

QX200™ Droplet Reader and QuantaSoft™ Software

Instruction Manual

Catalog #186-4001, 186-4003



BIO-RAD

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This instrument has been tested and found to be in compliance with all applicable requirements of the following safety and electromagnetic standards:

- IEC 61010-1:2010 (3rd ed.), EN61010-1:2010 (3rd ed). Electrical Equipment for Measurement, Control, and Laboratory Use — Part 1: General requirements
- EN 61326-1:2006 (Class A). Electrical equipment for measurement, control, and laboratory use. EMC requirements, Part 1: General requirements
- UL 61010-1:2004, Laboratory equipment, Test & Measurement Equipment and Industrial Process Controls
- CAN/CSA 22.2 No 61010-1-04, Safety Requirements for Electrical. Equipment for Measurement, Control, and Laboratory Use, Part I: General. Requirements

This equipment generates, uses, and can radiate radiofrequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference, in which case the user will be required to correct the interference at his own expense.



The CE mark indicates that the manufacturer ensures the product conforms with the essential requirements of the applicable EN directives.



The CSA mark indicates that a product has been tested to Canadian and U.S. standards, and it meets the requirements of those applicable standards.

This equipment has been tested and found to comply with the limits for a Class A digital device pursuant to Part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment.



The Waste Electrical and Electronic Equipment Directive symbol indicates that when the end-user wishes to discard this product, it must be sent to separate collection facilities for recovery and recycling.

Instrument Safety Warnings

Alteration of this instrument voids the warranty and safety certification and creates a potential safety hazard. This instrument is intended for laboratory use only. Bio-Rad Laboratories is not responsible for any injury or damage caused by use of this instrument for purposes other than those for which it is intended, or by modifications of the instrument not performed by Bio-Rad Laboratories or an authorized agent. Follow the safety specifications listed here and throughout this manual. Use only the power cord supplied with the instrument, using only the plug adaptor that corresponds to the electrical outlets in your region. Use of unapproved supermixes may harm the instrument and voids the warranty.

PPE (Personal Protective Equipment) Training

Proper use of gloves is recommended with use of oils and sample plates. OSHA requirements for PPE are set forth in the Code of Federal Regulations (CFR) at 29 CFR 1910.132 (General requirements); 29 CFR 1910.138 (Hand protection); 29 CFR 1926.95 (Criteria for standard personal protective equipment). Any gloves with impaired protective ability should be discarded and replaced. Consider the toxicity of the chemicals and factors such as duration of exposure, storage, and temperature when deciding to reuse chemically exposed gloves. Features to aid glove selection for handling of machines, assays, oils, and cleaning solvents:

- Butyl gloves are made of a synthetic rubber and protect against peroxide, hydrofluoric acid, strong bases, alcohols, aldehydes, and ketones
- Natural (latex) rubber gloves are comfortable to wear and feature outstanding tensile strength, elasticity, and temperature resistance
- Neoprene gloves are made of synthetic rubber and offer good pliability, finger dexterity, high density, and tear resistance; they protect against alcohols, organic acids, and alkalis
- Nitrile gloves are made of copolymer and provide protection from chlorinated solvents such as trichloroethylene and tetrachloroethene; they offer protection when working with oils, greases, acids, and caustic substances

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1

QX200™ Droplet Digital™ PCR System

1.1 Introduction

The QX200 Droplet Digital PCR (ddPCR™) system performs accurate and precise digital PCR. The system consists of two instruments, the QX200 droplet generator and the QX200 droplet reader, and their associated consumables. The QX200 droplet generator partitions samples into ~20,000 nanoliter-sized droplets and, after PCR on a thermal cycler, droplets from each sample are analyzed individually on the QX200 droplet reader. PCR-positive and PCR-negative droplets are counted to provide absolute quantification of target DNA in digital form. Alternatively, amplified products can be extracted from droplets following PCR for downstream applications, such as sequencing or cloning.

The ddPCR system lets you:

- Detect rare DNA target copies with unmatched sensitivity
- Determine copy number variation with unrivaled accuracy
- Measure gene expression levels with precision

Applications and uses include:

- Copy number variation
- Rare sequence detection
- Gene expression analysis
- Next-generation sequencing (NGS) library quantification
- Viral load determination
- Single cell gene expression analysis
- Absolute quantification
- Rare mutant detection
- miRNA analysis
- NGS sample preparation
- GMO detection

For instructions on use of the QX200 droplet generator, please refer to the QX200 Droplet Generator Instruction Manual (bulletin 10031907).

1.2 System Components

The system consists of two instruments and associated software, consumables, and reagents (Table 1.1):

- **QX200 droplet generator** — utilizes proprietary reagents and microfluidics to partition samples into ~20,000 nanoliter-sized droplets
- **QX200 droplet reader** — following PCR amplification of the nucleic acid target in the droplets, this instrument analyzes each droplet individually using a two-color detection system (set to detect FAM and either VIC, HEX, or EvaGreen); PCR-positive and PCR-negative droplets are counted to provide absolute quantification of target DNA in digital form using QuantaSoft™ software

Additional materials required for performing ddPCR are listed in Table 1.2.

Table 1.1. QX200 Droplet Digital PCR system components. Items shipped with the QX200 ddPCR system (catalog #186-4001). Catalog # refers to replacement items (quantities may be different).

Component	Description	Catalog #
QX200 droplet generator		
QX200 droplet generator	Instrument used for droplet generation	186-4002
DG8™ droplet generator cartridges and gaskets (24)	Microfluidic cartridge used to mix sample and oil to generate droplets; gaskets seal the cartridge to prevent evaporation and apply pressure required for droplet formation	186-4007
Droplet generator cartridge holder	Positions and holds the droplet generator cartridge in the instrument for droplet generation	186-3051
Power cord	Connects QX200 droplet generator to power source	Call technical support
QX200 droplet reader		
QX200 droplet reader	Instrument used for droplet reading, data collection	186-4003
Laptop PC and QuantaSoft software	Connects to QX200 droplet reader for data collection and analysis	186-3007
Droplet reader plate holders (2)	Position 96-well PCR plate in the droplet reader	29711024
USB 2.0 cable	Connects QX200 droplet reader to PC	Call technical support
Power cord	Connects QX200 droplet reader to power source	Call technical support



QX200 Droplet Digital PCR system. Left, QX200 droplet reader. Right, QX200 droplet generator.

Table 1.2. Additional materials required.

Component	Recommended	Catalog # and Supplier
QX200 droplet generator		
<i>Reagents for probe detection</i>		
PCR supermix	ddPCR supermix for probes (no dUTP)	186-3023, 186-3024, 186-3025
	ddPCR supermix for probes	186-3010, 186-3026, 186-3027, 186-3028
	One-Step RT-ddPCR supermix for probes	186-3021, 186-3022
Droplet generation oil	Droplet generation oil for probes	186-3005
Control	ddPCR buffer control kit for probes	186-3052
<i>Reagents for EvaGreen detection</i>		
PCR supermix	QX200™ ddPCR™ EvaGreen® supermix	186-4033, 186-4034, 186-4035, 186-4036
Droplet generation oil	Droplet generation oil for EvaGreen	186-4005, 186-4006
Control	QX200 buffer control kit for EvaGreen	186-4052
<i>Consumables and other materials</i>		
Pipets	20 µl pipet for sample loading	Rainin L-20
	50 µl pipet for droplet transfer	Rainin L-50, L8-50
	8-channel, 200 µl pipet for oil	Rainin L8-200
Pipet tips	Filtered	Rainin GP-L10F, GP-L200F
96-well PCR plates	twin.tec semi-skirted 96-well plate	Eppendorf 951020362
Reagent trough	Any	
Foil plate seals	Pierceable foil plate seals	181-4040
Plate sealer	PX1™ PCR plate sealer	181-4000
8-cap strips	Any	
QX200 droplet reader		
Droplet reader oil	ddPCR droplet reader oil	186-3004
Droplet reader waste bottle	Empty waste bottle	N/A

1.3 Droplet Digital PCR Workflow

Droplet Digital PCR involves the following steps (4.5–5.5 hours for the complete workflow):

- **Prepare PCR-ready samples** — combine nucleic acid sample (DNA or RNA), primers, and probes (FAM, VIC, or HEX) or intercalating dye (EvaGreen) with Bio-Rad ddPCR supermix (see Table 1.2)
- **Make droplets** — load 20 μ l of the ddPCR reaction into the DG8 droplet generator cartridge, then load the cartridge into the QX200 droplet generator to partition the sample into droplets. The QX200 droplet generator uses microfluidics to combine oil and aqueous sample to generate the nanoliter-sized droplets required for ddPCR analysis. It processes up to eight samples at a time in about 2 minutes
- **Perform PCR** — pipet droplets from the cartridge to a 96-well PCR plate and seal the plate with foil using a PX1 plate sealer (see Table 1.2). Perform PCR to end point (~40 cycles) using a thermal cycler
- **Read droplets** — load the plate into the QX200 droplet reader and start your run. The droplet reader sips each sample, singulates the droplets, and streams them in single file past a two-color detector. The detector reads the droplets to determine which contain a target (+) and which do not (-)

If reading or quantifying droplets and recovering material from droplets in parallel, prepare two sets of reactions, one for each application. For example, a set of eight wells in a single DG8 cartridge can be generated: four of these will be read after thermal cycling, and four will not be read

- **Analyze results** — the droplet reader connects to a laptop computer running QuantaSoft software. The software provides a complete set of tools for setting up and naming samples, running and controlling the instrument, and analyzing results. It also reads the positive and negative droplets in each sample and plots the fluorescence droplet by droplet. The fraction of positive droplets in a sample determines the concentration of target in copies/ μ l

The QX200 ddPCR system is compatible with hydrolysis probe (TaqMan) chemistry and can detect up to two probes at a time (FAM/VIC or FAM/HEX), using a dye deconvolution matrix in the software to ensure target specificity. It is also compatible with EvaGreen chemistry. Use only the approved Bio-Rad supermixes listed in Table 1.2 with this system; using unapproved supermixes may harm the instrument and voids the warranty.

1.4 System Setup and General Operation Instructions





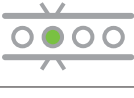
- Connect the QX200 droplet generator and QX200 droplet reader to a power source using the power cords and power adapter provided. Leave 10" (5 cm) clear space behind and 5" (2.5 cm) clear to the right and left of each instrument for proper ventilation. Position the instrument such that it can be easily disconnected from the power source should that become necessary for servicing the equipment.
- Connect the QX200 droplet reader to the laptop PC using the USB 2.0 cord provided. QuantaSoft software is preinstalled on the laptop
- Power on the QX200 droplet reader using the switch at the back. The status indicator turns solid green to indicate power is on. The QX200 droplet generator is powered on by plugging it into a power source
- The droplet reader requires droplet reader oil (catalog #186-3004). Please refer to Section 4.2.2 for instructions on replacing droplet reader oil

2

Using the QX200™ Droplet Reader

1. Power on the QX200 droplet reader using the switch at the back. Allow it to warm up for 30 min, then switch on the PC and launch QuantaSoft™ software.
2. Check the indicator lights on the front of the droplet reader (Table 2.1). The first two lights at left should be solid green, indicating power is on, there is sufficient oil in the designated oil reservoir, and there is <700 ml in the waste bottle. If the lights are flashing amber, the run cannot be started; clean out the waste bottle or replace the oil (see Section 4.2.2).

Table 2.1. Status indicator lights on the QX200 droplet reader.

				
Solid green	Power on	Bottle levels OK*	Plate in place	Run complete
Flashing green	—	Oil <30% or waste >70%**	—	Run in progress
Flashing amber	—	Oil <10% or waste >90%***	—	Error during run
Off	Power off	—	No plate	Idle

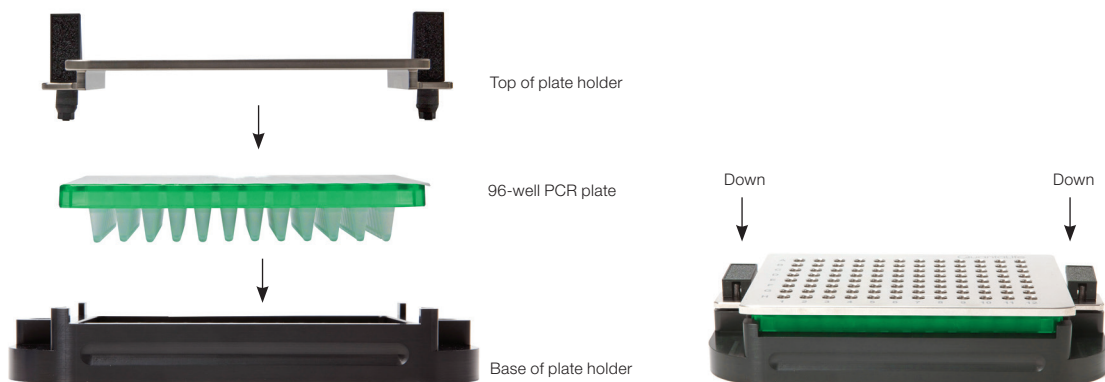
* There is sufficient oil and room in the waste bottle to run 96 wells.

** The run can be started if <96 wells are run (for example, only 19% oil is required for 24 wells); if there is not enough oil for the run, the software will not allow you to start the run.

*** The software will not allow you to start the run.

3. Place the 96-well PCR plate into the plate holder:

- a. Place the 96-well PCR plate containing the amplified droplets into the base of the plate holder. Well A1 of the PCR plate must be in the top left position.
- b. Move the release tabs of the top of the plate holder into the “up” position and place the top on the PCR plate. Firmly press both release tabs down to secure the PCR plate in the holder.

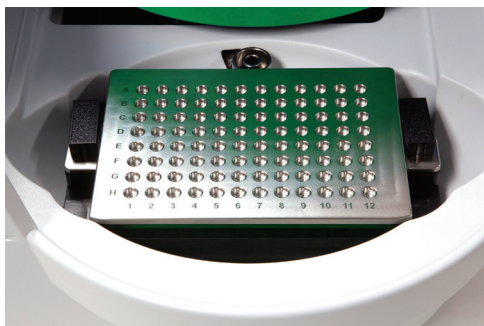


Placing the 96-well plate into the plate holder.

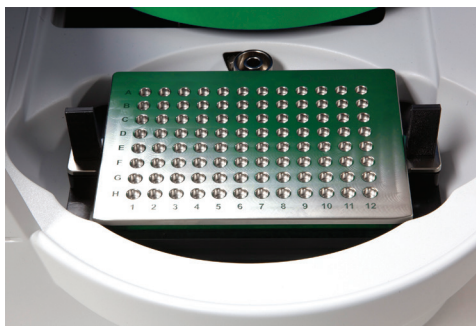
4. Press the button on the green lid to open the droplet reader. Load the plate holder into the droplet reader, and press the button on the lid again to close the cover. Confirm the first three indicator lights are green (Table 2.1).



Placing the plate holder into the droplet reader.



Correct



Incorrect

Correct and incorrect placement of the plate holder. Note the release tabs are in the “up” position in the incorrect placement at right.

5. In QuantaSoft software, click **Setup** in the left navigation bar to define your experiment (see Chapter 3), then click **Run**. The run indicator light (far right) flashes green to indicate droplet reading is in progress.
6. When droplet reading is complete, all four indicator lights are solid green. Open the door and remove the plate holder from the unit. Remove the 96-well PCR plate from the holder and discard it.

3

Using QuantaSoft™ Software

QuantaSoft software (v. 1.4 and later) organizes and provides one-click access to the three main steps of droplet analysis in the left navigation bar, moving you through the entire workflow:

- **Setup** — enter information about the samples, assays, and experiments (see Section 3.1)
- **Run** — start the run and control the instrument, if needed (see Section 3.2)
- **Analyze** — compute nucleic acid concentration (see Sections 3.3 and 3.4)

QuantaSoft software uses the following file types:

- Template (*.q!t) — user-defined plate layout settings (no data) for reading a ddPCR™ plate
- Raw data (*.q!b) — unprocessed data from each well in a ddPCR plate
- Results (*.q!p) — user-defined plate layout settings and processed data for a ddPCR plate
- Comma-separated values (*.csv) — analyzed data in a format that can be assessed using other programs, such as Microsoft Excel

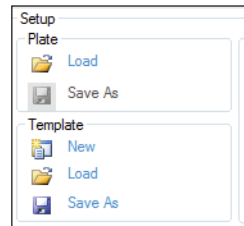
3.1 Setup

The screenshot displays the QuantaSoft software interface for a 96-well plate. The plate map is organized into rows (A-H) and columns (01-12). Each well contains a small thumbnail image representing the assay type and concentration. For example, row A contains wells with 'RED' and 'CNV' assays, while row B shows 'Mutant (hom.)' assays. Annotations with arrows point to various parts of the interface: 'File name' points to the top bar; 'Load settings and data from previous experiments' points to the 'Load' button in the 'Setup' panel; 'Options for advanced data analysis and setup' points to the 'Options' button; 'Open experiment editor' points to the 'Open' button; 'Define experiment settings' points to the 'Setup' button; 'Start the run' points to the 'Run' button; 'View and analyze data' points to the 'Analyze' button; and 'Abort run or exit program' points to the 'About' button. On the right side, there are additional panels for 'Instrument Readiness' and 'Plate map'.

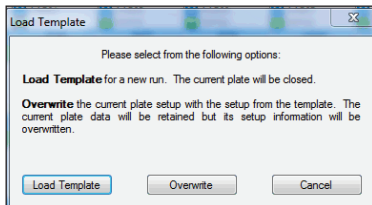
QuantaSoft software Setup interface. The plate map is a diagram of the wells in the 96-well plate and contains information about the type of analysis, sample, and assay represented by that well. After a run, it also contains concentration data.

1. Click **Setup** to enter information about the samples, assays, and experiments.

- To open saved details (settings and data) from another experiment, click **Plate > Load** and select the file
- To open a saved template for a plate map (settings only, no data), click **Template > Load** and select the file
- To create a new template, click **Template > New**



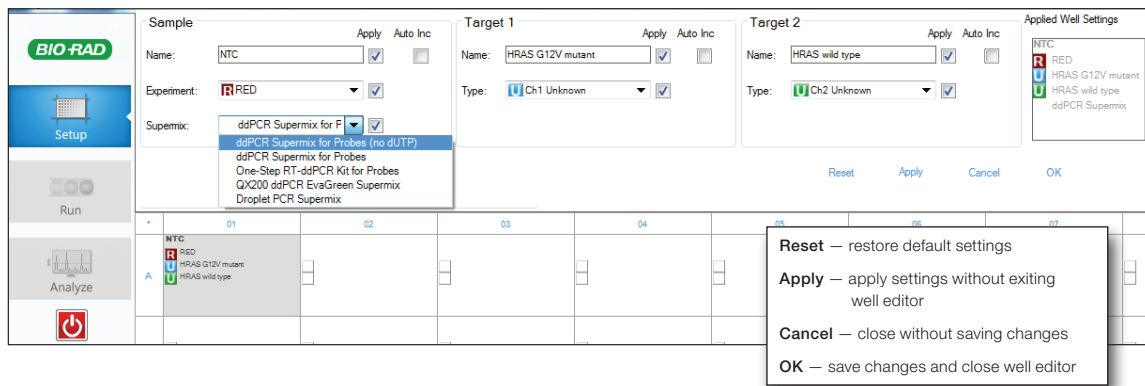
To overwrite the setup information for a plate that is open (experiment type and name, sample name, etc.), click **Template > Load**. In the Load template window, click **Overwrite**.



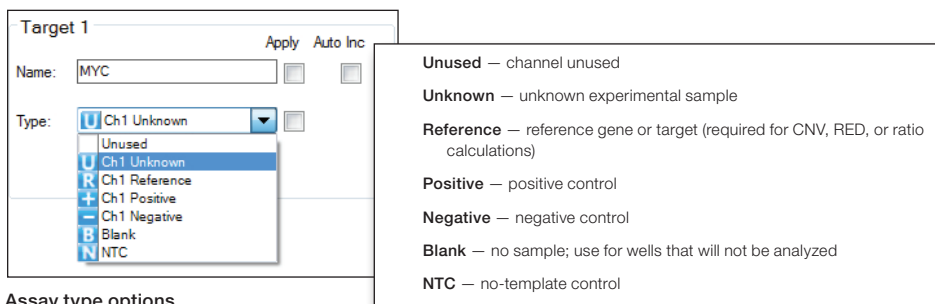
2. Enter the file name, then use the well editor (see Section 3.1.1) and experiment editor (see Section 3.1.2) to adjust the settings for your experiment. Click **Options** to access advanced options data analysis and setup (see Section 3.1.3).

3.1.1 Using the Well Editor

Use the well editor to define the settings (samples, experiment type, and detection type) for the plate. Sample and experiment types are color-coded and can be customized for easy reference in the plate map.



Well editor. Settings for absolute quantification of two unknowns in a single sample are shown.



Assay type options.

- To open the well editor, double-click on the well(s) you wish to edit. Selected wells are highlighted in gray, and the well editor appears across the top of the interface.
 - To select multiple wells, hold **Ctrl** and select the wells
 - To select wells in a continuous series (horizontal or vertical), hold **Shift** and select the first and last wells
 - To select all wells in the plate, double-click in the top left corner of the plate
 - To select a row or column, double-click the letter or number for that row or column
- In the Sample panel, enter the sample **Name** and select the **Experiment** from the drop-down menu.
 - All saved experiments appear in the drop-down menu, along with the option to **add experiment...**
 - The sample name is case-sensitive; only wells with identical sample names can be treated as merged wells during data analysis
 - To create or edit an experiment, use the experiment editor (see Section 3.1.2)
- Select the **Supernix** from the drop-down menu (required; selection cannot be changed after data collection).

- Define Target 1 (channel 1, the FAM channel) and Target 2 (channel 2, the VIC or HEX channel). Assign each assay a **Name** and sample **Type**.

Settings appear in the Applied Well Settings box as you enter them. When you are done, click **Apply** or **OK** to save the information. The settings appear in the well in the plate map.

Tips:

You can change the selected well using the plate map without exiting the well editor.

To append sample or assay names with numbers incrementally through selected wells, select the **Auto Inc** check box next to **Name**.

Use standard Windows keyboard shortcuts for copying, pasting, and deleting selections (for example, use **Ctrl+c** to copy a selection, **Ctrl+z** to undo an operation, etc.).

3.1.2 Using the Experiment Editor

Use the experiment editor to define the experiment type. To open the experiment editor, select **Experiment > add experiment...** in the well editor or select **New** or **Edit** (double-click on an experiment name) in the Experiments window under Setup. Four types of experiments are possible: Absolute Quantification (ABS), Rare Event Detection (RED), Copy Number Variation (CNV), and Gene Expression (GEX). A default list of experiments is supplied at installation, but you can create and save custom experiments; upgrade installations will preserve current experiment lists.

Settings are summarized in the Applied Well Settings box as you enter them. Click **Apply** or **OK** to save the experiment information. The settings appear in the well in the plate map.

If the Sample Name, Experiment, Target Name, Target Type, and Supermix all match across multiple wells (all are case-sensitive), the software can identify these as “merged” and provide an option to view merged data during analysis (see Section 3.3).

The screenshot shows the Experiment Editor dialog box with the following details:

- Name:** CNV
- Type:** Radio buttons for Absolute Quantification (ABS), Rare Event Detection (RED), Copy Number Variation (CNV) (selected), and Gene Expression (GEX).
- Reference Copies:** Input field containing the number 2.
- Comments:** Empty text area.
- Background Color:** Dropdown menu set to DarkOrange.
- Foreground Color:** Dropdown menu set to White.
- Buttons:** Remove, Apply, OK, Cancel.
- Experiment List:** A list on the right side containing: ABS, RED, CNV1, CNV2, CNV3, CNV4, GEX, CNV.

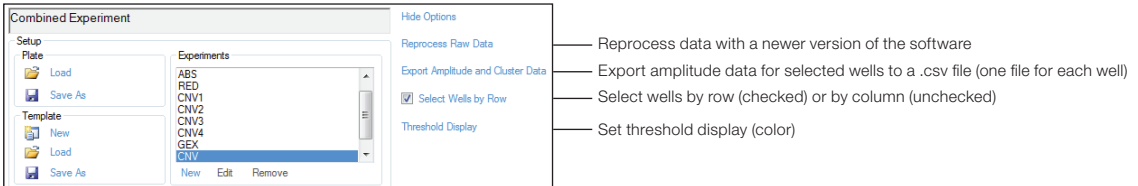
Annotations in the image:

- A line points from the text "Copy number options appear for CNV experiments only" to the Reference Copies input field.
- A line points from the text "Customize the color codes for experiments (for example, by experiment type, user, etc.)" to the experiment type list.

Experiment editor. Options for a CNV experiment are shown.

3.1.3 Using the Advanced Options

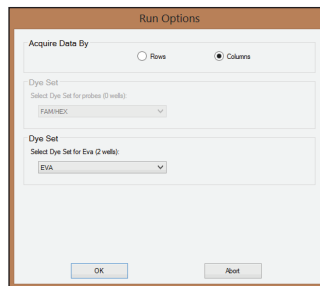
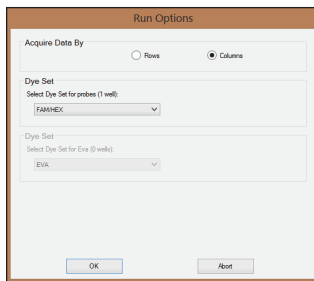
Click **Options** in the Setup window to see all advanced options for data collection and analysis.



Advanced options.

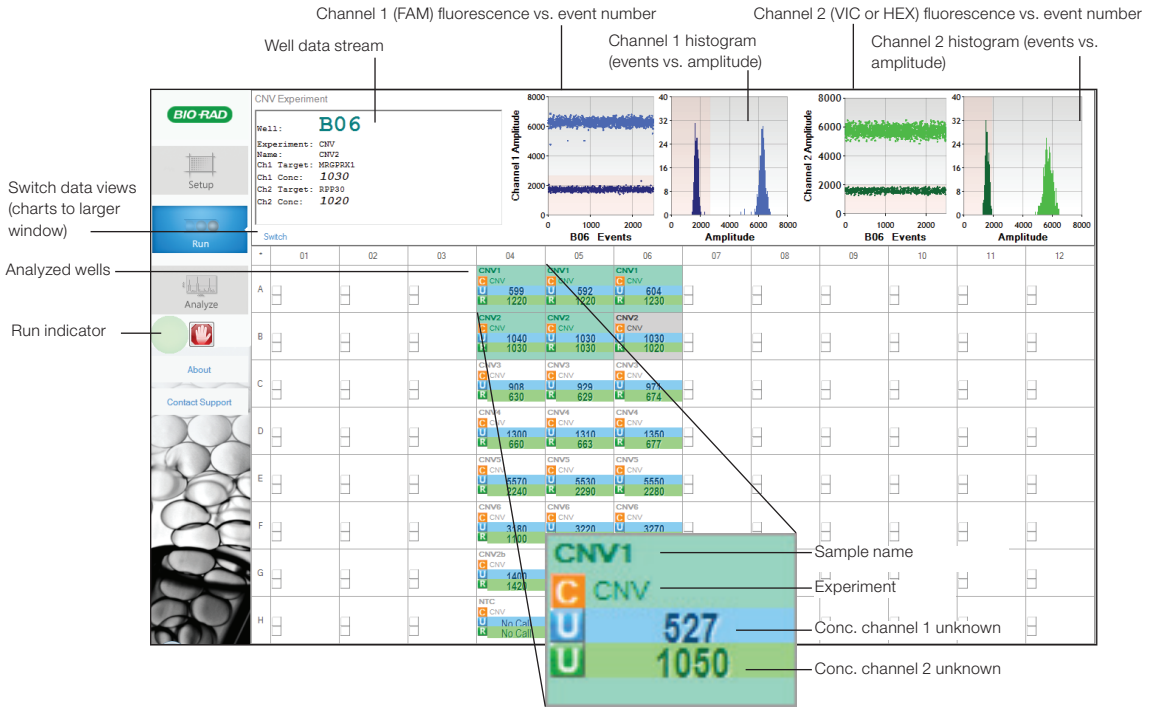
3.2 Run

1. Click **Run** in the left navigation bar to start the run.
2. In the Run Options window, select the detection chemistry:
 - If a probe supermix is selected in the well editor, the probe dye sets appear. Select FAM/HEX or FAM/VIC
 - If an EvaGreen supermix is selected, the EvaGreen dye set appears; the screen confirms the number of EvaGreen wells configured on the plate



Run options.

3. Up to 1 minute later, a green circle appears next to the abort button and flashes periodically to indicate the run is in progress. Active and analyzed wells are also highlighted in green in the plate map.
4. As each well is analyzed, the data appear across the top navigation area. Once the run is complete, all data are reanalyzed for the final data file.



Interface during an active run. Data for both detector channels are shown for the well being read.

3.3 Analyze

In the **Setup** window, load a plate (*filename.qip*), then click **Analyze** to open and analyze the data. The data analysis interface is separated into three windows:

- Results table — summarizes results for wells selected in the well selector
- Well selector — enables selection of wells for targeted analysis
- Processed data/graphical display — allows visualization of graphical data from selected wells

Results table

Well selector

Graphical data display

Y-axis display options

Copy graph to clipboard or print

Options

Table Export CSV Ch1,Ch2 Sequential Single

1D Amplitude 2D Amplitude Concentration Copy Number Ratio Events Heat Map

Ch1+Ch2+-50762 Ch1+Ch2-28206 Ch1-Ch2+18956 Ch1-Ch2-29284

Channel 1 Amplitude

Channel 2 Amplitude

Ch1 Ch2 Set Thresholds

Data analysis interface. Data from a CNV analysis are shown.

Well	E	Experiment	Sample	T	Assay	Status	Concentration	T
A04	C	CNV	CNV1	U	MRGPRX1	OK	599	
B04	C	CNV	CNV2	U	MRGPRX1	OK	1040	
C04	C	CNV	CNV3	U	MRGPRX1	OK	908	
D04	C	CNV	CNV4	U	MRGPRX1	OK	1300	
E04	C	CNV	CNV5	U	MRGPRX1	OK	5570	
F04	C	CNV	CNV6	U	MRGPRX1	OK	3180	
G04	C	CNV	CNV2b	U	MRGPRX1	OK	1400	

Table Export Ch1,Ch2 Sequential Single

Status options:

- OK** — automatic analysis successful
- CHECK** — automatic analysis unsuccessful; to view concentration, use manual analysis tools
- Multi** — data automatically analyzed as part of a multi-well selection
- Manual** — droplets analyzed manually

View table in graphical display window

Export data to .csv file

Select data from detector channel(s)

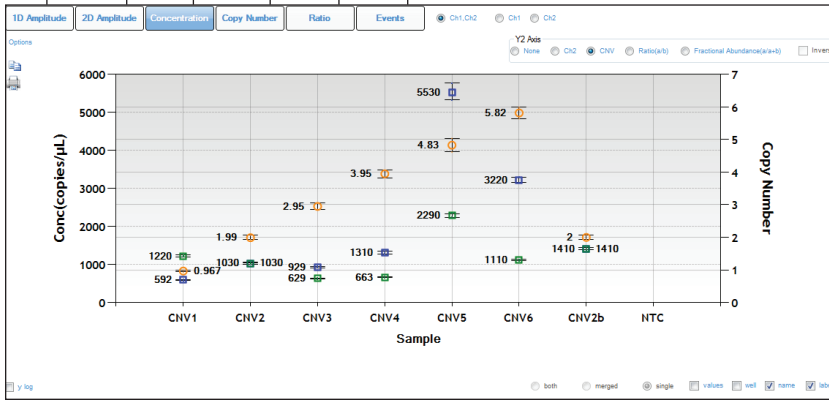
Toggle order in which channel data are displayed in the table

Display replicates separately, as merged wells (if Sample Name, Experiment, and Assay Name and Type all match across the wells), or both

Results table options. Data from a CNV analysis are shown.

Channel 1 vs. channel 2 clustering plot

Channel data | Concentration data | Plot of ratio of unknown:reference (a/b or a/[a+b]) | Plot of measured copy numbers | Plot of # droplet events counted




Graphical data display options. A concentration plot from a CNV analysis is shown, with display options across the top.



3.3.1 Viewing Channel Data (1D Amplitude)

Click **1D Amplitude** to visualize the data collected from each channel of selected wells. Use the radio buttons to select the channels to be displayed. This tab also provides options for adjusting the thresholds used in assigning positives and negatives for each channel.

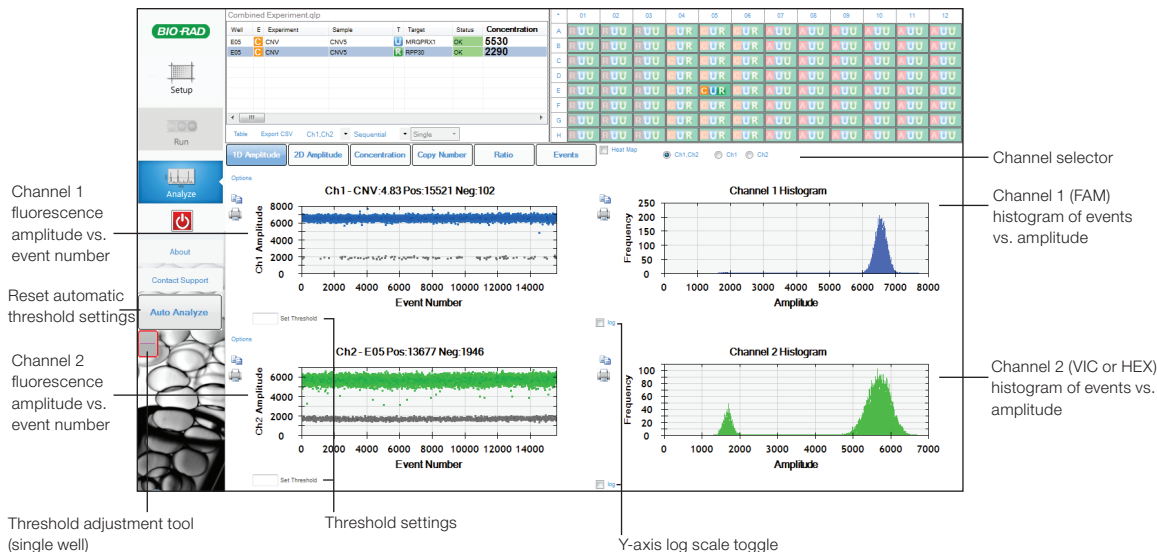
When viewing a single well, change the threshold using one of the following options:

- Use the single-well threshold tool . The assigned threshold appears as a horizontal pink line
- Or-
- Enter threshold values in the Set Threshold field

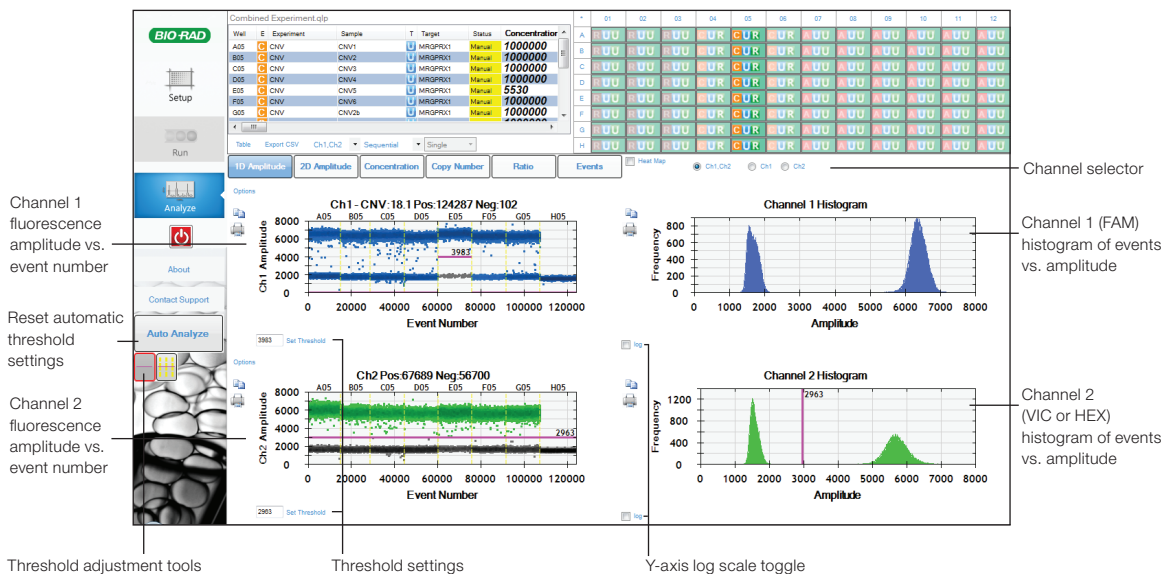
When viewing multiple wells, change the thresholds as follows:

- Use the single-well threshold tool  to change the threshold in a single well. Vertical yellow lines in the processed data plots show where droplet data from each well start and end, and the assigned threshold appears as a horizontal pink line
- Use the multi-well threshold tool  to change the threshold in all the wells (appears as a pink line in the plots)
- To manually set threshold values for single or multiple wells, enter the values in the Set Threshold field below the plot and click **Set Threshold** or **Enter**

Click **Auto Analyze** to revert to automatic threshold settings and calculations. Threshold adjustments can also be made in the 2D Amplitude clustering plots (see Section 3.3.2).



Viewing channel data for a single well. Processed data from both channels of a single well are shown. In channel 1, the single-well threshold tool is enabled (the threshold is shown by the pink line and the value in the **Set Threshold** field).



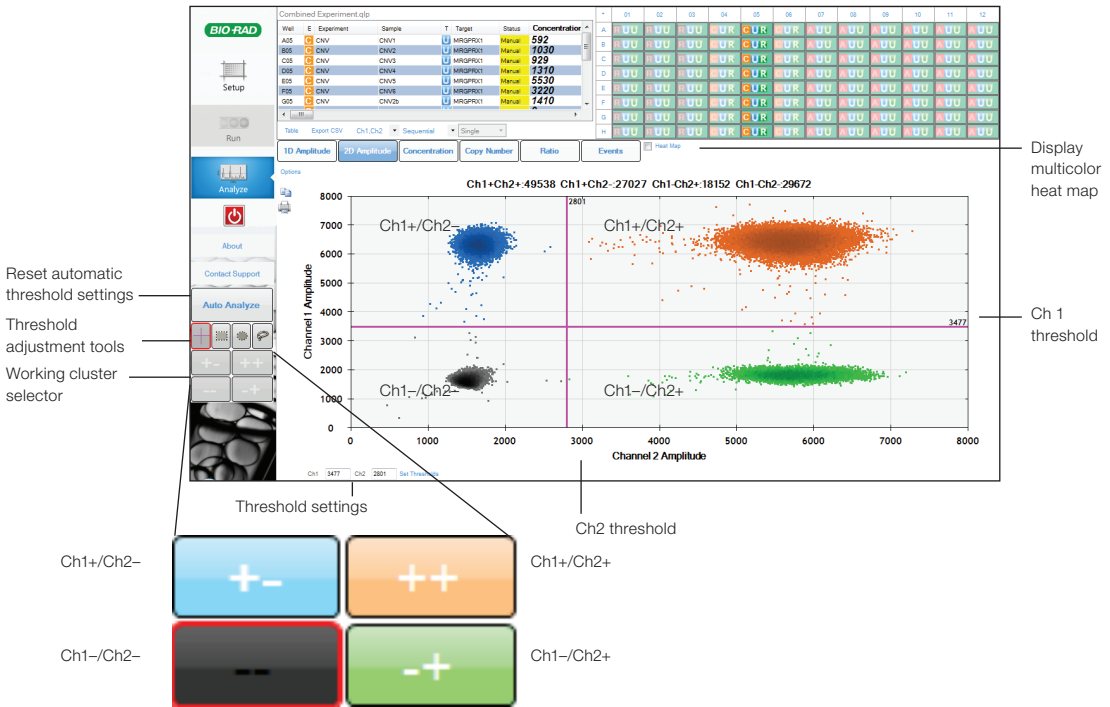
Viewing channel data for multiple wells. Processed data from both channels of multiple wells are shown. In channel 1, the single-well threshold tool is enabled (the threshold is indicated by the pink line and the value in the **Set Threshold** field; the status of that well in the results also shows Manual). In Channel 2, the multiple threshold tool is enabled.

3.3.2 Viewing Clustering Plots (2D Amplitude)

Click **2D Amplitude** to view the channel 1 vs. channel 2 clustering plot and enable options for manually or automatically adjusting the thresholds used in assigning positives and negatives for each detection channel.

- To reset automatic thresholds for positives and negatives, click **Auto Analyze**
- To manually assign thresholds:
 - Use the thresholding crosshair to assign classification regions for the whole plot (the clustering tool is disabled when viewing the plot in heatmap mode)
 - Use the ellipse, rectangle, or lasso threshold adjustment tool to classify a region of the plot. Click the tool, then click the region type in the working cluster selector. Use the tool to select the region within the plot

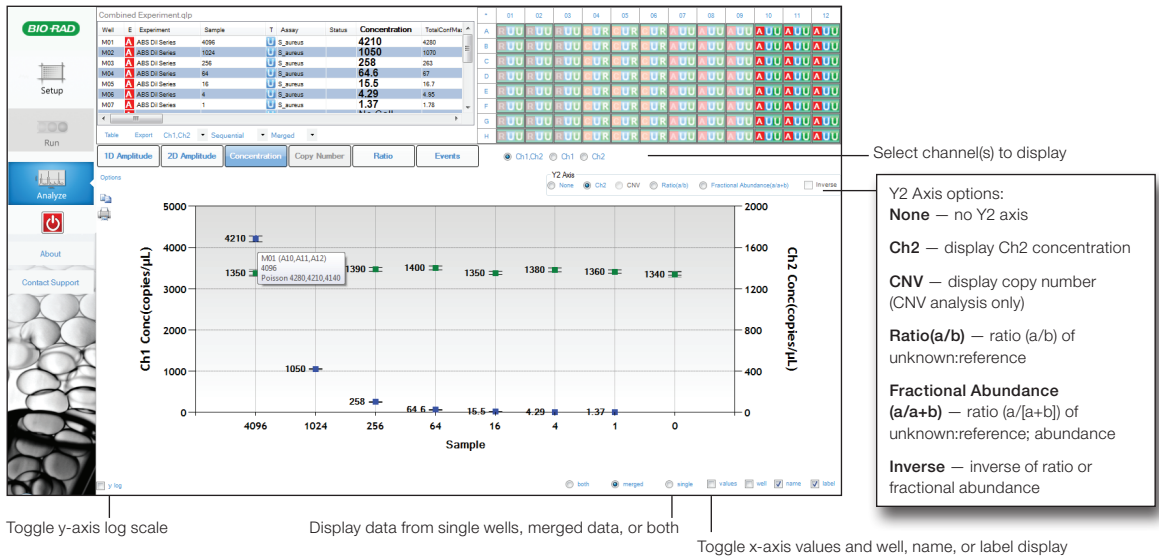
Tip:
 Mouse over any well in the well selector to preview data from that well in the clustering plot.



Viewing clustering plots. Threshold adjustment options available in the clustering plot are shown. Threshold values are indicated by the pink lines in the plot.

3.3.3 Viewing Concentration Data (Concentration)

Concentration data for each target appear in the wells in the plate map and are tabulated in the results table. Click **Concentration** to visualize data in concentration plots. Use the radio buttons to select the channels displayed. Error bars reflect total error or Poisson 95% confidence limits. These data can be exported for analysis in other spreadsheet applications (for example, Microsoft Excel).



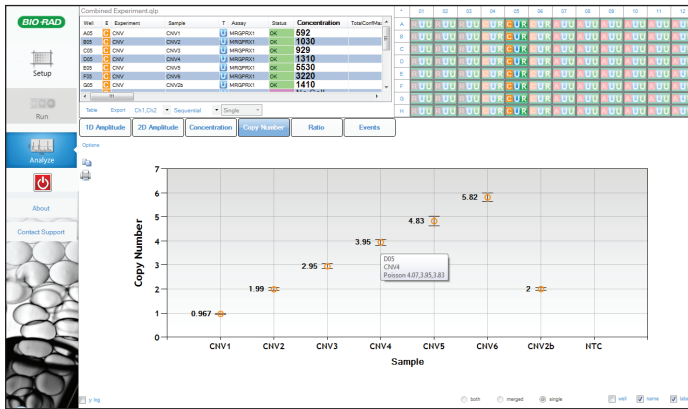
Viewing concentration data. Data from absolute quantification are shown. Hover over data points to reveal well identity, concentration, and Poisson confidence limits. Solid data points (shown) indicate merged data; open data points (not shown) indicate data from single wells.

The Copies/μl Well column displays the total amount of starting material in the ddPCR sample. The values shown reflect the product of the concentration (in copies per μl) multiplied by the 20 μl ddPCR reaction input used to make droplets. This value does not appear for merged well data.

This column is also exported in the *.csv file. The original concentration column and calculations remain unchanged.

3.3.4 Viewing Copy Number Data (Copy Number)

Click **Copy Number** to view copy number for selected wells/samples.



Toggle well, name, or label display

Viewing copy number data. Hover over data points to reveal well identity, concentration, and Poisson confidence limits. Solid data points (not shown) indicate merged data; open data points (shown) indicate data from single wells.

3.3.5 Viewing Ratio Data (Ratio)

Click **Ratio** to view ratio data for selected wells/samples. Use the radio buttons to select a plot of the **Ratio** (unknown:reference) or **Fractional Abundance** (% of sample); select **Inverse** to apply the inverse of either.



Select ratio or abundance plot, inverse ratios

Toggle well, name, or label display

Viewing ratio data. Data from absolute quantification are shown. Hover over data points to reveal well identity, concentration, and Poisson confidence limits. Select **y log** to convert the y-axis to logarithmic scale (shown). Solid data points (not shown) indicate merged data; open data points (shown) indicate data from single wells.

3.3.6 Viewing Events

Click **Events** to view the number of droplet events counted for selected wells/samples. Use the radio buttons to select the channels displayed. View positive, negative, or total droplet counts, or any combination of these.

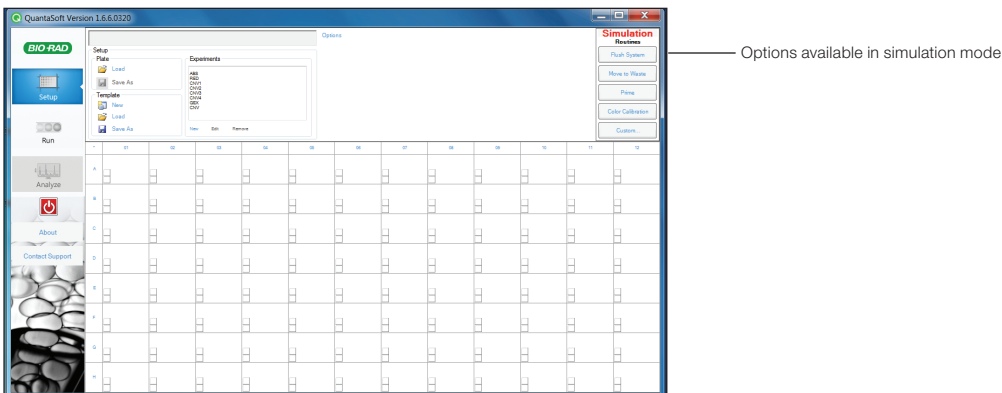


Viewing event data. Data from absolute quantification are shown.

3.3.7 Simulation Mode

When installing QuantaSoft v. 1.5 or later, a second program — QuantaSoft-Simulation — will appear in the Windows Start menu (but not on the desktop). Open this mode from the Start menu; otherwise, files automatically open in the regular version of QuantaSoft.

Simulation mode allows you to perform functions from the top right menu that are otherwise enabled only when connected to a droplet reader. For example, you can preview common functions such as a system flush or a color calibration run. The simulation mode also allows you to load a demo plate or create a new template and simulate a ddPCR run.

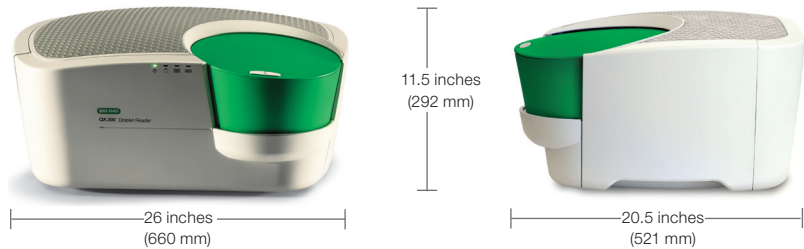


Simulation mode (main screen).

4

Specifications and Maintenance

4.1 Specifications



Weight	56.6 lb. (26 kg)
Size (W x D x H)	26 x 20.5 x 11.5" (660 x 521 x 292 cm)
Electrical requirements	100–240 V, 50/60 Hz, 1 A; voltage fluctuations not to exceed +10% of ratings (for external power supply provided)
Temperature	18–30°C
Altitude	0–6,560 ft (0–2,000 m)
Humidity	85% max (noncondensing)
Pollution degree	2 (indoor use)
Installation category	II (external power supply plugs into standard AC receptacle)
Ventilation requirements	5" (12 cm) left and right of machine and 10" (25 cm) behind should be unobstructed for proper ventilation

4.2 Maintenance

4.2.1 General Maintenance Procedures

Surfaces of the instrument may require general cleaning. Use deionized/distilled water for general wipe down with a slightly dampened cloth. For decontamination, 10% bleach followed by 70% ethanol and/or deionized/distilled water may be used. Do not use acetone or tap water.

Inspect equipment regularly for damaged external components or wiring. Do not use if damaged.

Apply standard MSDS (Material Safety Data Sheet) and OSHA practices when handling and disposing of generated waste.

Bio-Rad droplet generation and reader fluids are based on fluorinated hydrocarbon chemistry and should be disposed of in accordance with institutional, state, and local regulations. These nonflammable fluids are inert and have low environmental impact and low toxicity. Collect waste in a polyethylene container and discard within one month.

Droplets made with Bio-Rad master mix have antimicrobial properties, but microbial growth is possible. The waste profile should contain the following: fluorinated hydrocarbons, water, fluorescent dye (from probes), protein, and nucleic acids. Do not replace detachable power cord with an uncertified or an inadequately rated cord.

4.2.2 Replacing Droplet Reader Oil and Removing Waste

Replace the droplet reader oil and empty the waste receptacle as needed. Use the handle built into the side compartment to slide the carriage out:

- Use empty oil supply bottles as new waste bottles. Add 50 ml 10% bleach to the waste bottle to prevent microbial growth, and place a label on the waste bottle at this time
- Place the new bottle of oil in the oil position and screw the cap into place. In QuantaSoft™ software, under Instrument Routines in the Setup window, click **Prime** to fill the lines with oil before the system is run
- If the instrument has been unused for longer than a week, prime the system before running a plate



Replacing droplet reader oil and removing waste. Slide out the carriage containing the droplet reader oil and waste receptacle to remove and replace the bottles (left). After replacing oil, click **Prime** (right) to fill the lines; other instrument maintenance routines are for use by field specialists only.

Appendix A

Ordering Information

QX200™ ddPCR™ System

Catalog #	Description
186-4001	QX200™ Droplet Digital™ PCR System , includes droplet generator, droplet reader, laptop computer, software, associated component consumables
186-4002	QX200 Droplet Generator , includes droplet generator, 1 box of 24 cartridges, 1 pkg of 24 gaskets, 2 cartridge holders, 1 power cord
186-4003	QX200 Droplet Reader , includes droplet reader, ddPCR manual, 2 plate holders, USB cable, power cord
186-4007	Droplet Generator Cartridges and Gaskets , includes 5 pkg of 24 DG8™ cartridges, 5 pkg of 24 DG8 gaskets
186-4008	DG8 Cartridges for QX100™/QX200 Droplet Generator , 1 pkg of 24 cartridges
186-3009	DG8 Gaskets for QX100/QX200 Droplet Generator , 1 pkg of 24 gaskets
186-3051	DG8 Cartridge Holder
29711024	Droplet Reader Plate Holder

186-3005	Droplet Generation Oil for Probes , 10 x 7 ml
186-4005	Droplet Generation Oil for EvaGreen , 2 x 7 ml
186-4006	Droplet Generation Oil for EvaGreen , 10 x 7 ml
186-3004	ddPCR droplet Reader Oil , 2 x 1 L

ddPCR Reagents

186-3023	ddPCR Supermix for Probes (no dUTP) , 2 ml (2 x 1 ml), 2x supermix
186-3024	ddPCR Supermix for Probes (no dUTP) , 5 ml (5 x 1 ml), 2x supermix
186-3025	ddPCR Supermix for Probes (no dUTP) , 25 ml (5 x 5 ml), 2x supermix
186-3026	ddPCR Supermix for Probes , 2 ml (2 x 1 ml), 2x supermix
186-3010	ddPCR Supermix for Probes , 5 ml (5 x 1 ml), 2x supermix
186-3027	ddPCR Supermix for Probes , 25 ml (5 x 5 ml), 2x supermix
186-3028	ddPCR Supermix for Probes , 50 ml (10 x 5 ml), 2x supermix

186-3021	One-Step RT-ddPCR Kit for Probes , 2 ml (2 x 1 ml), 200 x 20 µl reactions, 2x RT-ddPCR mix, includes 1 manganese acetate tube	Thermal Cyclers and Plate Sealer
186-3022	One-Step RT-ddPCR Kit for Probes , 5 ml (5 x 1 ml), 500 x 20 µl reactions, 2x RT-ddPCR mix, includes 2 manganese acetate tubes	185-1196 C1000 Touch™ Thermal Cycler with 96-Well Fast Reaction Module , includes C1000 Touch thermal cycler chassis, 96-well reaction module, USB flash drive
186-4033	QX200™ ddPCR™ EvaGreen® Supermix , 2 ml (2 x 1 ml), 200 x 20 µl reactions	185-1197 C1000 Touch™ Thermal Cycler with 96-Deep Well Fast Reaction Module , includes C1000 Touch thermal cycler chassis, 96-deep well reaction module, USB flash drive
186-4034	QX200 ddPCR EvaGreen Supermix , 5 ml (5 x 1 ml), 500 x 20 µl reactions	181-4000 PX1™ Plate Sealer , includes heat sealing instrument, plate support block that holds 96-well and 384-well plates, sealing frame, power cord
186-4035	QX200 ddPCR EvaGreen Supermix , 25 ml (5 x 5 ml), 2,500 x 20 µl reactions	181-4040 Pierceable Foil Heat Seal , pkg of 100
186-4036	QX200 ddPCR EvaGreen Supermix , 50 ml (10 x 5 ml), 5,000 x 20 µl reactions	
186-3052	ddPCR Buffer Control Kit for Probes , 2 x 4.5 ml bottles, 2x buffer	
186-4052	ddPCR Buffer Control Kit for EvaGreen , 2 x 4.5 ml bottles, 2x buffer	



**Bio-Rad
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