in the boxes at the end of each threshold line.
 - Tap the up and down arrows to incrementally adjust the numbers.



- To adjust thresholds using the cluster option, select a cluster type () and draw the chosen shape around the cluster of droplets to classify.



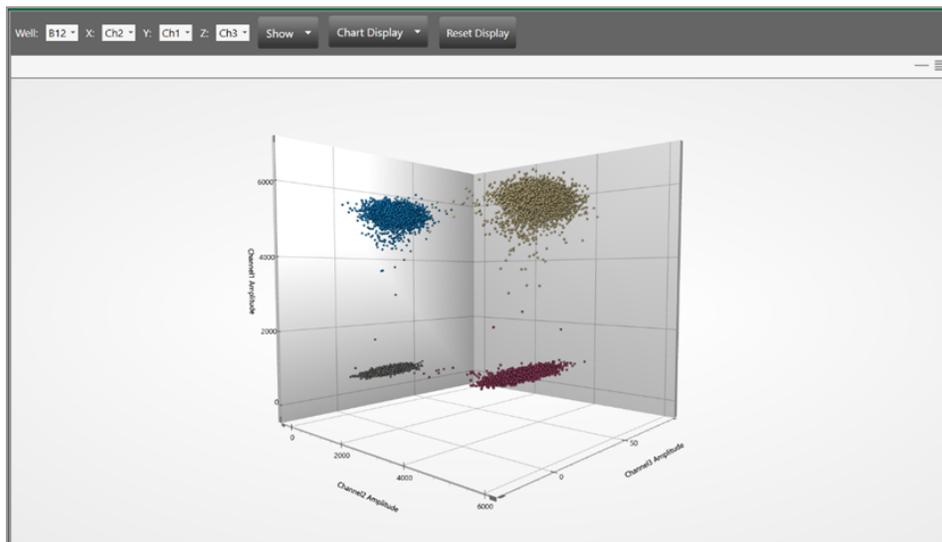
3. (Optional) To reset to the automatic threshold, tap Auto.
4. (Optional) To adjust the automatic threshold to consider tilt correction, tap Auto with Tilt.
5. (Optional) To reset everything to the original display, tap Reset.

3D Amplitude

When you select the 3D Amplitude tab, the toolbar options that allow you to select the well and choose which channels to display appear above the chart.

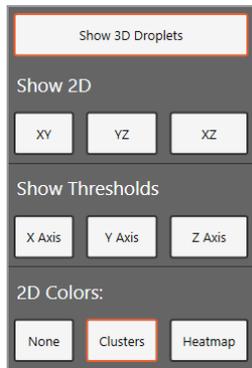


For the well selected from the toolbar, the 3D amplitude plot illustrates three targets in a single chart.



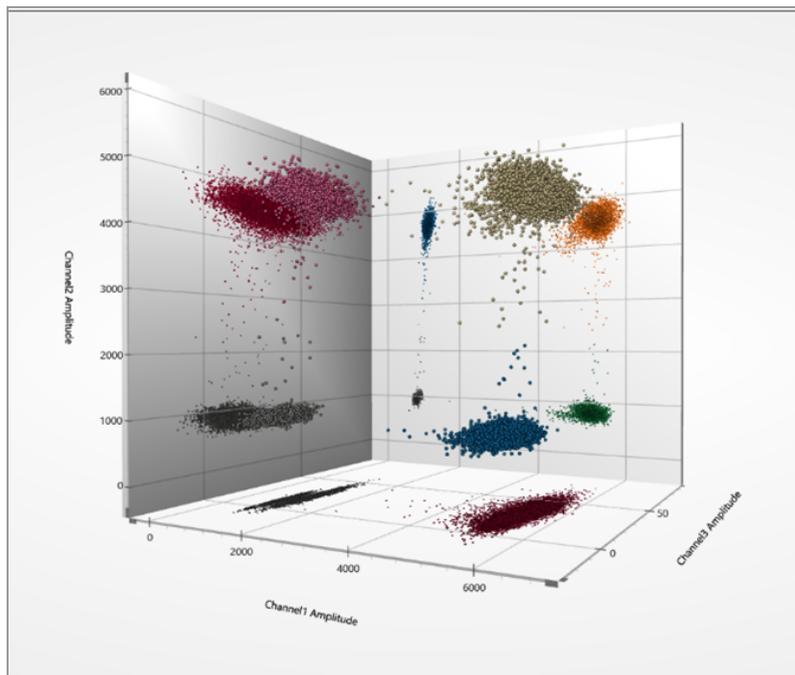
To use the different 3D amplitude viewing tools

1. Select Well.
2. Select the axis and color channels.
3. Tap Show and a dropdown menu will appear.



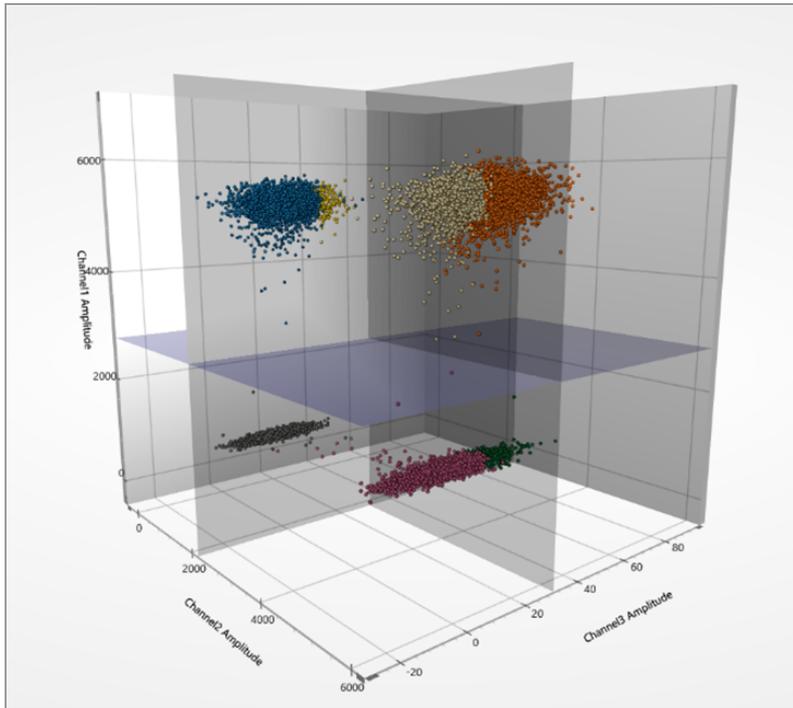
4. Select one or more of the Show 2D options to display 2D amplitude data behind the 3D droplets.

Note: You can display data in clusters or as a heat map.



5. Select one or more of the Show Threshold options to display thresholds previously set in the 1D or 2D amplitude display.

Note: You must set thresholds in the 1D or 2D Amplitude charts.



6. To restore the default orientation of the chart, tap Reset Display.

Statistical Probability Chart Analysis Options

QX ONE Software also provides chart displays where you can view the data from the original calculations, or choose different options to recalculate the results.

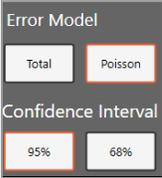
In charts reflecting statistical probability distributions, you can

- Show individual or merged well data.
- Change the error model and confidence level.

Note: For event counts, you can also alter the view mode to see positive, negative, or all droplet counts.

Table 25 explains the available options.

Table 25. Chart tool options for statistical probability distribution charts

Icon	Mode	Description
	Show both	Shows the calculated total or percentage as a merged calculation for all selected wells and as an individual calculation for each selected well
	Show merged	Shows the calculated total or percentage as a combined number for all selected wells with the same parameters Note: Well setup must match for each well before the software can merge them.
	Show individual	Shows the calculated total or percentage as an individual calculation for each selected well
	X-axis well	Shows the well number on the x axis
	X-axis sample	Shows a partial sample name on the x axis Note: You can select Well and Sample simultaneously.
	Error model and confidence interval	Select an error model and confidence level. The default choices are Poisson and 95%, to yield the highest confidence in the results. 

Important: The software uses the following multipliers in its calculations:

- For 95% confidence interval, the sigma multiplier is 1.96.
- For 68% confidence interval, the sigma multiplier is 1.0.

Table 26 identifies the additional options available in the Event Count window. These options are available for event counts only.

Table 26. Additional options for event counts

Icon	Mode	Description
<div style="border: 1px solid gray; padding: 5px; width: fit-content;">Positive</div>	View mode positive	Displays in a bar chart the number of positive droplets for each target in each well
<div style="border: 1px solid gray; padding: 5px; width: fit-content;">Negative</div>	View mode negative	Displays in a bar chart the number of negative droplets for each target in each well
<div style="border: 1px solid gray; padding: 5px; width: fit-content;">Total</div>	View mode total	Displays in a bar chart the number of total droplets in each well

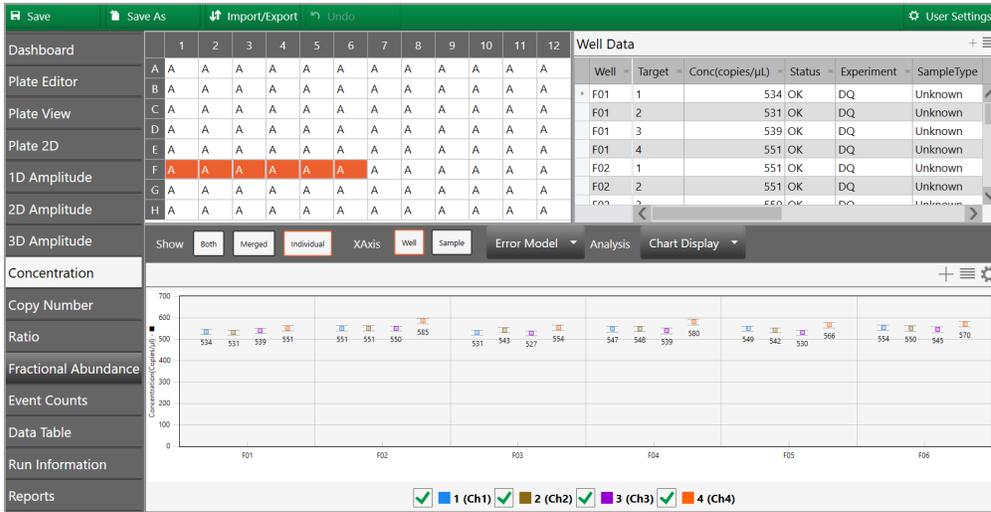
Concentration

Concentration measures the number of copies of the target molecules in each μl of sample based on the default settings. Use the toolbar to merge or separate the calculated results for each well, and to recalculate your results based on a different error model and confidence interval.

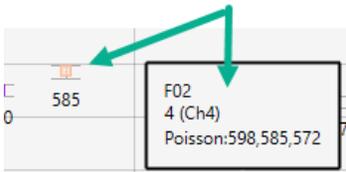
For information on toolbar options, see [Statistical Probability Chart Analysis Options on page 101](#).



The following image displays concentration data, across a sequence of wells.



QX ONE Software plots the averaged concentration for each target as a data point. Pause on a data point to see the high, median, and low numbers.



The software uses the formula and variables shown below.

Concentration Calculation

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

Where:

-ln	Negative natural logarithm
$V_{droplet}$	Volume of droplet
N_{neg}	Number of negative droplets
N	Total number of droplets

Confidence Interval Calculation

Note: If you select a 68% confidence interval, the multiplier is changed from 1.96 to 1.00.

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

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

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

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

Where:

$V_{droplet}$	Volume of droplet
N_{neg}	Number of negative droplets
N	Total number of droplets
p	p-value (probability of finding the target in the sample)
∂	Delta

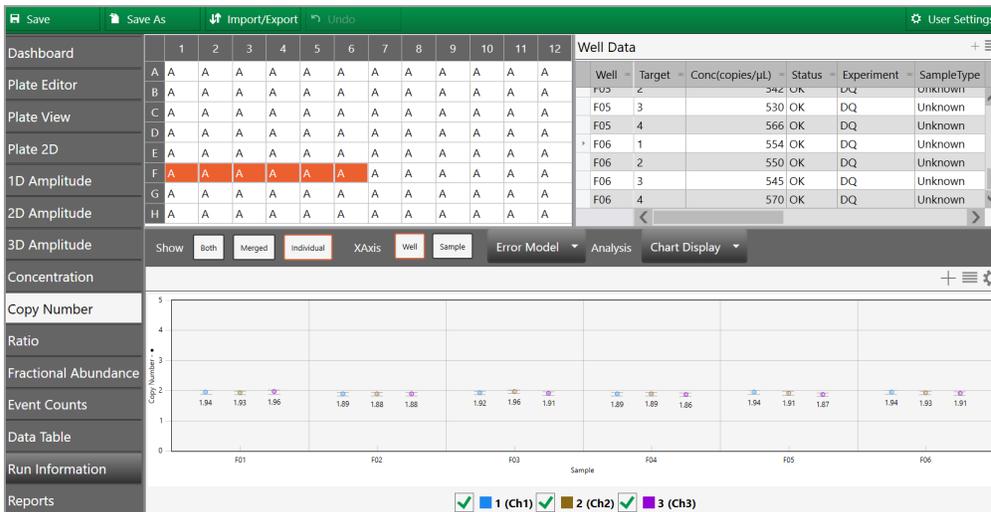
Copy Number

Important: To see copy number variation data, you must run a copy number variation (CNV) ddPCR experiment.

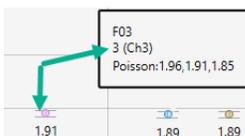
Copy number measures the probable count of the target gene, relative to the reference gene, to calculate the number gene targets per genome. Use the toolbar to merge or separate the calculated results for each well, and to recalculate your results based on a different error model and confidence interval. For information on toolbar options, see [Statistical Probability Chart Analysis Options on page 101](#).



The following image displays copy number data, across a sequence of wells.



QX ONE Software plots each copy number variation as a data point. Pause on a data point to see the high, median, and low numbers.



The software uses the formula and variables shown below.

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

Where:

A Target concentration

B Reference concentration

N_B Number of reference copies in genome (usually 2)

Notes:

Copy Number analysis involves determining the number of copies of a given target DNA sequence as compared to an invariant reference DNA sequence. Use the CNV analysis to determine structural variations, such as ploidy changes (addition or deletion of chromosomes), deletions (missing partial chromosomes) or duplications (repeated target sequences) in the genome.

The ability to discriminate, with statistical confidence, between consecutive CN states has been a technical challenge in copy number assessment. Fundamentally, as CN state increases, the percentage difference in target genomic material between states decreases. For example, for a given target locus, a CN of 3 is 50% more abundant in concentration per genome than a CN of 2, while a CN of 5 is only 25% more abundant per genome than a CN of 4, and so on.

Due to the massive partitioning of the reaction across up to 20,000 droplets per well, and the absolute nature and precision of the ddPCR concentration measurement, ddPCR CNV analysis enables the quantitative discrimination required to resolve consecutive copy number states beyond CN 3. For example, a 20% difference in target concentration between a CN 5 versus CN 6 sample can be reproducibly resolved using ddPCR. This discrimination is both a function of concentration differences between consecutive CN states, as well as the amount of DNA assayed per well.

Ratio

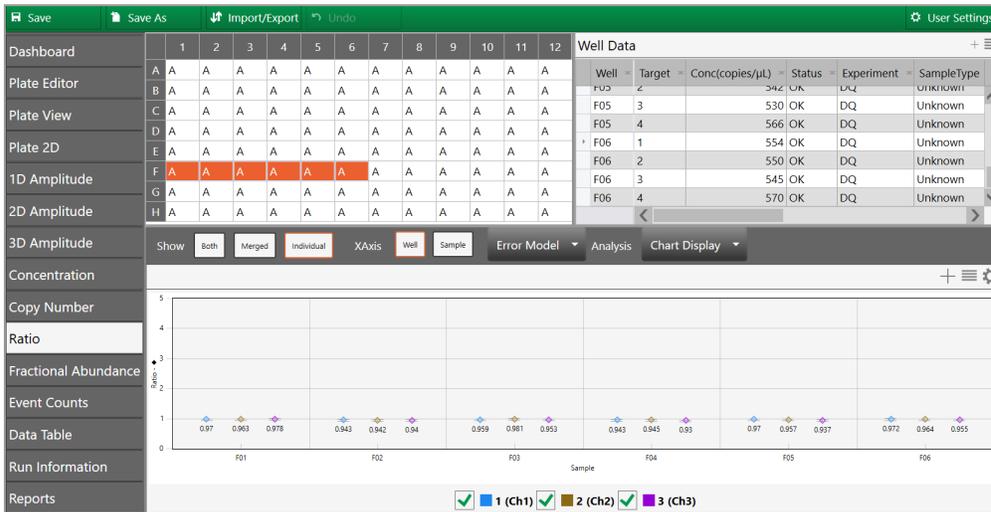
Ratio determines the quotient of two target molecules in each μl of sample based on the default settings.

T1:T2

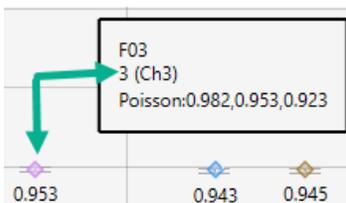
Use the toolbar to merge or separate the calculated results for each well, and to recalculate your results based on a different error model and confidence interval. For information on toolbar options, see [Statistical Probability Chart Analysis Options on page 101](#).



The following image displays ratio data, across a sequence of wells.



QX ONE Software plots each ratio as a data point. To see the confidence level numbers, pause on the data point.



The software uses the formula and variables shown below.

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

Where:

A	Target concentration
B	Reference concentration

Note: If no reference is selected, the target with the highest dye concentration in the highest channel will automatically be considered as the reference. If multiple references are selected, the geometric mean is used.

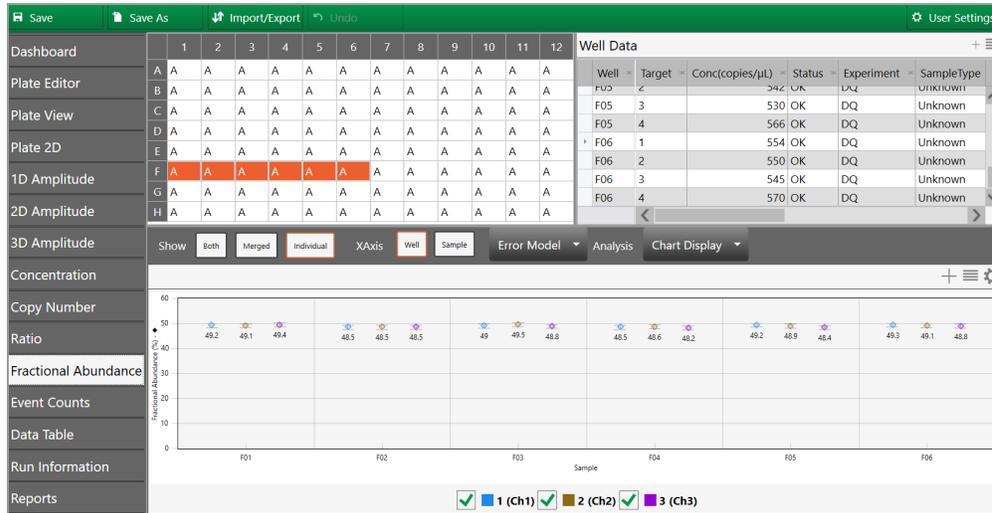
Fractional Abundance

Fractional Abundance determines the relative abundance of your targets in each µl of sample based on the default settings. Use the toolbar to merge or separate the calculated results for each well, and to recalculate your results based on a different error model and confidence interval. For information on toolbar options, see [Statistical Probability Chart Analysis Options on page 101](#).

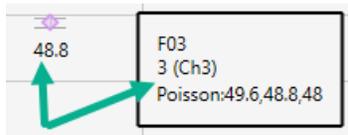


The following image displays fractional abundance data across a sequence of wells.

Chapter 6 Data Analysis Methodology



QX ONE Software plots the fractional abundance as a data point. To see the high and low confidence interval numbers, pause on the data point.



The software uses the formula and variables shown below.

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

Where:

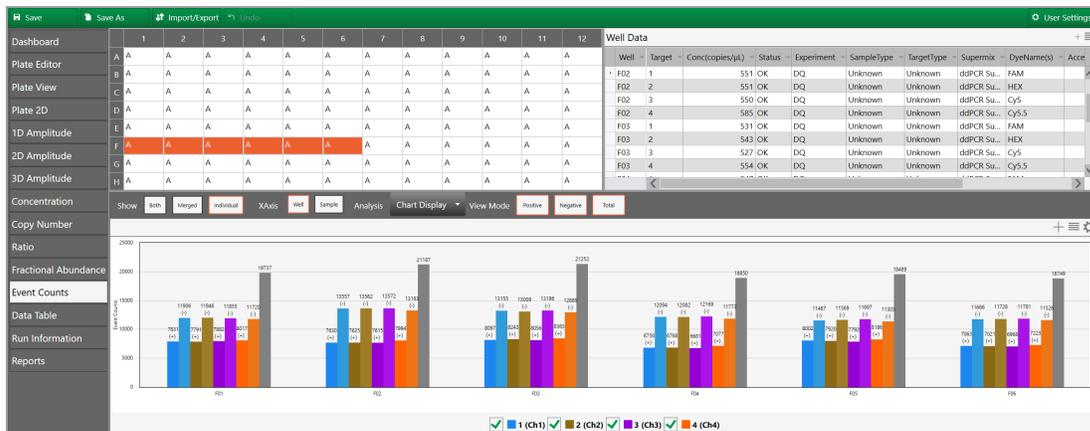
A Target concentration

B Reference concentration

Note: If no reference is selected, the target with the highest dye concentration in the highest channel will automatically be considered as the reference. If multiple references are selected, the geometric mean is used.

Event Counts

The Event Counts tab shows a bar chart view of the number of positive and negative droplets for each of the targets in each of the selected wells.



Use the toolbar to change the display by combining or separating data for each well. You can also show data by well name or by sample name, and you can show any combination of positive, negative, or combined droplet counts.



Chapter 7 Analysis Module Reporting

Use the Reporting function to create a variety of reports in the QX ONE Software Analysis module.

You can create reports on the fly from any analysis file or, if you need to generate specific reports on a regular basis, you can set up report templates for reuse.

This section contains information on available report elements, how to create analysis reports, and how to create report templates.

Report Elements

QX ONE Software allows you to generate reports for any or all of the following elements of the run and data.

Run Setup

- Plate Setup
 - Well
 - Perform droplet generation
 - Perform droplet reading
 - Experiment type
 - Sample description 1, 2, 3, and 4
 - Sample type
 - Supermix name
 - Plex mode
 - Target name
 - Target type
 - Signal channel 1, 2, 3, and 4
 - Reference copies
 - Well notes

- Plot?
- Rdq conversion factor
- Plate name
- GCR type
- Supermix information
- Thermal cycling protocol name
- Number of wells read
- Selected wells for report
- Username of the person who did the run
- Run start time
- Run complete time
- Initial wait elapsed time
- DG elapsed time
- Droplets wait time
- TC elapsed time
- DR elapsed time
- Errors
- Instrument serial number
- Firmware version
- Software version
- Software
- Run notes
- Lot information

1D Amplitude

- Threshold value
- Tilt correction
- Amplitude 1D chart

- Amplitude 1D chart for each well
- Amplitude 1D histogram chart
- Amplitude 1D histogram chart for each well

2D Amplitude

- Threshold value
- Tilt correction
- Amplitude 2D chart
- Amplitude 2D chart for each well

3D Amplitude

- X axis
- Y axis
- Z axis
- Show 3D droplets
- Show 2D XY
- Show 2D YZ
- Show 2D XZ
- Show X axis threshold
- Show Y axis threshold
- Show Z axis threshold
- Show 2D colors
- Amplitude 3D chart for each well

Thermal Cycling Protocol

- Temperature and time per step
- Number of steps

Concentration

- Error model
- Confidence model

- Chart

Copy Number

- Error model
- Confidence model
- Chart

Ratio

- Error model
- Confidence model
- Inverse
- Chart

Fractional Abundance

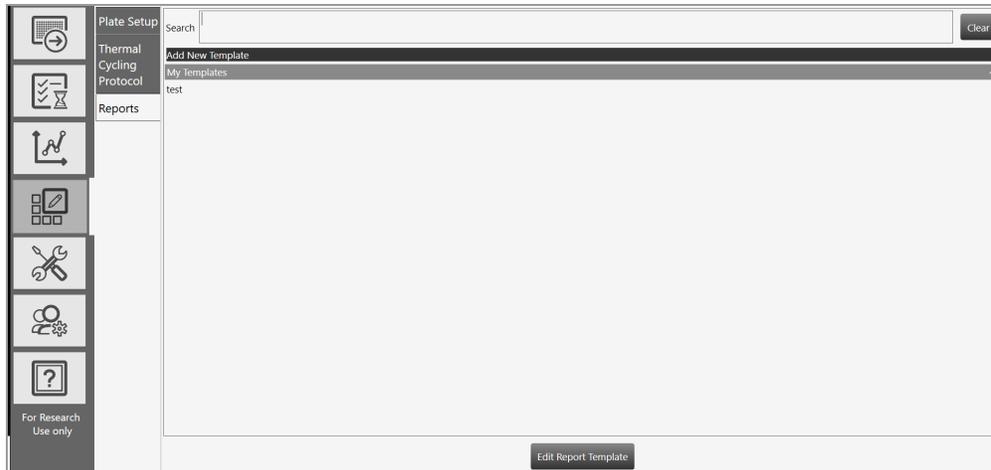
- Error model
- Confidence model
- Inverse
- Chart

Event Counts

Analysis Results

Creating Report Templates

If you are assigned the Create New Templates user privilege, you can create and save a new report template by editing the default report structure or by opening and editing an existing report, and then saving the modified structure as a new template.



Note: For information on creating reports, see [Report Elements on page 113](#).

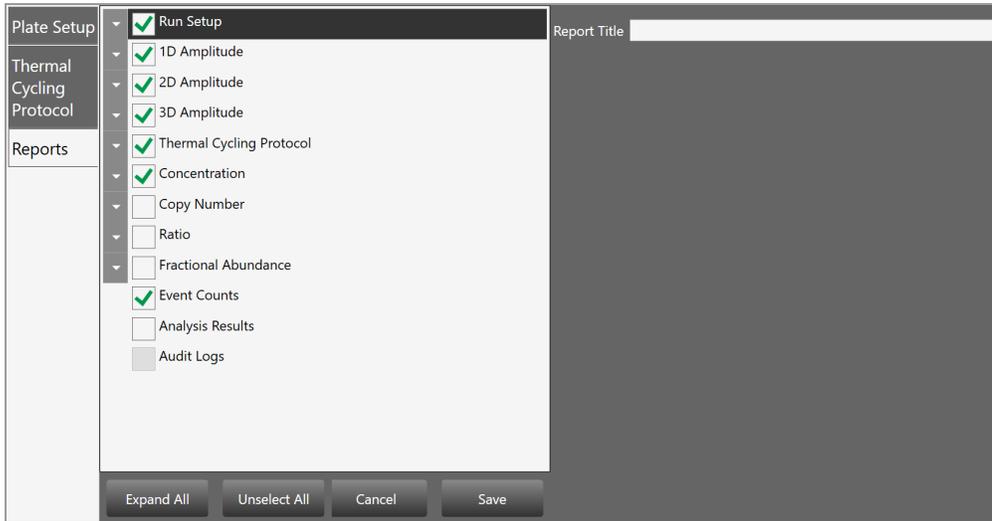
To create a report template

1. Select the Template Setup tab and tap Reports.

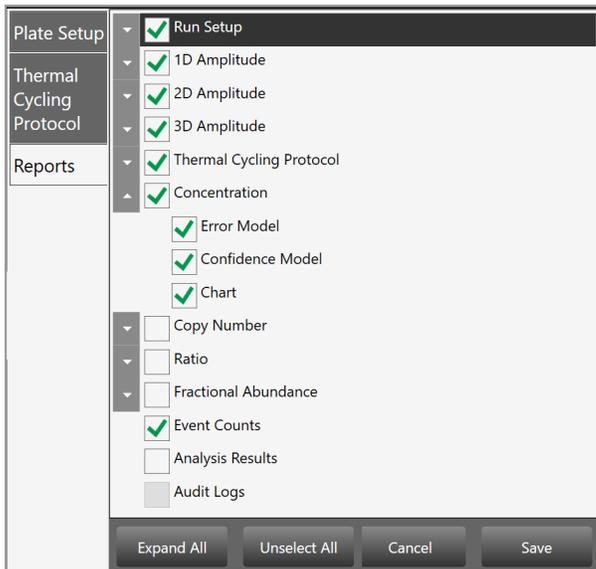


2. Do one of the following:
 - To create a new template, tap Add New Template.
 - To open an existing template, select the template and then tap Edit Report Template.

The default or selected layout appears.



3. Tap the arrow to the left of each item to expand the list of reportable items.



4. Select and clear checkboxes to create the report structure.

Tip: Use the buttons at the bottom of the screen to expand or collapse all categories at once, or to select or clear all checkboxes at once. You can also drag and drop the section to rearrange the order of appearance in the report, and drag and drop items in the section to reorder them.



5. In the Report Title field, enter a name for your report template.
6. Tap Save.
7. In the Save dialog box, enter a name for your template file.
8. Select a storage location.

Choose from the following:

- System Templates or My Templates

Important: Only one of these locations will be valid. If your administrator has specified preferred Locations in System Settings, you can save your templates to the System Templates folder or the Shared folder. If preferred locations are not specified, you can save them to the My Templates folder or the Shared Templates folder.

- Shared Templates

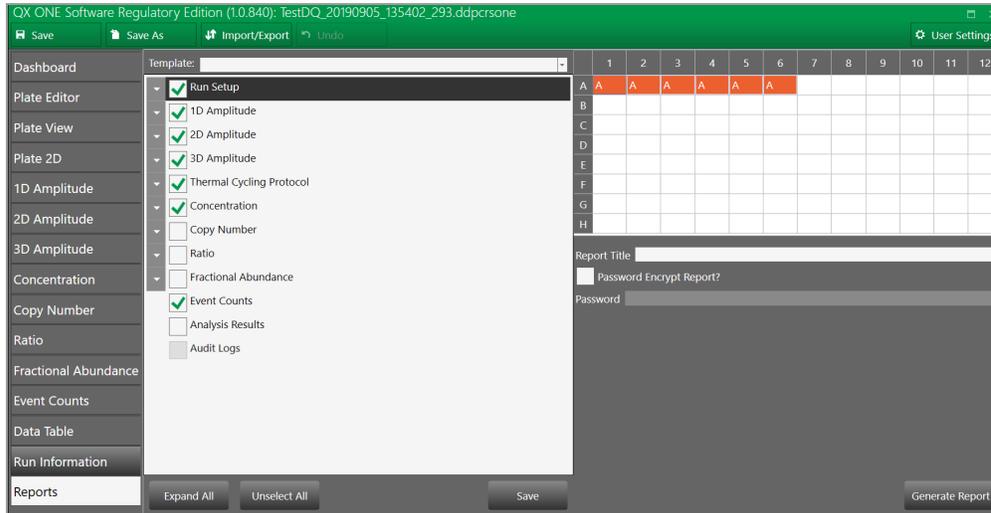
You have the option to save your templates to this folder to make them available to all users.

9. Tap Save again to save the report format as a template.

Creating Analysis Reports

The reporting function allows you to run reports on elements of a run and corresponding analysis data from the Analysis module.

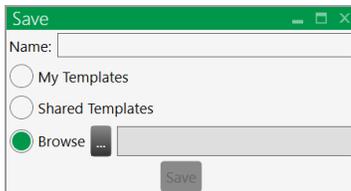
Chapter 7 Analysis Module Reporting



1. Open an analysis data file and tap Reports.
2. Do one of the following:
 - Work in the default report layout, which appears automatically.
 - Select a report template from the Template dropdown list.
3. Tap the arrow to the left of each item to expand the list of reportable items.
4. Keep the original configuration, or select and clear check boxes to create a new report format.
5. Change the order of categories and items in a report. Drag the options to the required position. Items can be reordered only within the categories to which they belong. You can also drag and drop the section to rearrange the order of appearance in the report, and drag and drop items in the section to reorder them.
6. (Optional) Use the options located in the lower portion of the window to expand or collapse the tree, and to select or clear all checkboxes.



7. In the Report Title field, enter a name for your report.
8. (Optional) To save the structure as a new template, tap Save and then enter a name, and select a storage location.



Choose from:

- Templates you created and saved, which are automatically saved to one of the following paths:
 - The path specified in your user preferences; these appear under My Templates
 - The path specified under Preferred Locations in System Settings, if your system administrator has specified the path for all users, these appear under System Templates

Important: If your administrator has specified the Preferred Location for all users, the path overrides the path specified in user preferences. Preferred locations can be enabled only by users with the System Settings user privilege.

- Templates created and saved by any user and designated as shared; these appear under Shared Templates.

9. To generate the report, tap Generate Report.

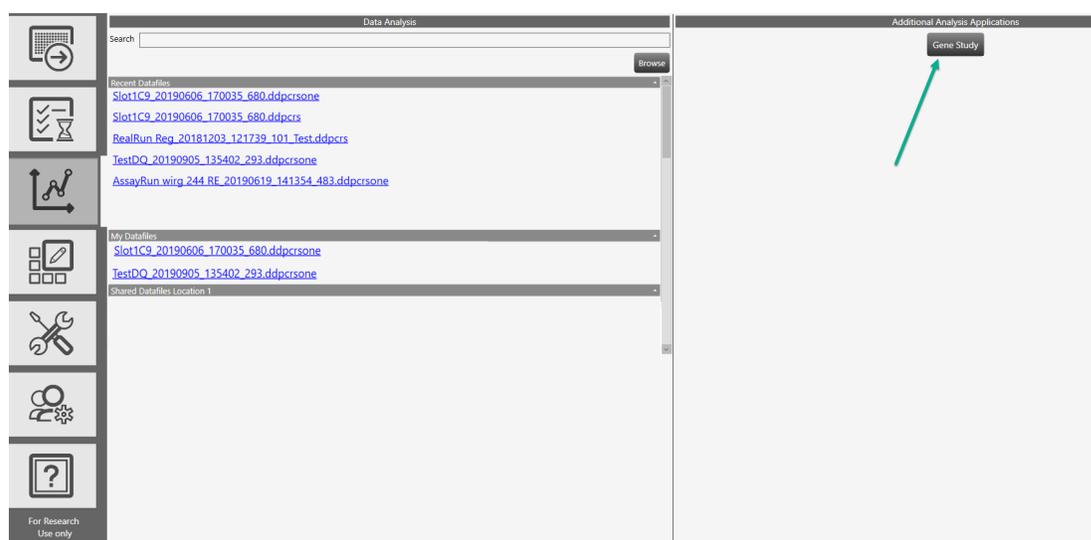
QX ONE Software creates a PDF report that opens immediately.

In each page header, the software automatically displays the user name of the person creating the report, date and time stamps, and the name of the analysis file used to generate the report.

Chapter 8 Gene Study Module

You access the Gene Study module from the Data Analysis window.

Note: The Gene Study module is not available from the touch screen computer.

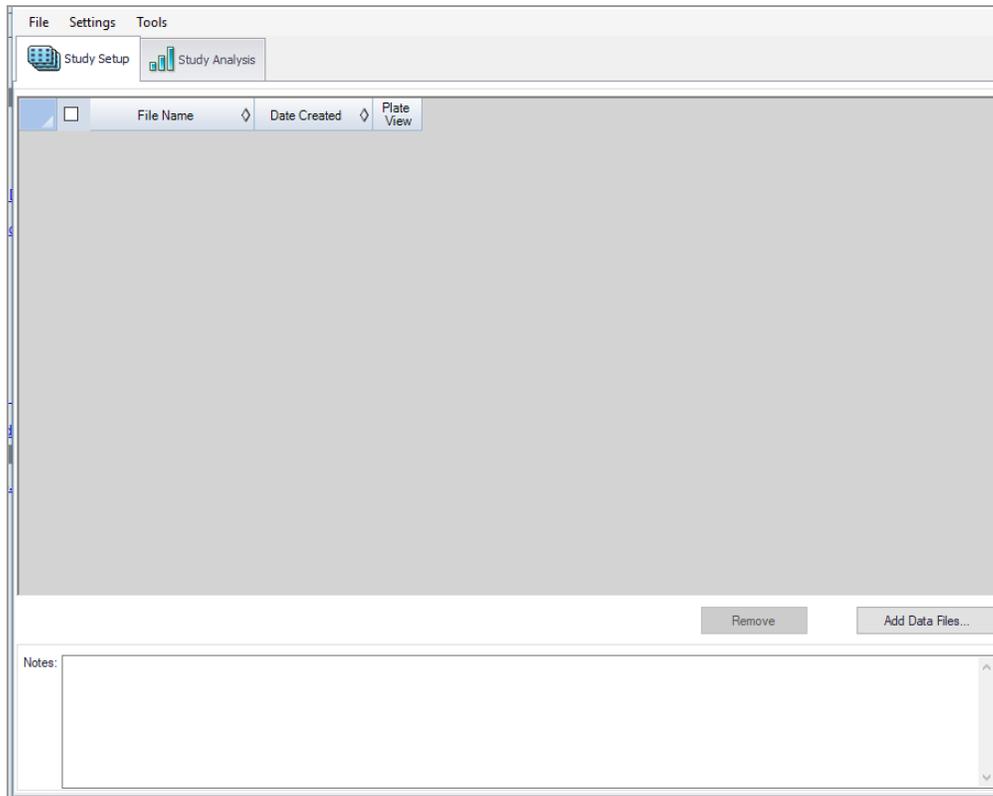


To create a gene study, you add one or more data files and then compare gene expression data from one or more ddPCR experiments.

Note: The maximum number of samples you can analyze in a gene study is limited by the size of the computer's RAM and virtual memory.

To open the Gene Study module

- ▶ Click Gene Study.



The Gene Study dialog box includes two tabs:

- **Study Setup** — manages the run files in the gene study

Important: Adding or removing data files in a gene study does not change the data in the original file.

- **Study Analysis** — displays the gene expression data for the combined runs.

From these tabs you can add files to set up a gene study, analyze your data in charts and plots, and run reports on the gene study data.

Gene Study Options

The Gene Study module provides the menus and corresponding options described below.

- **File** — Open, save, and close the gene study, and add files
- **Settings** — Restore the default layout
- **Tools** — Create a report

To open a gene study file

1. Tap File → Open.
2. In the Open dialog box, navigate to and select the data file, and then tap Open.
The file opens in the Gene Study module.

To save a gene study file

1. Tap File and select Save or Save As.
2. If prompted, enter a file name and tap Save.
3. If prompted to overwrite an existing file, tap Yes or No.

To close a gene study file

- ▶ Tap File → Close.
Note: If you have not saved the file, an advisory prompt appears.

To add data files

1. Tap File → Add Data Files.
2. In the Open dialog box, navigate to the data files to be added to the study.
3. Select one or more files and then tap Open.
The files are added to the Study Setup window.

To restore default settings

- ▶ Tap Settings → Restore default settings.
Note: At least one modified data file must be open.

To generate a report

- ▶ See [Gene Study Reports on page 133](#).

Setting up the Gene Study

Use the Study Setup window to

- Add data files to include in the study
- Remove data files from the study
- Select data files for viewing and analysis
- Show the plate view for a data file

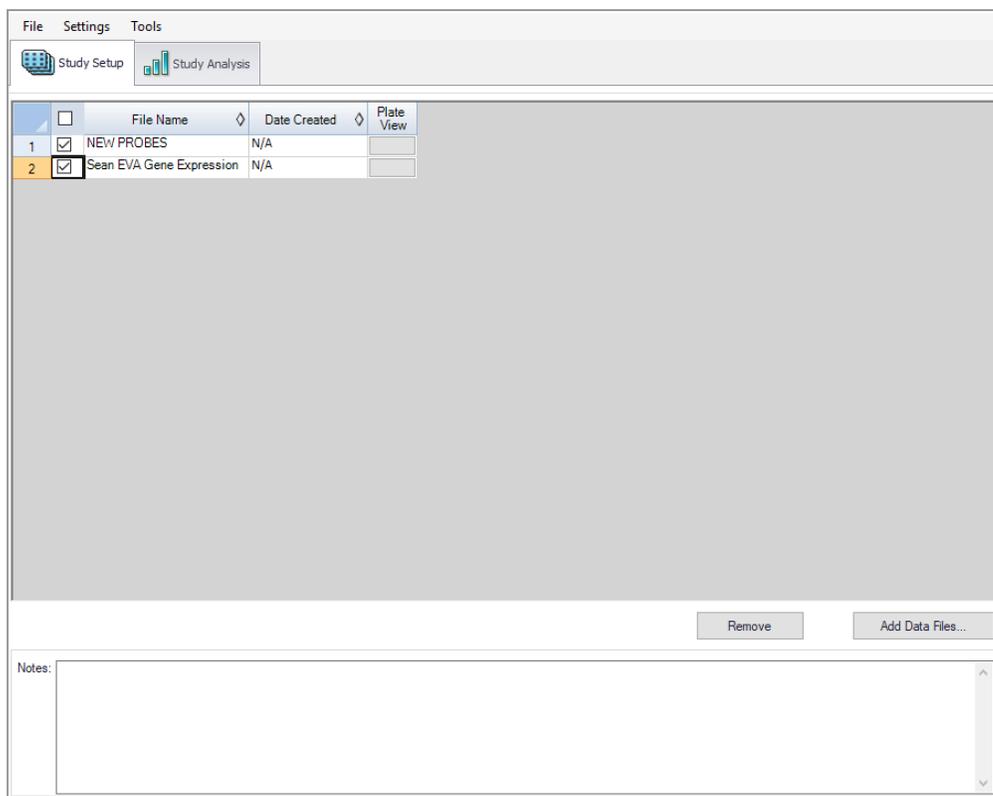


Table 27 defines each column in Study Setup.

Table 27. Gene Study Setup Fields

Column Title	Description
File Name	Name of the data file
Date Created	Date the file was imported into the Gene Study module
Plate View	Opens a map of the plate with the data for each dye in each well included in the Gene Study

Gene Study Plate View

	1	2	3	4	5	6	7	8	9	10	11	12
A	FAM	FAM	FAM	FAM	FAM	FAM	FAM					
	8.014	7.889	6.696	9.344	7.082	9.086	0.000					
	1	1	1	1	1	1	NTC					
B	Unk	Unk	Unk	Unk	Unk	Unk	Unk					
	FAM	FAM	FAM	FAM	FAM	FAM	FAM					
	4.705	3.816	3.995	4.353	4.447	4.647	0.000					
C	2	2	2	2	2	2	NTC					
	Unk	Unk	Unk	Unk	Unk	Unk	Unk					
	FAM	FAM	FAM	FAM	FAM	FAM	FAM					
D	2.369	2.169	2.223	1.885	2.357	1.747	0.000					
	3	3	3	3	3	3	NTC					
	Unk	Unk	Unk	Unk	Unk	Unk	Unk					
E	FAM	FAM	FAM	FAM	FAM	FAM	FAM					
	1.000	1.226	1.011	1.298	1.175	0.929	0.000					
	4	4	4	4	4	4	NTC					
F	Unk	Unk	Unk	Unk	Unk	Unk	Unk					
	FAM-1	FAM-1	FAM-1	FAM-1	FAM-1	FAM-1	FAM-1					
	32.904	31.401	29.440	32.486	31.268	33.993	0.000					
G	1	1	1	1	1	1	NTC					
	Unk	Unk	Unk	Unk	Unk	Unk	Unk					
	FAM-1	FAM-1	FAM-1	FAM-1	FAM-1	FAM-1	FAM-1					
H	33.789	33.305	33.518	32.269	30.299	32.104	0.000					
	2	2	2	2	2	2	NTC					
	Unk	Unk	Unk	Unk	Unk	Unk	Unk					
I	FAM-1	FAM-1	FAM-1	FAM-1	FAM-1	FAM-1	FAM-1					
	32.904	31.401	29.440	32.486	31.268	33.993	0.000					
	1	1	1	1	1	1	NTC					

Navigation: |< >| FAM / HEX

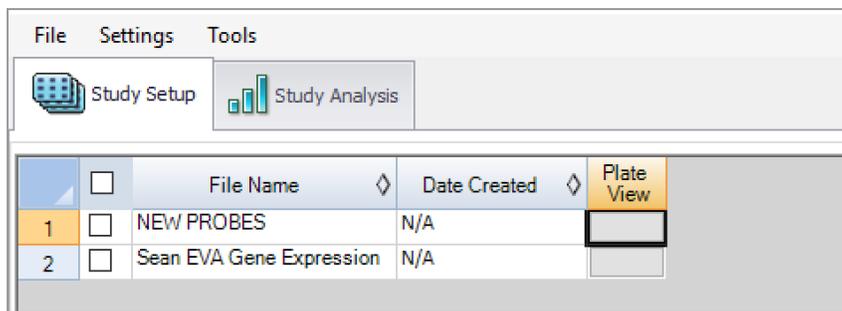
Close

Adding Data Files

To add data files

1. On the Study Setup tab, tap Add Data Files.
2. In the Open dialog box, navigate to the data files to be added to the study.
3. Select one or more files and then tap Open.

The files are added to the Study Setup window.



The screenshot shows the 'Study Setup' window with a menu bar (File, Settings, Tools) and two tabs: 'Study Setup' (active) and 'Study Analysis'. Below the tabs is a table with the following data:

	<input type="checkbox"/>	File Name	Date Created	Plate View
1	<input type="checkbox"/>	NEW PROBES	N/A	<input type="text"/>
2	<input type="checkbox"/>	Sean EVA Gene Expression	N/A	<input type="text"/>

Removing Data Files

To remove data files

- ▶ Select the checkbox next to the file and then tap Remove.

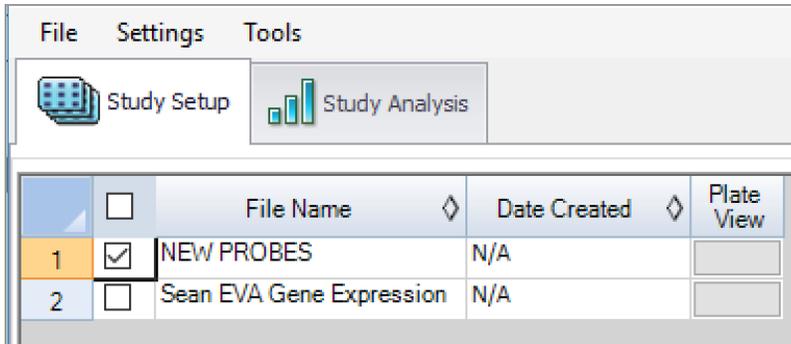
The file is removed from the Study Setup window.

Note: The Remove button is disabled until you select a file.

Selecting Data Files for Analysis

To select data files for analysis

1. Select the checkboxes next to the files to be included in the analysis.

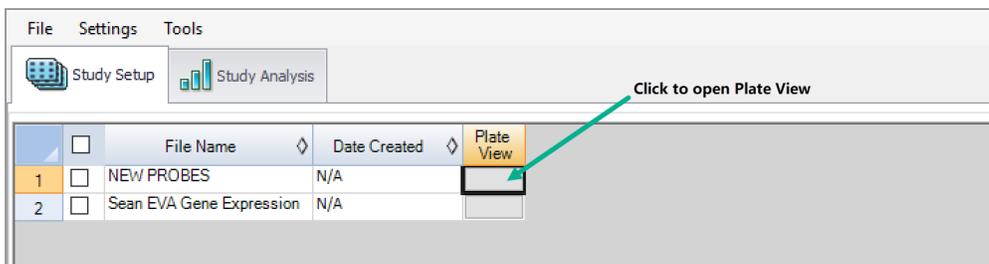


2. Select the Study Analysis tab to see the analysis results. For information, see [Using the Study Analysis Features on page 131](#).

Showing the Plate View

To show the plate view

1. In the Plate View column, tap the button for the plate view to be displayed.



The Plate view appears.

Chapter 8 Gene Study Module

Gene Study Plate View

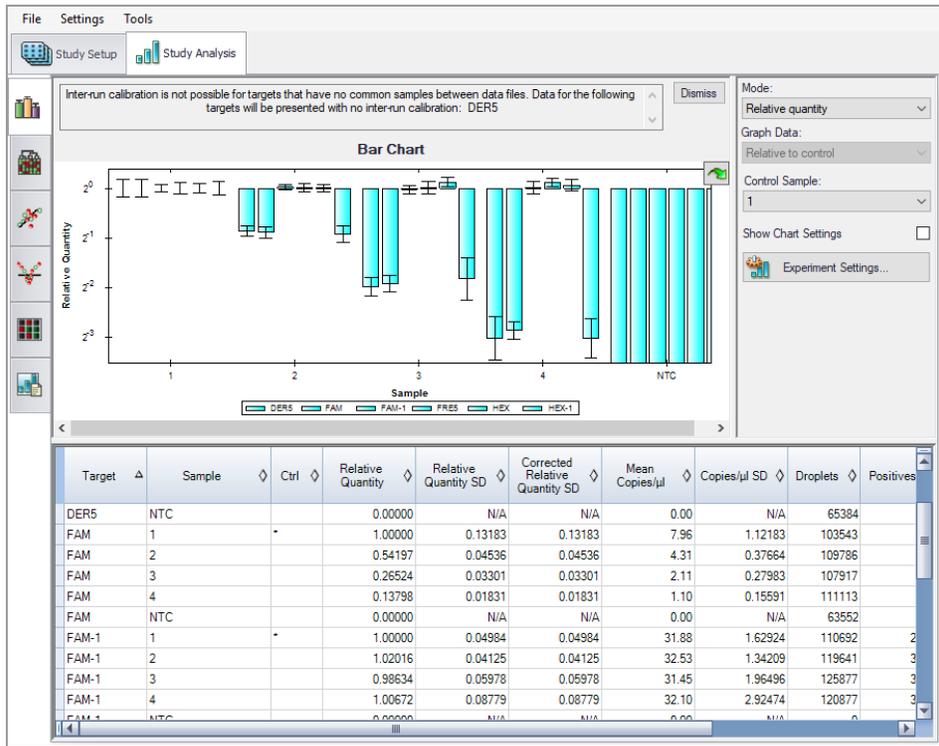
	1	2	3	4	5	6	7	8	9	10	11	12
A	FAM	FAM	FAM	FAM	FAM	FAM	FAM					
	8.014	7.889	6.696	9.344	7.082	9.086	0.000					
	1	1	1	1	1	1	NTC					
	Unk	Unk	Unk	Unk	Unk	Unk	Unk					
B	FAM	FAM	FAM	FAM	FAM	FAM	FAM					
	4.705	3.816	3.995	4.353	4.447	4.647	0.000					
	2	2	2	2	2	2	NTC					
	Unk	Unk	Unk	Unk	Unk	Unk	Unk					
C	FAM	FAM	FAM	FAM	FAM	FAM	FAM					
	2.369	2.169	2.223	1.885	2.357	1.747	0.000					
	3	3	3	3	3	3	NTC					
	Unk	Unk	Unk	Unk	Unk	Unk	Unk					
D	FAM	FAM	FAM	FAM	FAM	FAM	FAM					
	1.000	1.226	1.011	1.298	1.175	0.929	0.000					
	4	4	4	4	4	4	NTC					
	Unk	Unk	Unk	Unk	Unk	Unk	Unk					
E	FAM-1	FAM-1	FAM-1	FAM-1	FAM-1	FAM-1	FAM-1					
	32.904	31.401	29.440	32.486	31.268	33.993	0.000					
	1	1	1	1	1	1	NTC					
	Unk	Unk	Unk	Unk	Unk	Unk	Unk					
F	FAM-1	FAM-1	FAM-1	FAM-1	FAM-1	FAM-1	FAM-1					
	33.789	33.305	33.518	32.269	30.299	32.104	0.000					
	2	2	2	2	2	2	NTC					
	Unk	Unk	Unk	Unk	Unk	Unk	Unk					
	FAM-1	FAM-1	FAM-1	FAM-1	FAM-1	FAM-1	FAM-1					

Navigation: FAM HEX

Close

2. Click Close to close the view.

Using the Study Analysis Features



The Study Analysis tab displays the data from all runs in the gene study in the views described in [Table 28](#).

Table 28. Analysis Toolbar

Button	Description
	Size of bars correspond to normalized expression or relative quantity
	Data is arranged in a hierarchy based on the degree of similarity of expression for different targets and samples

Table 28. Analysis Toolbar, continued

Button	Description
Scatter Plot 	Dot plot of normalized expression of targets for a control versus an experimental sample
Volcano Plot 	Dot plot of change in expression (regulation) of a target for an experimental sample compared to a control
Heat Map 	Plate layout grid showing target regulation for an experimental compared to a control sample based on relative normalized expression Note: If the same targets are in the same location on multiple plates but with different samples, use the dropdown menu to select a particular plate for analysis.
Results Table 	Provides results in a tabular format

Gene Study Reports

Use the Gene Study Report dialog box to arrange the gene study data into a report. Table 34 lists all the options available for a gene study report.

Table 29. Gene Study Report Categories

Category	Option	Description
Header		
	Report Information	Date, user name, data file name, data file path, and the selected well group
	Gene Study File List	List of all the data files in the gene study
	Notes	Notes about the data report
Study Analysis Bar Chart		
	Analysis Settings	List of the selected parameters
	Chart	Gene expression bar chart showing the data
	Target Names	List of targets in the gene study
	Sample Names	List of samples in the gene study
	Data	Spreadsheet that shows the data
	Target Stability	Target stability data
	Inter-run Calibration	Inter-run calibration data
Study Analysis Clustergram, Scatter Plot, Volcano Plot, and Heat Map		
	Analysis Settings	Settings for each chart type
	Chart	Gene expression chart showing the data
	Data	Spreadsheet listing the data in each target

Creating a Gene Study Report

Before creating a report, make any needed adjustments to the gene study data and charts.

To create a gene study report

1. Select Tools → Reports in the Gene Study menu to open the Report dialog box.
2. Select the options you want to include in the report.

The report opens with default options selected. Select or clear the checkboxes to change whole categories or individual options within a category.

For information on the available options to display, see [Gene Study Reports on page 133](#).

3. Change the order of categories and items in a report.

Drag the options to the required position. Note that Items can be reordered only within the categories to which they belong.

4. Click Update Report to update the Report Preview window with any changes.
5. Print or save the report:
 - a. Click the Print Report button on the toolbar to print the current report.
 - b. Select File → Save to save the report in PDF, MHT or MHTML file format and select a location to save the file.

Note: MHT and MHTML are Microsoft formats.
 - c. Select File → Save As to save the report with a new name or in a new location.
6. (Optional) To save the current report settings as a template, select Template → Save or Save As.

Appendix A Experiment Examples

This section contains setup and analysis information for the following sample experiments:

- Direct Quantification, using the Amplitude Multiplex assay type
- Copy Number Variation, using the Probe Mix Triplex assay type
- Drop-Off, using the Basic Drop-off Assay type for genome edit detection
- Direct Quantification, using the Advanced Classification Method assay type

Direct Quantification Multiplexed

You can increase the number of targets to be evaluated by using the Amplitude Multiplex option.

Setting Up Experiment Parameters

To set experiment parameters

1. Open the analysis data file and select the Plate Editor tab.

The screenshot shows the 'Plate Editor' interface. On the left is a 96-well plate grid with columns 1-12 and rows A-H. The 'A1' well is highlighted in red. On the right is the configuration panel. Under 'Experiment Type', 'Direct Quantification (DQ)' is selected. Under 'Sample Description', 'DNA1' and 'DNA2' are entered. Under 'Sample Type', 'Unknown' is selected. Under 'Assay Type', 'Amplitude Multiplex' is selected. The 'Target Info' table is as follows:

Target Name	Target Type	Signal Ch1	Signal Ch2	Signal Ch3	Signal Ch4
1	Unkn	FAM Lo	None	None	None
2	Unkn	FAM Hi	None	None	None
3	Unkn	None	HEX Lo	None	None
4	Unkn	None	HEX Hi	None	None
5	Unkn	None	None	Cy5 Lo	None
6	Unkn	None	None	Cy5 Hi	None
7	Unkn	None	None	None	Cy5.5 Lo
8	Unkn	None	None	None	Cy5.5 Hi

2. Select one or more wells to be assigned well analysis attributes.
 3. Change the experiment type to Direct Quantification (DQ), and then tap Apply.
 4. (Optional) Change the sample description identifiers.
 5. (Optional) Change the sample type. The default type is Unknown.
- Note:** You cannot change the supermix that was used during data collection.
6. Under Assay Type, select Amplitude Multiplex and tap Apply.
Eight target rows appear.
 7. Change the target name and type (unknown or reference) for each target in the well.
 8. If you select Reference, you can change the number of reference copies. The default is 2.

If no reference is selected, the target with the highest dye concentration in the highest channel is used as the reference. For example, if there are targets in all four channels, the target in Channel 4 is used; however if there are targets only in the first three channels, then Channel 3 is the reference target.

If more than one reference is selected in the well, checkboxes appear to the right for using the reference in the plot.

Note: Each Reference target enables ratio calculations in the results.

9. (Optional) Change the high and low target identifiers.

Amplitude Multiplex allows for up to two assays in each channel, indicated by Lo and Hi labels. In this multiplex mode, each target may have a signal for only one channel. You can

- Select a different dye, if the channel supports two dyes (for example change HEX to VIC or VIC to HEX)
- Select a different channel for the target (for example, change FAM to HEX)
- Change Hi to Lo or Lo to Hi

10. Tap Apply.

Tip: Optionally, to expedite plate setup you can copy and paste data from one well to another, and you can tap Undo to undo an applied well setting.

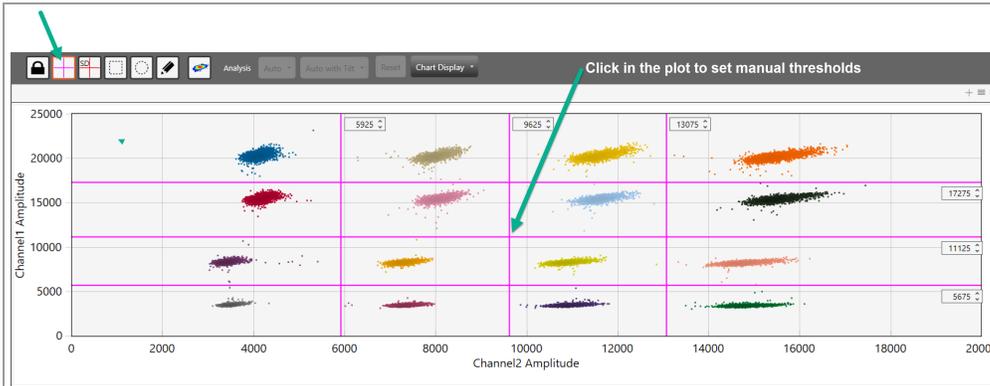
Viewing and Adjusting Results

Automatic thresholding is not available for Amplitude Multiplex experiments.

For clusters that are easily identifiable across the chart, use Threshold Lines mode to set manual thresholds. If the data is not orthogonal and the threshold lines do not adequately divide the populations of droplets, use Threshold Clusters mode to set manual thresholds.

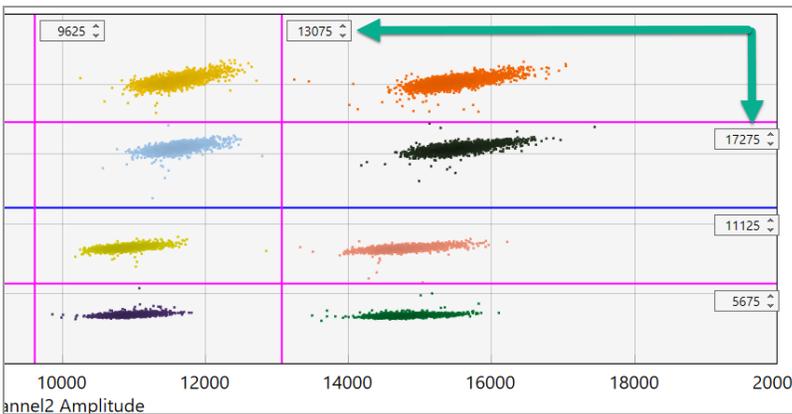
To set thresholds in the 2D Amplitude window

1. Select the 2D Amplitude tab.



2. On the toolbar, tap the Threshold Line Mode button.
3. Tap anywhere on the plot to apply “best fit” threshold lines to the data.
4. To adjust the thresholds, drag the lines horizontally or vertically in the plot area.

Tip: You can also enter a specific threshold value in the boxes at the end of each threshold line.



To apply and adjust manual clusters

1. Tap any of the three cluster mode buttons (square, circle, or freehand).
2. Press the left mouse button and draw the chosen shape around the cluster of droplets to be classified first.

Tip: When you release the mouse button, a pop-up tool appears to help you select the correct target combination. Target names are shown along the two axis based on the signal values assigned during plate setup.



3. To classify droplets as positive or negative for each given target, select the colored box that best represents the location of the cluster of droplets.

Copy Number Variation Triplexed

A CNV experiment with probe mix triplexing uses two targets and a reference in one or two groups to produce analysis data.

Setting Up Experiment Parameters

To set experiment parameters

1. Open the analysis data file and select the Plate Editor tab.
2. Select one or more wells to be assigned well analysis attributes.
3. Set the experiment type to Copy Number Variation (CNV), and tap Apply.
4. (Optional) Change the sample description identifiers.
5. (Optional) Change the sample type. The default type is Unknown.

Note: You cannot change the supermix that was used during data collection.

6. Tap Apply.
7. From the Assay Type dropdown list, select Probe Mix Triplex and tap Apply.

QX ONE Software displays three targets.

Important: You cannot add or delete triplex targets.

8. Enter target names and select the target type (unknown or reference) for each target in the well.

This creates two groups of three targets each.

9. Assign the targets as follows:

Group 1

Target	Target Type	Signal Ch1	Signal Ch2	Ref Copies	Plot?
Target 1	Unknown	FAM	None	N/A	N/A
Target 2	Unknown	None	HEX/VIC	N/A	N/A
Target 3	Reference	FAM	HEX/VIC	2	Yes

Group 2

Target	Target Type	Signal Ch3	Signal Ch4	Ref Copies	Plot?
Target 4	Unknown	Cy5	None	N/A	N/A
Target 5	Unknown	None	Cy5.5	N/A	N/A
Target 6	Reference	Cy5	Cy5.5	2	Yes

10. Tap Apply.

Tip: Optionally, to expedite plate setup you can copy and paste data from one well to another, and you can tap Undo to undo an applied well setting.

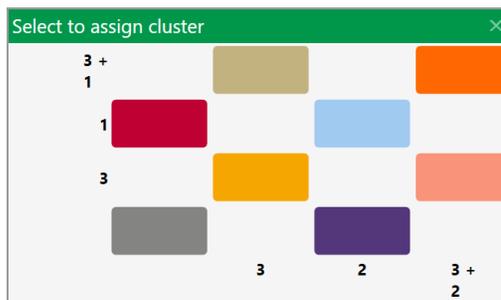
Viewing and Adjusting Results

Note: Thresholding is not available for Probe Mix Triplex experiments.

To see the results

1. After all wells are configured, select the 2D Amplitude tab to evaluate the data and manually set clusters.
2. Tap any of the three cluster mode buttons (square, circle, or freehand).
3. Press the left mouse button and draw the chosen shape around the cluster of droplets to be classified first.

Tip: When you release the mouse button, a pop-up tool appears to help you select the correct target combination. Target names are shown along the two axis based on the signal values assigned during plate setup.



4. To classify droplets as positive or negative for each given target, select the colored box that best represents the location of the cluster of droplets.

Drop-Off Assays for Genome Edit Detection

This type allows two groups of two targets per well; Group 1, shown by default, with 1 target in FAM and HEX (reference), and 1 target in FAM or HEX (unknown). You can add a second group with 1 target in Cy5 and Cy5.5 (reference) and 1 target in Cy5 or Cy5.5 (unknown).

Note: The channels within each group are not interchangeable therefore FAM is always paired with HEX/VIC and Cy5 with Cy5.5.

Setting Up Experiment Parameters

To set experiment parameters

1. Open the analysis data file and select the Plate Editor tab.
2. Select one or more wells to be assigned well analysis attributes.
3. Set the experiment type to Drop-Off (DOF) and tap Apply.

Target Name	Target Type	Signal Ch1	Signal Ch2	Signal Ch3	Signal Ch4	Ref Copies
1	Ref	FAM	HEX	None	None	2
2	Unkn	FAM	None	None	None	
3	Ref	None	None	Cy5	Cy5.5	2
4	Unkn	None	None	Cy5	None	

4. (Optional) Change the sample description identifiers.
5. (Optional) Change the sample type. The default type is Unknown.

Note: You cannot change the supermix that was used during data collection.
6. Tap Apply.

QX ONE Software automatically assigns the assay method as Basic Drop-Off.
7. Enter the target names, and then designate one target as Reference and the other as Unknown.

The Reference target enables ratio calculation of results.
8. Tap Apply.

Tip: Optionally, to expedite plate setup you can copy and paste data from one well to another, and you can tap Undo to undo an applied well setting.

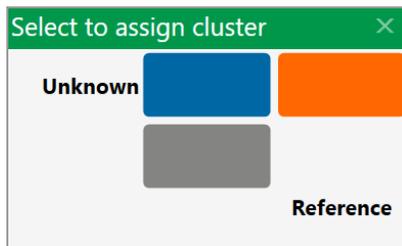
Viewing and Adjusting Results

Thresholding is not available for Drop Off experiments.

To apply and adjust clusters

1. After all wells are configured, select the 2D Amplitude tab to evaluate the data and manually set clusters.
2. Tap any of the three cluster mode buttons (square, circle, or freehand).
3. Press the left mouse button and draw the chosen shape around the cluster of droplets to be classified first.

Tip: When you release the mouse button, a pop-up tool appears to help you select the available cluster assignments. Target names are shown along the two axis based on the signal values assigned during plate setup.



4. To classify droplets as positive or negative for each given target, select the colored box that best represents the location of the cluster of droplets.

Direct Quantification with the Advanced Classification Method

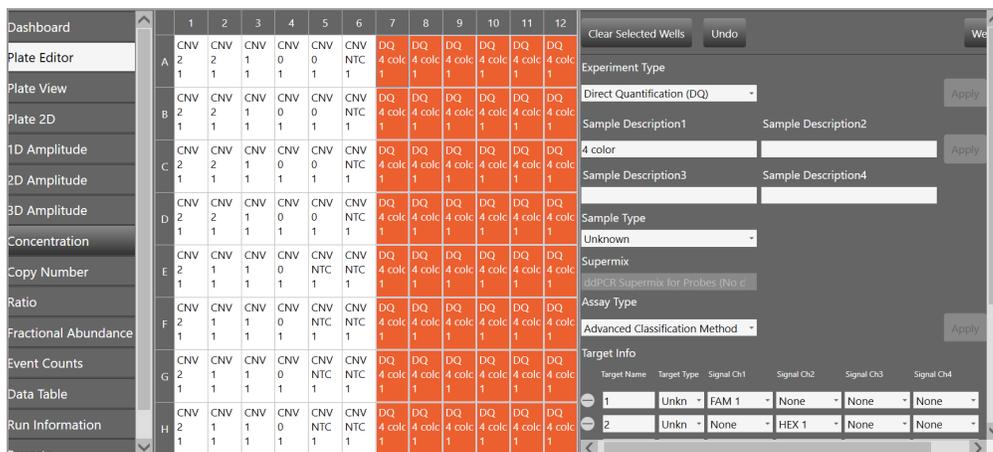
The Advanced Classification Method analysis mode allows the user complete control of droplet identification, and does not force or preassign any clusters. This allows a higher degree of multiplexing through radial dye mixing.

Important: This mode is intended for experienced users of droplet digital PCR.

Setting Up Experiment Parameters

To use the advanced classification method

1. Open the analysis file and select the Plate Editor tab.



2. Select one or more wells to be assigned well analysis attributes.
3. Set the experiment type to Direct Quantification (DQ) or RED and tap Apply.
4. (Optional) Change the sample description identifiers.
5. (Optional) Change the sample type. The default type is Unknown.

Note: You cannot change the supermix that was used during data collection.
6. Tap Apply.
7. Under Assay Type, select Advanced Classification Method and tap Apply.
8. Use the + icon to add up to ten targets.

9. Enter the target name and type (unknown or reference) for each target in the well.
10. (Optional) If you select Reference, you can change the number of reference copies. The default is 2.
 - If no reference is selected, the target with the highest dye concentration in the highest channel is used as the reference. For example, if there are targets in all four channels, the target in Channel 4 is used; however if there are targets only in the first three channels, then Channel 3 is the reference target.
 - If more than one reference is selected in the well, checkboxes appear to the right for using the reference in the plot.

Note: Each Reference target enables ratio calculations in the results.

11. Assign the first target an expected signal in a channel.
12. Assign your last target a signal in a channel that was not selected for your first target.
Each target in between will be assigned a signal depending on mixture ratio of the target.
13. Tap Apply to confirm all well settings.

Tip: Optionally, to expedite plate setup you can copy and paste data from one well to another, and you can tap Undo to undo an applied well setting.

Viewing and Adjusting Results

1. Tap the 2D Amplitude tab.
All droplets are unclassified by default and are colored red. Only the Threshold Cluster mode is available for setting thresholds in Advanced Classification experiments in the 2D plot.
2. Use one of the Threshold Cluster mode tools (box, circle or freehand) to assign droplet clusters to targets.

When you select a droplet cluster, a cluster assignment box appears.

Select to assign cluster			
1	<input type="radio"/>	+	<input checked="" type="radio"/>
2	<input checked="" type="radio"/>	+	<input type="radio"/>

Reset Cluster Assign Cluster

3. Select a radio button to identify the composition of the cluster.

For example, if the cluster is composed of positive droplets for Target-2, select the positive radio button for Target-2 and the negative radio buttons for the remainder.

Important: For calculations to occur, you must have at least one positive cluster for each target, as well as the assigned negative cluster.

Note: The software sets aside unassigned for calculation purposes. If you are unsure of droplet/cluster composition, leave it unassigned (red).

You can view the concentration results in the data table and in the Concentration tab.

If one of your samples was identified as a Reference under AssayType, you can view a ratio of concentration for unknown versus reference in the Ratio tab.

Appendix B Additional Computers

This section explains the recommended standalone computer requirements for QX ONE Software, and provides instructions for installing or updating the software.

Computer Requirements

Standalone computers are for analysis purposes only. The computers should meet the requirements specified in [Table 30](#).

Table 30. Standalone computer requirements

System Component	Minimum	Recommended
Operating system	Windows 10 64-bit	Windows 10 64-bit
CPU	Intel i5 chip set	Intel i7 chip set
Hard disk space	500 GB	1 TB
System memory	8 GB	8 GB
Display resolution	1920 x 1080	1920 x 1080
Ports	1 USB	1 USB

Notes:

(1) The software is compatible with Windows Defender and Trend Micro Office Scan anti-virus applications.

Important: Do not update the anti-virus or perform a scan while the instrument is acquiring wells or data may be lost.

(2) Enabling FIPS security on your Windows 10 computer does not interfere with instrument communication or application functionality.

Installing or Updating the Software

The QX ONE Droplet Digital PCR System must complete all runs and be in an inactive state when you upgrade the software on the installed touch screen computer, or the instrument firmware.

Appendix B Additional Computers

QX ONE Software can also be installed on additional computers.

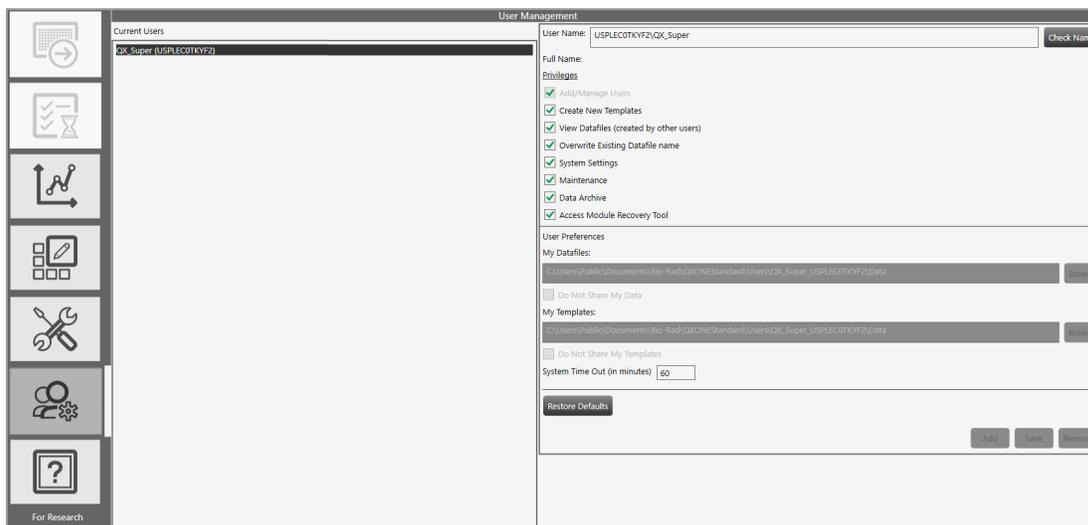
If you are already logged into QX ONE Software and you have open windows, save your data and close the windows before proceeding with the software update.

When the software is updated, the new version overwrites the existing version. If you are reverting to an older version, you must uninstall the newer version before installing the older version.

For more information on installing QX ONE Software, call Bio-Rad Technical Support.

Appendix C Managing Users

Use the User Setup and Preferences window to add, change, or remove QX ONE Software users, and set or change user privileges and preferences.



The Bio-rad engineer sets up an initial user with all privileges, including Add/Manage users, on the touch screen. Your software administrator can use this account to set up additional users with appropriate privileges..

Note: Users who are not assigned any of the specified privileges can still execute runs and use the Analysis module.

You can set up local or domain accounts. If the instrument touch screen computer

- Can access your company's network, QX ONE Software can recognize the Active Directory user names.
- Cannot access your company's network, your IT department can set up user names as local Windows accounts.

You can create user accounts with any user name convention and password character structure, as long as it complies with your organizational requirements. The software is designed to validate each user against the name it finds in either the Active Directory or local domain, so users must log in with the proper domain and user name.

However, QX ONE Software must be able to validate the user in either the local or domain user group before you can add the user in the software. Note the following:

- For local IDs, you must set up each user as a local Windows user account on each computer where the software is installed.
- For Active Directory IDs, your IT department must connect the QX ONE Droplet Digital PCR System instrument touch screen, as well as any separate computers running the software, to your network.

Adding Users

Only a user who is assigned the Add/Manage Users privilege can add QX ONE Software users.

Before you begin, ensure that all users have IDs set up in Active Directory or as local accounts.

To add a user

1. In the User Name field, enter the user name and then tap Check Name.

One of the following occurs:

- If the software recognizes the user name, the software displays a validation message. Continue to Step 2.
 - If the user is not recognized, an error message appears. Do one of the following:
 - For network users, ensure there is a working connection to the network and then verify your entry is accurate.
 - For local users, verify the entry is accurate. If the user is not set up locally or on the network, contact your system administrator for assistance.
2. When the successful validation message appears, tap OK, and then tap Add.
The software displays a confirmation message.
 3. Tap Yes to add the user, and then tap OK to close the dialog box.

Adding or Removing User Privileges

Only a user assigned the Add/Manage users privilege can add or remove user privileges.

By default, individual users without the privileges described in [Table 31](#) can set up and execute a run, open files, view and analyze results.

Table 31. User Privileges

Privilege	Description
Add and manage users	Add or remove users, set privileges, and change preferences. Note: Only the superuser can remove this privilege from other users.
Create new templates	Save a plate, thermal cycling protocol, or analysis report design as a template.
View data files created by other users	View files created by other users in the storage folders.
Overwrite existing data file name	Use Save or Save As capability. <ul style="list-style-type: none"> ■ Selecting Save replaces the original file content with any changes made by the user, without changing the file name. ■ Selecting Save As saves the file with a new file name or location. Note: Users without this permission can open files and perform analysis but cannot save their changes.
System settings	View event logs, and view and modify the shared data file and template locations. Two locations can be assigned for data files and one for templates. Note: You can also set preferred locations, which override all paths in individual preferences.
Maintenance	View the event log. Note: All users can view the maintenance log and maintenance reports.
Data archive	Move raw data from the computer installed on the QX ONE Droplet Digital PCR System to free up disk space needed for runs on the instrument.
Access the Module Recovery Tool	Rerun a plate in which droplet generation or droplet reading failed.

Table 32 contains a sample set of privileges assigned by user roles common to a laboratory. User roles can be defined and used in any scenario, but may be required in regulatory environments.

Table 32. Example User Roles

Privilege	Superuser	Lab Manager	Group Lead	Technician/ Student Intern
Add/manage users	✓	✓		
Create new templates*	✓	✓	✓	✓*
View data files created by other users	✓	✓	✓	✓
Override existing ddPCR file name	✓	✓	✓	
System settings	✓			
Maintenance	✓			
Archive data files	✓	✓	✓	✓
Access the module recovery tool	✓	✓	✓	✓

*You can assign this privilege to non-administrative roles if anyone can create and save a template. If template formats are restricted, then you should assign this permission to administrative roles only.

Note: Bio-Rad recommends defining at least one additional superuser as a backup, as well as other administrative and standard roles based on your needs. Guest users can open shared templates, execute runs, and perform analysis but are not typically assigned other privileges.

To add or modify privileges

1. Tap the Add/Manage Users tab to open the User Management window.
2. Select a user from the Current Users list. The name appears in the User Name field.
Note: A network or computer name and backslash may appear before the user name.
3. Select or clear the checkbox for each privilege in accordance with the user's role in using the software.

You can assign user privileges in any combination.

4. When the confirmation message appears, tap Yes to save the changes, and then tap OK to close the pop-up.

Modifying User Preferences

Users assigned the Add/Manage Users privilege can change the preferences for other users. Individual users can also change their own preferences.

To modify a user's preferences

1. Tap the Add/Manage Users tab, and then enter the user name.
2. Change any of the following preferences for the user:
 - Enter a different file path for user data files and templates.
Important: You can also specify Preferred Locations in System Settings, which override all file paths specified in individual preferences. For information, see [System Settings Tab on page 155](#).
 - Select or clear the checkbox to change user data file and template privacy settings.
 - Enter a different default system timeout period.
 - Enter a different number of completed plates to show in the Status section, up to a maximum of 100.
3. Tap Save.
QX ONE Software displays a confirmation message.
4. Tap Yes to save the changes, and then tap OK to close the dialog box.

Removing Users

Only a user assigned the Add/Manage Users privilege can remove users. Removing a user from the software does not remove the user from other systems or databases. The user still exists on your network, or in the Windows local user list.

To remove a user

1. Tap the Add/Manage Users tab to open the User Management window.
2. In the Current Users pane, select the user and tap Remove.
QX ONE Software displays a confirmation message.
3. Tap Yes to remove the user, and then tap OK to close the dialog box.

Appendix C Managing Users

Appendix D System Utilities

From the System Utilities tab, you can access the following tabs for managing tasks and data related to the instrument, software, data, and storage:

- **System Settings** — View shared template and data file locations
- **Event Log** — View logged data about all software activities
- **Maintenance Log** — Record maintenance activities
- **Maintenance Reports** — PDF maintenance records from Bio-Rad maintenance
- **Archive Data** — Clear up space in your primary storage areas

Notes:

- The Instrument Calibration tab is enabled only for the Bio-Rad service engineer user account.
- If you are not using the instrument touch screen, only the System Settings and Event Log tabs are available.

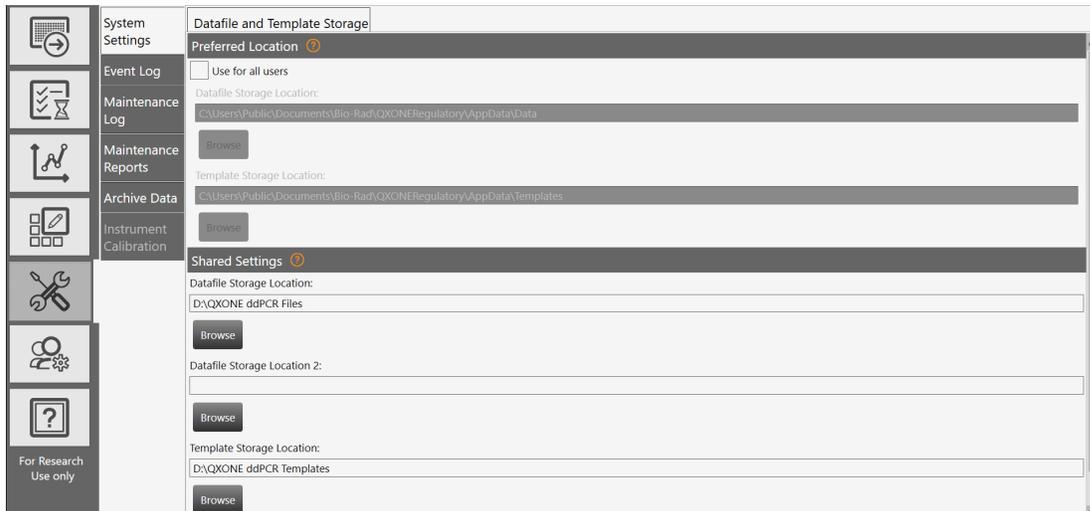
System Settings Tab

The System Settings window opens by default when you select the System Utilities tab, and contains fields where you can enable and enter storage file paths.

In System Settings, you can set up the following storage areas:

- Preferred Location
- Shared Settings

Important: Primary storage locations are either the file paths specified in the user's individual preferences, or the file paths specified under Preferred Locations. If a system administrator enables the Preferred Location settings for all users, files paths in individual user preferences are overridden.



Preferred Location

If Preferred Location file paths are used, then data files for all users are automatically saved to the specified folders on the instrument.

Important: Only users assigned the System Settings user privilege can set preferred locations. This setting overrides individual user storage settings.

Note: Preferred location restrictions apply to data files only; users are able to choose a location for template storage.

To specify preferred locations

1. Select the Use for all users checkbox and tap Save.

The file paths are identified in the Datafile Storage Location and Template Storage Location fields.

2. (Optional) Modify the file paths, and then tap Save.

Shared Settings

Under Shared Settings, you can specify two shared data file storage folders and one shared template storage folder. Because data files are often very large, the software allows you to set a primary storage folder and a backup storage folder. Bio-Rad recommends routinely checking available storage space and if necessary, using the Archive function.

Important: Only users assigned the System Settings user privilege can change storage locations in the System Settings window.

To specify shared folders

1. In the Datafile Storage Location field, enter a file path or tap Browse to search for the folder.
2. In the Datafile Storage Location 2 folder, enter a file path or tap Browse to search for the folder.

Note: These paths point to secondary storage locations. Data files are always saved initially to the preferred location (if specified by your administrator), or to the path specified in the user's preferences.

3. In the Template Storage Location field, enter a file path or tap Browse to search for the folder.
4. Tap Save.

System Log Files

From the System Utilities tab, you can access log files for system and software events (event log), and maintenance activities (maintenance log).

Event Log

Users who are assigned the Maintenance user privilege can view the system event log, which contains timestamped information on all software activities.

System Settings		Log File: QXONEStandard.History.log		Open Log...	PDF Password	PDF Report
Event Log		Search	Previous	Next		
Maintenance Log	Date Time	Message	Severity	User Name		
	8/29/2019 5:34:43 PM	There was valid instrument info when comparing information acquire...	Info	USPLECOTKYF2\QX_Super		
	8/29/2019 5:34:43 PM	There was valid instrument info when comparing information read fr...	Info	USPLECOTKYF2\QX_Super		
	8/29/2019 5:34:43 PM	Connected to instrument (Try 1) - Serial Number - SIMULATOR, FW V...	Info	USPLECOTKYF2\QX_Super		
	8/29/2019 5:34:37 PM	User Logged in. User Name: QX_Super, User Full Name:	Info	USPLECOTKYF2\QX_Super		
	8/29/2019 5:34:25 PM	Application StartUp. Application Version: 1.0.840, OS Version: Microso...	Info			
	8/29/2019 4:16:30 PM	Application Closed	Info	USPLECOTKYF2\QX_Super		
	8/29/2019 4:16:10 PM	User Logged in. User Name: QX_Super, User Full Name:	Info	USPLECOTKYF2\QX_Super		
	8/29/2019 12:32:14 PM	Imported plate file:C:\Users\Public\Documents\Bio-Rad\QXONEStand...	Info	USPLECOTKYF2\QX_Super		
	8/29/2019 12:22:11 PM	There was valid instrument info when comparing information acquire...	Info	USPLECOTKYF2\QX_Super		
	8/29/2019 12:22:11 PM	There was valid instrument info when comparing information read fr...	Info	USPLECOTKYF2\QX_Super		
	8/29/2019 12:22:11 PM	Connected to instrument (Try 1) - Serial Number - SIMULATOR, FW V...	Info	USPLECOTKYF2\QX_Super		
	8/29/2019 12:22:04 PM	User Logged in. User Name: QX_Super, User Full Name:	Info	USPLECOTKYF2\QX_Super		
	8/29/2019 12:21:58 PM	Application StartUp. Application Version: 1.0.840, OS Version: Microso...	Info			
	8/28/2019 4:50:07 PM	Application Closed	Info			
	8/28/2019 4:50:03 PM	Application StartUp. Application Version: 1.0.840, OS Version: Microso...	Info			
	8/28/2019 3:19:37 PM	Application Closed	Info			
	8/28/2019 3:19:28 PM	Application StartUp. Application Version: 1.0.840, OS Version: Microso...	Info			
Selected Row Message		There was valid instrument info when comparing information acquired from the QXONE instrument's Calibration Info and acquired from the QXONE instrument				

To view the Event Log

1. Select the System Settings tab.



2. Select Event Log and do one of the following:
 - Scroll through the list of events displayed in the Event Log window.
 - Tap Open Log and open the text file in Notepad.
3. (Optional) To automatically generate and display a PDF document, tap PDF Report.

Tip: To restrict access to the file, you can assign a password. Select the PDF Password check box and then enter a password in the field. The file is saved in the Log Reports folder and can be opened only by entering the password.

Maintenance Log

All users can view the maintenance log, which contains a list of maintenance records. The Maintenance Log tab is available from the instrument touch screen only.

Users who are assigned the Maintenance user privilege can set up maintenance records, specify associated activities, and generate PDF reports.

The screenshot displays the Maintenance Log interface. On the left is a navigation menu with icons for System Settings, Event Log, Maintenance Log (selected), Maintenance Reports, Archive Data, Instrument Calibration, and a 'For Research Use only' section. The main area shows a table of maintenance records with the following data:

Date Time	User	Product Version	Firmware Version	Activity
9/9/2019	USPLECOTKYF2\QX_Super	1.0.840	1.0.0.0	

Below the table is a form for adding a new record with the following fields:

Date Time	User	Product Version	Firmware Version:
9/9/2019 11:48:59 AM	USPLECOTKYF2\QX_Super	1.0.840	1.0.0.0
Activity			

Buttons for 'Cancel' and 'Save' are located at the top right of the interface. A 'PDF Report' button is located at the bottom right of the main area.

To view the Maintenance Log

1. Select the System Settings tab.



2. Select Maintenance Log.
3. To view the log entries, scroll through the list in the grid.

To add a new activity

1. Tap the plus sign (+) next to New Activity.

A line item appears in the grid and the Date Time, User, Product Version, and Firmware Version fields are automatically completed.

2. In the Activity field, enter a description of the planned activity.
3. Tap Save.
4. Repeat to add another activity.

After saving, you can create a PDF report of the added activities.

5. (Optional) Tap PDF Report.

Maintenance Reports

Reports generated from the Bio-Rad service engineer when performing maintenance on your instrument are stored on the Maintenance Reports tab. The Maintenance Reports tab is available from the instrument touch screen only.

- ▶ To open a report, tap the list item.

The screenshot shows the QX ONE Software Standard Edition interface. At the top, there's a header with the BIO-RAD logo, system name 'QX ONE ddPCR System', and status 'C1 Prototype (Ready)'. It also displays 'Runs' (1 In Progress, 4 Available), oil levels for DG, DR, and Waste, and a 'Disk Space (C:)' gauge showing 265 GB free (14%) and 1596 GB used (86%). A sidebar on the left contains icons for System Settings, Event Log, Maintenance Log, Maintenance Reports, Archive Data, Instrument Calibration, and Help. The main area shows a list of reports under 'Color Calibration Reports' and 'Volume Calibration Reports'.

Following is an example of a Color Calibration report:

The screenshot shows a 'Color Calibration and Carryover Report' document. It includes the following information:

- Report Title: Color Calibration and Carryover Report
- Username of the Person who did the Run: BBR&D
- Report Generation Date and Time: 7/22/2019 09:03:29 PM
- Instrument Serial Number: CHARLIE-1
- Firmware Version: 1.2790
- Software Version: 1.0.774.0628
- Software: QX ONE Software Standard Edition
- Lot Information: GC96 Cartridge: 142362; Droplet Generation Oil: BBD28101; BBD28101; Droplet Reader Oil: BBD28101; BBD28101; Calibration Effective Dates: 2/27/2019 03:20:11 PM

Color Calibration Result	Carryover Test Result	# of carryover events
Channel 1 (FAM)	Pass	0
Channel 2 (HEX/VIC)	Pass	0
Channel 3 (CY5)	Pass	0
Channel 4 (CY5.5)	Pass	0
Low Hex	Fail	0

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Archiving Data

When the storage space on the touch screen computer installed on the QX ONE Droplet Digital PCR System approaches a predefined limit, the software displays an advisory message at set intervals. Users can free up space by moving raw data to an archive storage folder in a different location. The Data Archive tab is available from the touch screen only.

Important: You must be assigned the Data Archive user privilege to archive data.

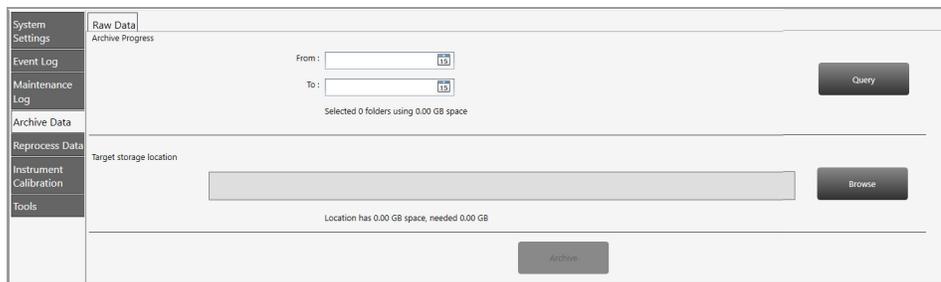
To archive files

1. Select the System Settings tab.



2. Select Archive Data.

You can archive raw data only.



The screenshot shows the 'System Settings' interface with a sidebar on the left containing options: System Settings, Event Log, Maintenance Log, Archive Data, Reprocess Data, Instrument Calibration, and Tools. The 'Archive Data' section is active, displaying 'Raw Data' and 'Archive Progress' tabs. Below the tabs are two date pickers labeled 'From:' and 'To:', both showing '13'. A 'Query' button is to the right. Below the date pickers, it says 'Selected 0 folders using 0.00 GB space'. Underneath is a 'Target storage location' section with a text input field, a 'Browse' button, and a status message: 'Location has 0.00 GB space, needed 0.00 GB'. At the bottom center is an 'Archive' button.

3. Under Raw Data Archive Progress, tap the calendar icons and select a From date and a To date to define a date span.
4. Tap Browse to search for a select the target storage location.
5. Tap Archive.

System Calibration

You cannot execute runs on the QX ONE Droplet Digital PCR System unless the instrument is properly calibrated.

Only a Bio-Rad service engineer can calibrate your instrument and use the Instrument Calibration options in QX ONE Software. The option is available from the touch screen only, and is disabled for all other users.

If your instrument requires service, contact Bio-Rad Technical Support.



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