QX200 Droplet Reader and QuantaSoft Software

Instruction Manual

Catalog #: 1864001
       1864003

BIO·RAD

Bio-Rad Technical Support Department

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

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

Legal Notices

Safety and Regulatory Compliance

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 61326-1:2013 (Class A). Electrical equipment for measurement, control, and laboratory use. EMC requirements, Part 1: General requirements

- UL 61010-1:2012, Laboratory equipment, Test & Measurement Equipment and Industrial Process Controls

- CAN/CSA 22.2 No 61010-1-12, 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.

This instrument is for use only by trained personnel.

Do not position the equipment so that it is difficult to operate the plug of the power supply.

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
- Genome edit detection

> For instructions on use of the QX200 Droplet Generator, please refer to the QX200 Droplet Generator Instruction Manual (bulletin 10031907). For information on QX200 AutoDG Droplet Digital PCR System and the Automated Droplet Generator, please refer to the Automated Droplet Generator Instruction Manual (bulletin 10043138).

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 #1864001). Catalog # refers to replacement items (quantities may be different).

| Component	Description	Catalog #
QX200 Droplet Generator		
QX200 Droplet Generator	Instrument used for droplet generation	1864002
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	1864007
DG8 Cartridge Holder	Positions and holds the droplet generator cartridge in the instrument for droplet generation	1863051
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	1864003
Laptop PC and QuantaSoft Software	Connects to QX200 Droplet Reader for data collection and analysis	1863007
Droplet Reader Plate Holders (2)	Position 96-well PCR plate in the droplet reader	12006834
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
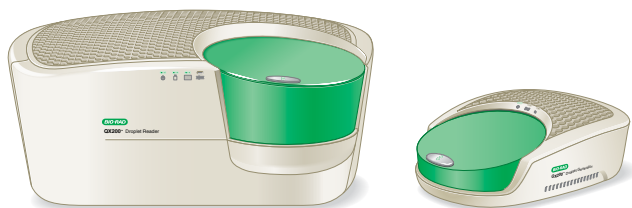
Fig. 1. QX200 Droplet Digital PCR System. Left, QX200 Droplet Reader. Right, QX200 Droplet Generator.

Table 1.2. Additional materials required.

Component	Recommended	Catalog # and Supplier
QX200 Droplet Generator		
Reagents for probe detection		
PCR supermix	ddPCR Supermix for Probes (No dUTP)	1863023, 1863024, 1863025
	ddPCR Supermix for Probes	1863026, 1863010, 1863027, 1863028
	ddPCR Supermix for Residual DNA Quantification	1864037, 1864038, 1864039, 1864040
	One-Step RT-ddPCR Advanced Kit for Probes	1864021, 1864022
Droplet generation oil	Droplet Generation Oil for Probes	1863005
Control	ddPCR Buffer Control for Probes	1863052
Reagents for EvaGreen® detection		
PCR supermix	QX200 ddPCR EvaGreen® Supermix	1864033, 1864034, 1864035, 1864036
Droplet generation oil	Droplet Generation Oil for EvaGreen®	1864005, 1864006
Control	QX200 Buffer Control Kit for EvaGreen®	1864052
Consumables and Other Materials		
Pipets	20 µl pipet for sample loading	Rainin L-20, L8-20
	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-LTS-A-10µL-/F-960/10, GP-LTS-A-200µL-/F-960/10
96-well PCR plates	ddPCR 96-Well Plates	12001925
Reagent trough	Any	
Foil plate seals	Pierceable foil plate seals	1814040
Plate sealer	PX1 PCR Plate Sealer	1814000
QX200 Droplet Reader		
Droplet reader oil	ddPCR Droplet Reader Oil	1863004
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 µl 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/µl

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 instruments such that they can be easily disconnected from the power source should that become necessary to service the equipment

- Connect the QX200 Droplet Reader to the laptop PC using the USB 2.0 cord provided

- 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 ddPCR Droplet Reader Oil (catalog #1863004). 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. 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; replace the oil or waste bottle (see Section 4.2.2).

Table 2.1. Status indicator lights on the QX200 Droplet Reader.

	Power on	Bottle levels	Plate in place	Run complete
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.

Top of plate holder

96-well PCR plate

Down   Down

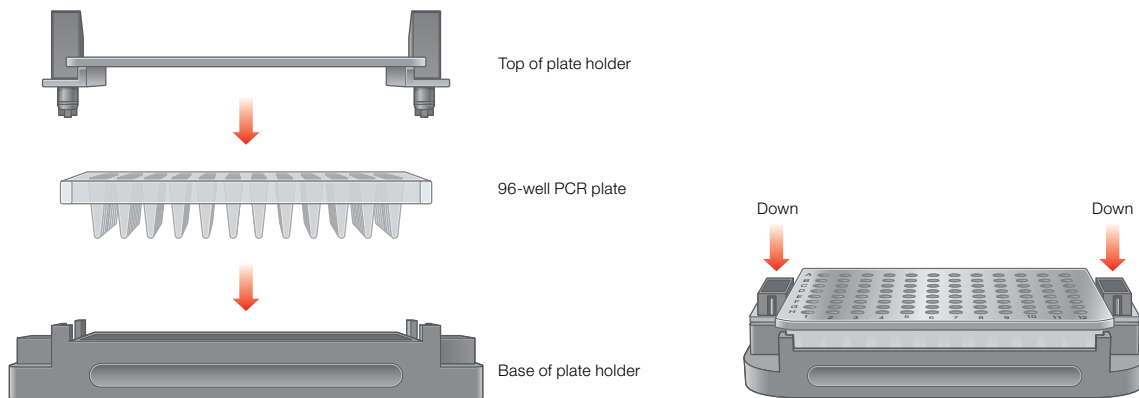Base of plate holder

Fig. 2. 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).

Push to open/close
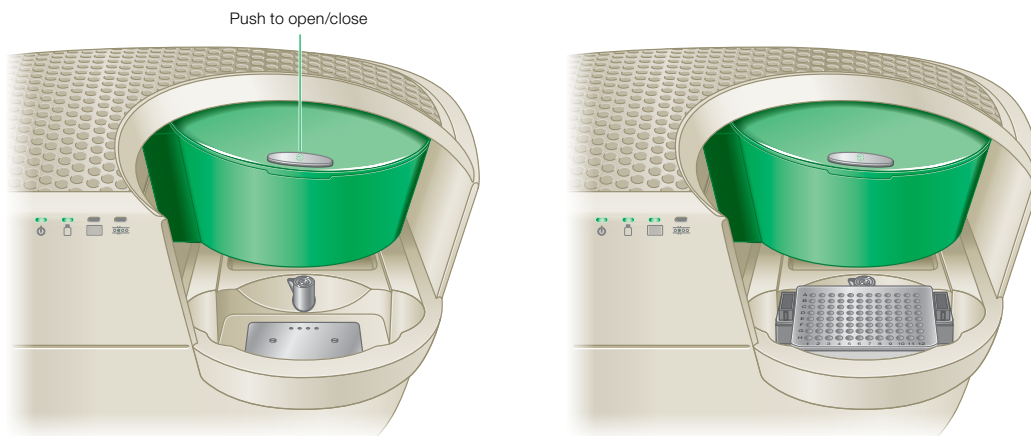
Fig. 3. Placing the plate holder into the droplet reader.

Tip: Make sure the plate holder lid is flat on top of the plate and the clamps are locked down. Failure to do so could cause the sampling needle to run into the lid, damaging the instrument.
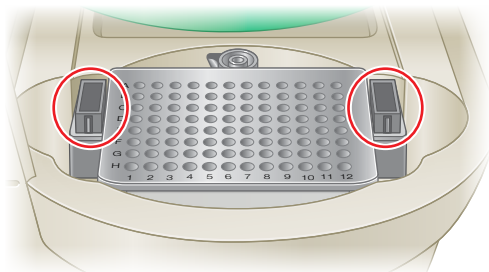
Correct
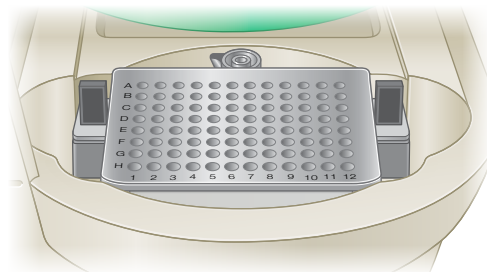
Incorrect

Fig. 4. 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 Section 3.3)

QuantaSoft Software uses the following file types:

- Template (*.qlt) — user-defined plate layout settings (no data) for reading a ddPCR Plate
- Raw data (*.qlb) — unprocessed data from each well in a ddPCR Plate
- Results (*.qlp) — 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

File name

Load settings and data from previous experiments

Load or create a template (settings only)

Options for advanced data analysis and setup

Open experiment editor

Define experiment settings

Start the run

View and analyze data

Abort run or exit program

Instrument maintenance options (appear only when connected to the droplet reader or when in simulation mode)
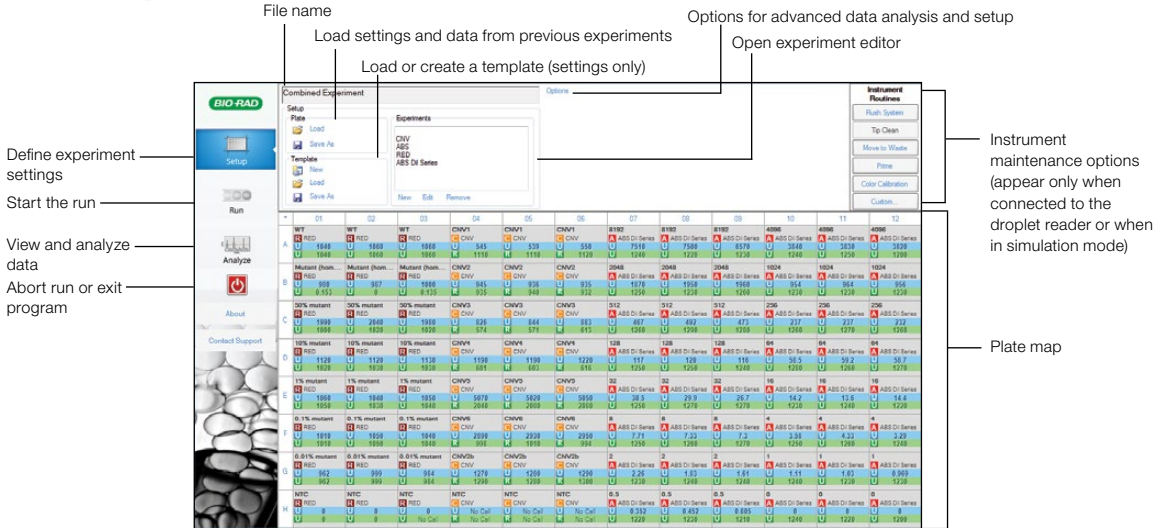
Plate map

Fig. 5. 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**
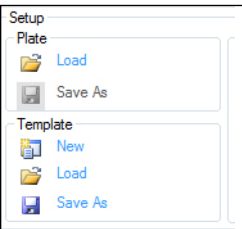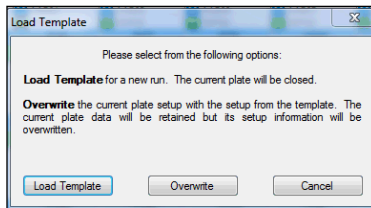
Fig. 6. Setup window.

Fig. 7. Load Template window.

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 for 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.
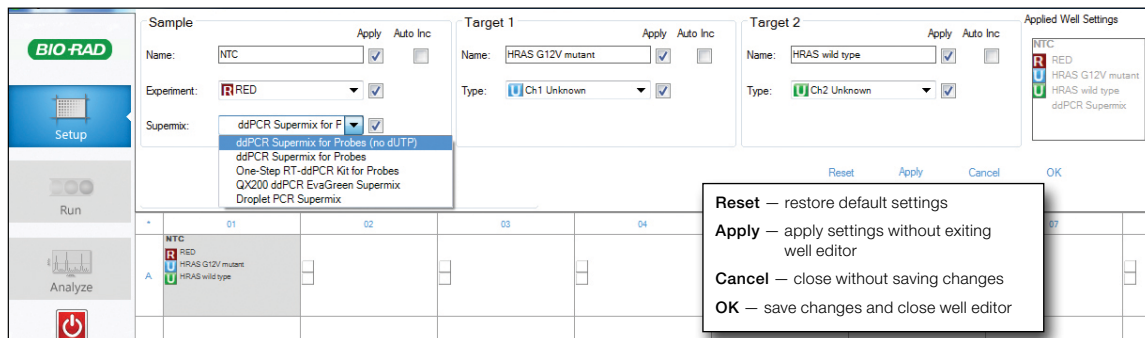

Fig. 8. Well editor. Settings for absolute quantification of two unknowns in a single sample are shown.
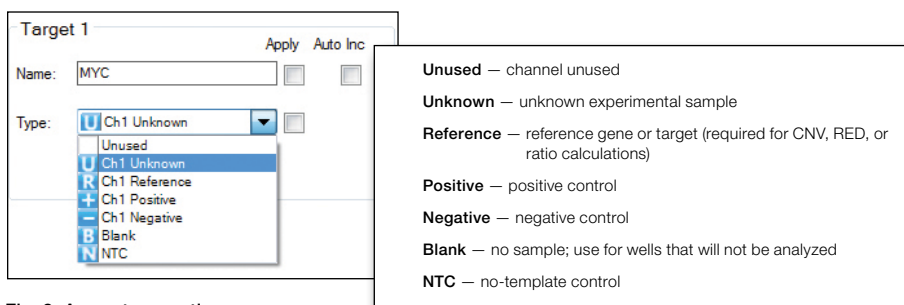
Fig. 9. Assay type options.

1. 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

2. In the Sample panel, enter the sample **Name** and select the **Experiment** from the dropdown menu.

   - All saved experiments appear in the dropdown 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)

3. Select the **Supermix** from the dropdown menu (required; selection cannot be changed after data collection).

4. 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:
Use the plate map to change the selected well 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. Three types of experiments are possible: Absolute Quantification (ABS), Rare Event Detection (RED), and Copy Number Variation (CNV). 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).

Copy number options appear for CNV experiments only

Customize the color codes for experiments (for example, by experiment type, user, etc.)

Fig. 10. 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.
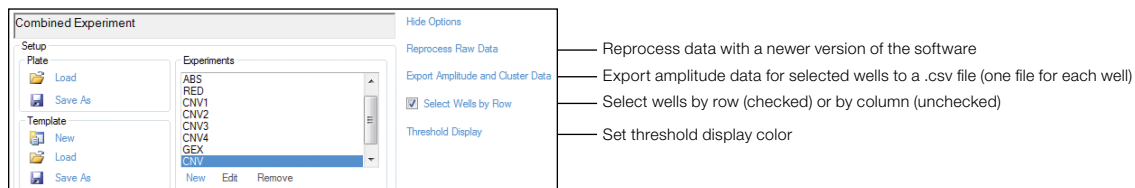
Fig. 11. Advanced options.

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
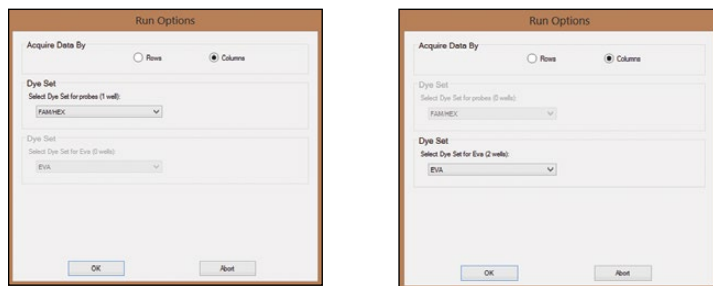
Fig. 12. Run options.

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.
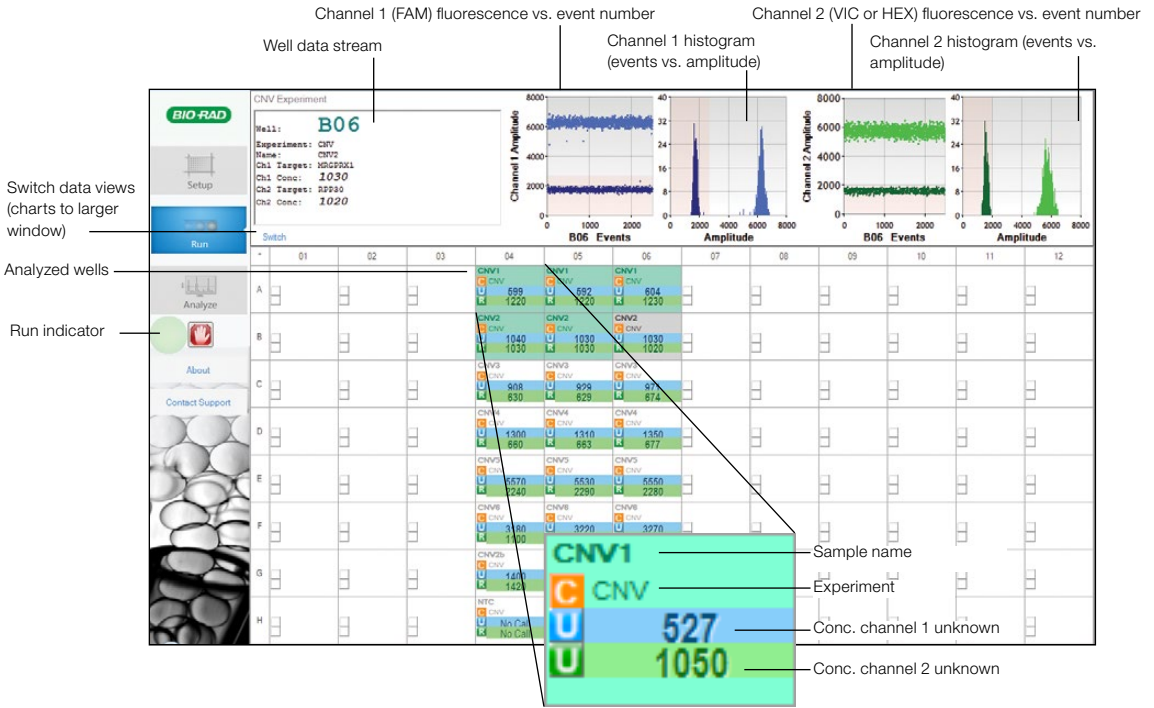
Fig. 13. Interface during an active run. Data for both detector channels are shown for the well being read.

3.3 Analyze

In the **Setup** window, load a plate (filename.qlp), 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
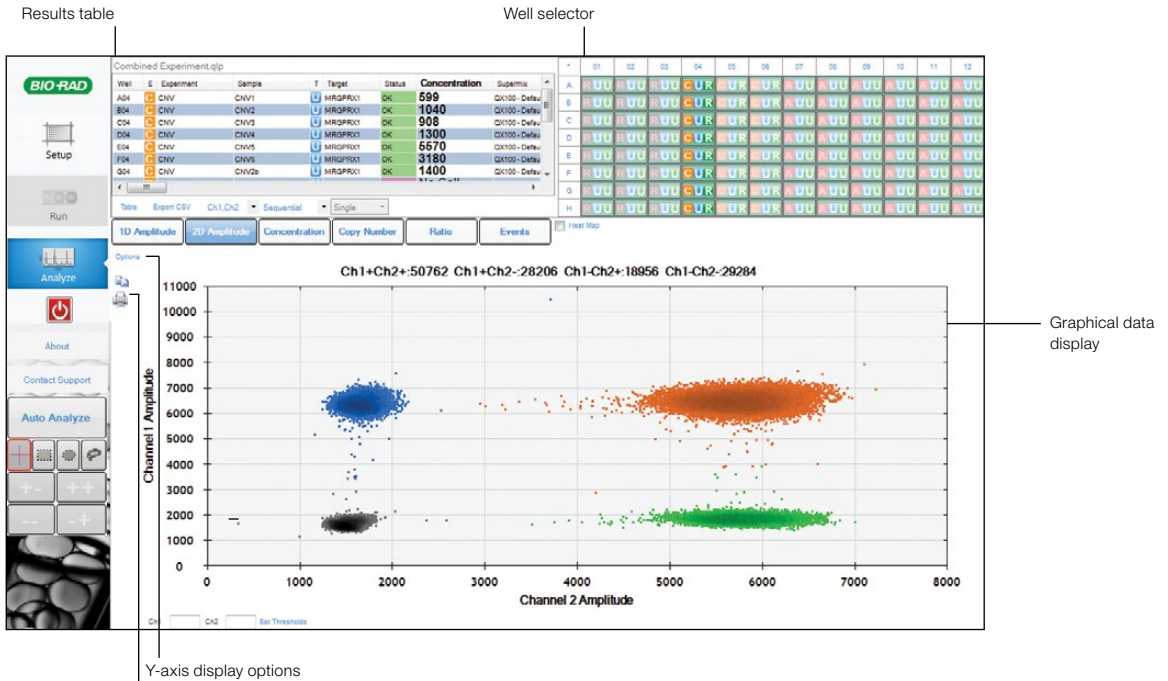
Copy graph to clipboard or print

Fig. 14. Data analysis interface. Data from a CNV analysis are shown.

Status options:

OK — automatic analysis successful

CHECK — automatic analysis unsuccessful; to view concentration, use manual analysis tools

To get more details of an error, hover over the check tab with the mouse arrow

Multi — data automatically analyzed as part of a multi-well selection

Manual — droplets analyzed manually

View table in graphical display window (toggle)

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
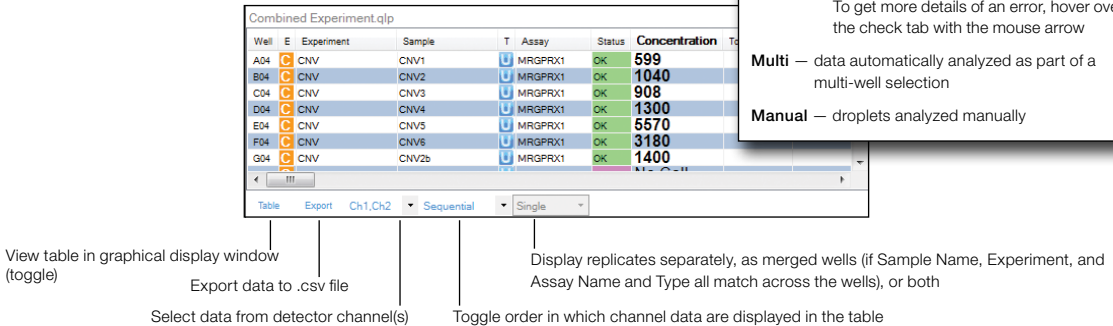
Fig. 15. Results table options. Data from a CNV analysis are shown.

Note: If a concentration estimate fails to appear in the concentration chart in QuantaSoft, this indicates the software could not auto-analyze or assign droplets to positive or negative populations using its auto-analysis algorithm, or the well had an unusually low droplet count (<10,000). Manually set a threshold and QuantaSoft Software will calculate a concentration, which will appear in the concentration chart.

Channel 1 vs. channel 2 scatter plot

Plot of ratio of unknown:reference (a/b or a/[a+b])

Channel data   Concentration data

Plot of measured copy numbers

Plot of # droplet events counted
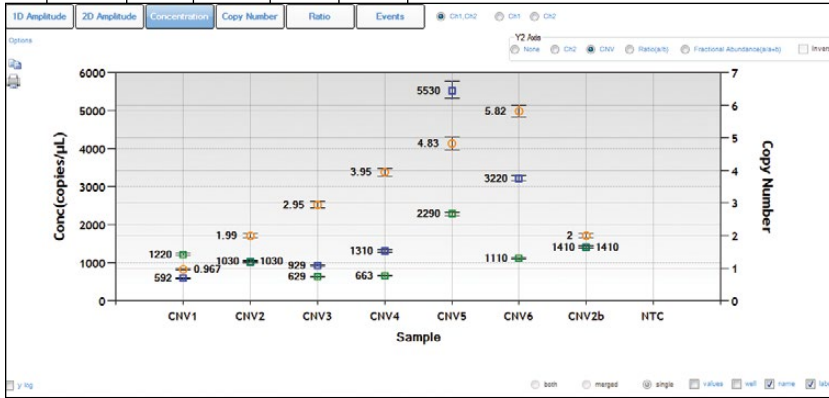
Fig. 16. Graphical data display options. A concentration plot from a CNV analysis is shown, with display options across the top.

3.3.1 Viewing Channel Data

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**

> **Tip:** It is recommended that researchers review the thresholds set automatically by the software and make changes as needed.

Click **Auto Analyze** to revert to automatic threshold settings and calculations. Threshold adjustments can also be made in the 2D Amplitude scatter plots (see Section 3.3.2).
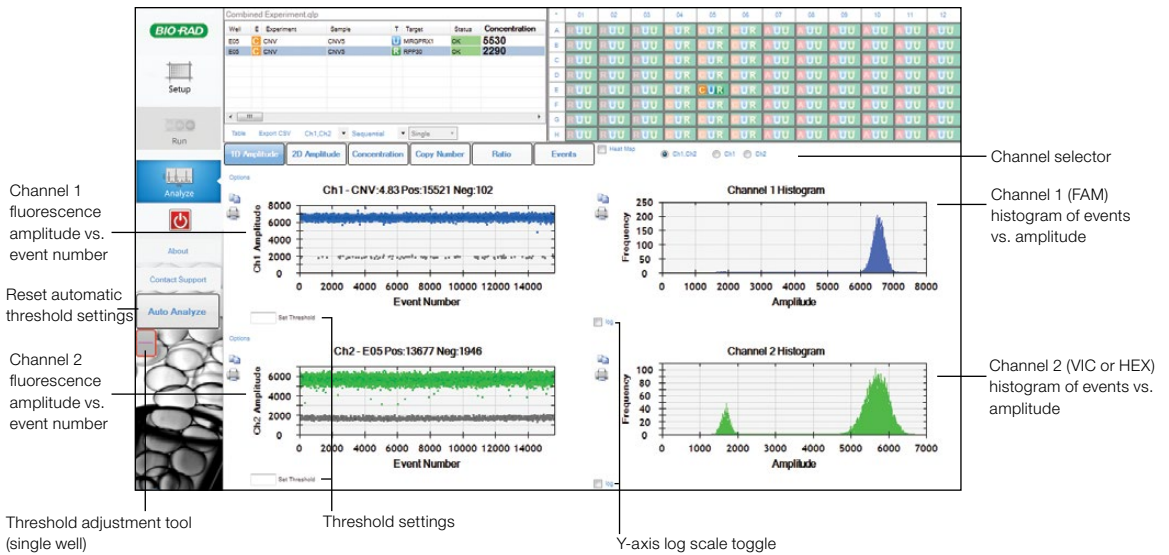
Fig. 17. Viewing channel data for a single well. Processed data from both channels of a single well are shown. In channel 1, the single-well threshold tool is enabled (the threshold is shown by the pink line and the value in the **Set Threshold** field).
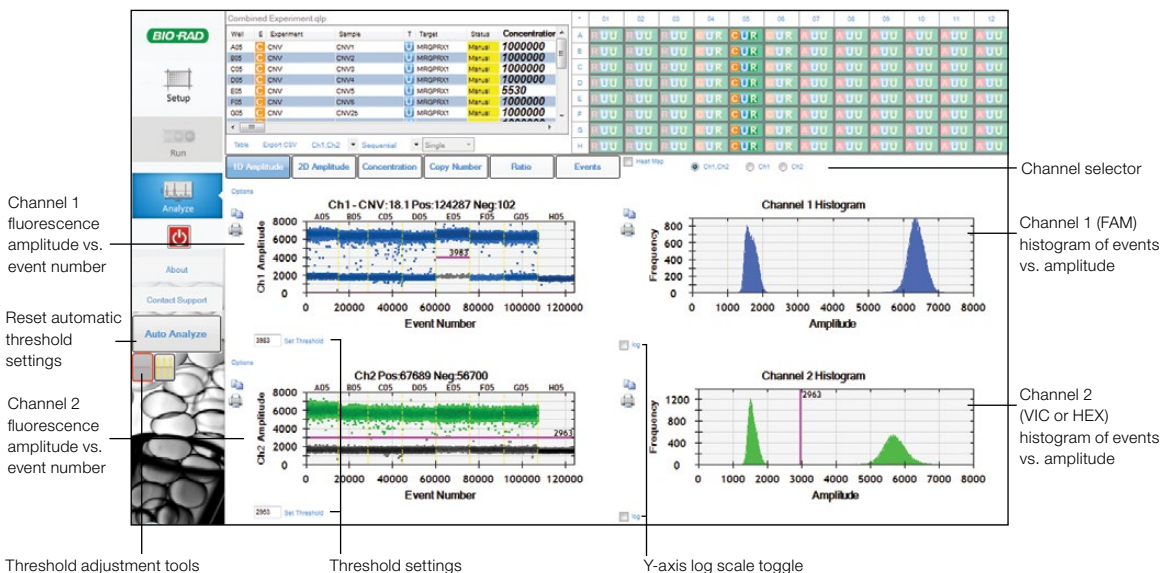
Fig. 18. Viewing channel data for multiple wells. Processed data from both channels of multiple wells are shown. In channel 1, the single-well threshold tool is enabled (the threshold is indicated by the pink line and the value in the **Set Threshold** field; the status of that well in the results table shows as Manual). In Channel 2, the multiple threshold tool is enabled.

3.3.2 Viewing Scatter Plots

Click **2D Amplitude** to view the channel 1 vs. channel 2 scatter 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 heat map 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

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Tip: Mouse over any well in the well selector to preview data from that well in the scatter plot.
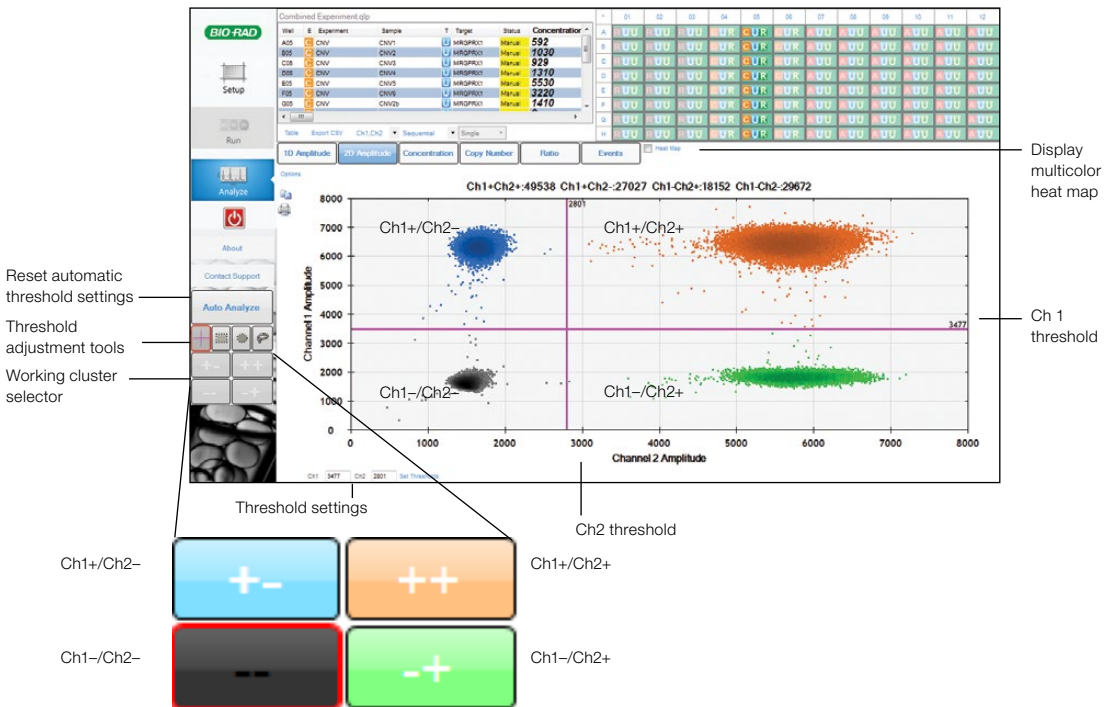
Fig. 19. Viewing scatter plots. Threshold adjustment options available in the scatter plot are shown. Threshold values are indicated by the pink lines in the plot.

3.3.3 Viewing Concentration Data

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).

Fig. 20. 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.

3.3.4 Viewing Copy Number Data

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

Note: Available only if CNV is selected as the experiment for selected wells.

Toggle well, name, or label display
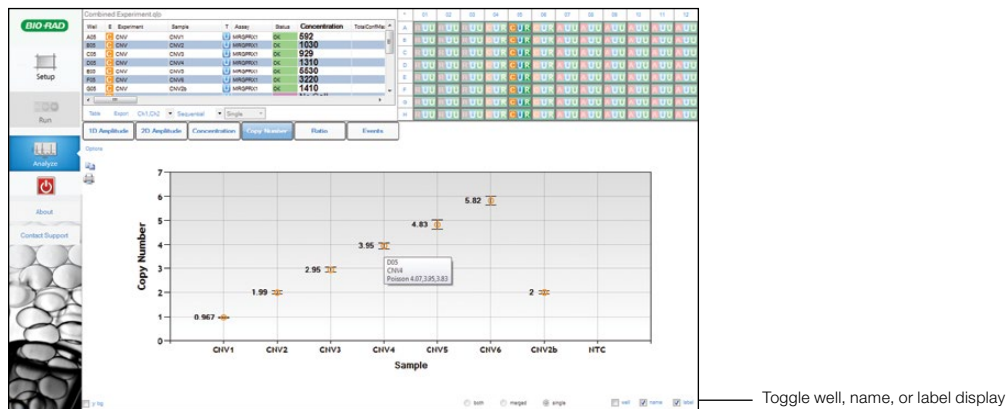
Fig. 21. Viewing copy number data. Hover over data points to reveal well identity, concentration, and Poisson confidence limits. Solid data points (not shown) indicate merged data; open data points (shown) indicate data from single wells.

3.3.5 Viewing Ratio Data

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

Note: Available only if there is a duplexed reaction.

Select ratio or abundance plot, inverse ratios
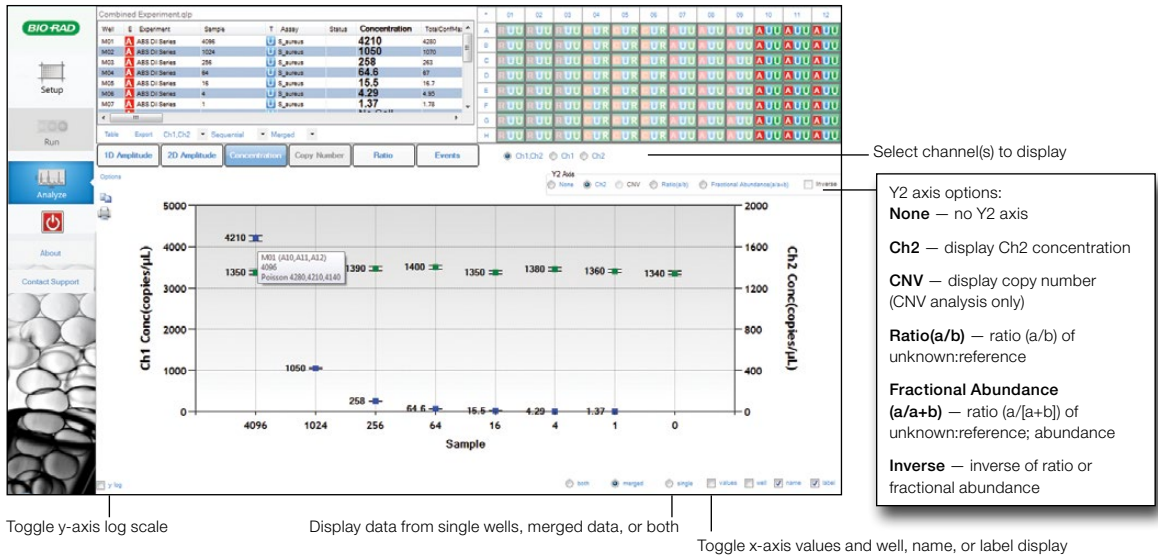
Toggle well, name, or label display
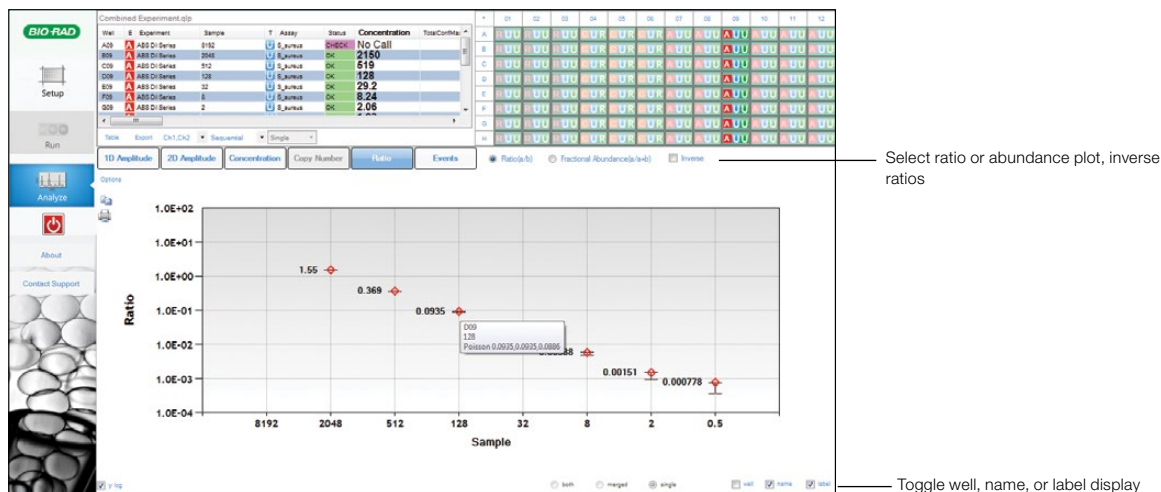
Fig. 22. Viewing ratio data. Data from absolute quantification are shown. Hover over data points to reveal well identity, concentration, and Poisson confidence limits. Select **y log** to convert the y-axis to logarithmic scale (shown). Solid data points (not shown) indicate merged data; open data points (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.

Fig. 23. 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.
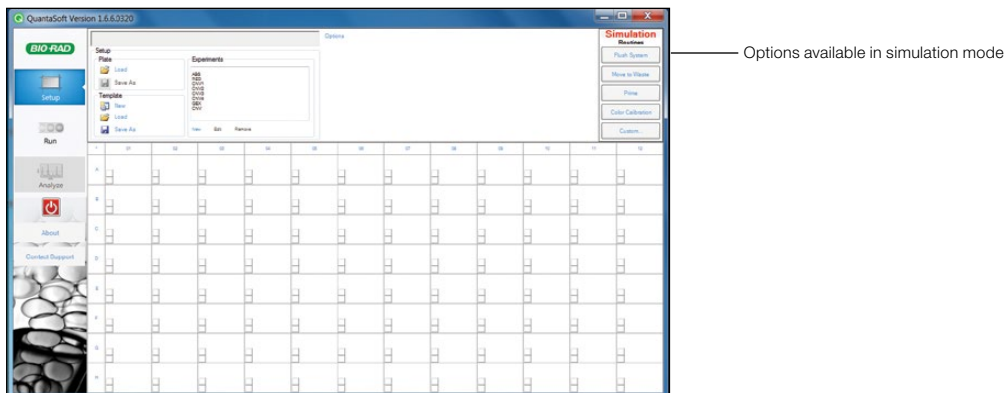
Fig. 24. Simulation mode (main screen).

4 | Specifications and Maintenance

4.1 Specifications
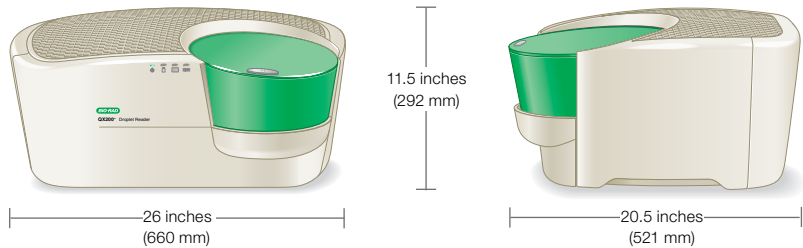
Weight	56.6 lb. (26 kg)
Size (W x D x H)	26 x 20.5 x 11.5" (660 x 521 x 292 mm)
Electrical requirements	100–240 V, 50/60 Hz, 1 A; voltage fluctuations not to exceed +10% of ratings (for external power supply provided)
Temperature	18–30ºC
Altitude	0–6,560 ft (0–2,000 m)
Humidity	85% max (noncondensing)
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. When disposing of the reader oil waste, consult with your local environmental health and safety specialists for guidance appropriate to disposal at your locality. You can find more MSDS available online. A typical waste profile should contain the following: fluorinated oils (95%), water (5%), bleach (<0.5%), proteins, nucleic acids, fluorescent dye (<0.1%).

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 supermix 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.

Since the QX200 Reader contains moving parts, fluids, tubing, and pumps, we strongly recommend annual preventive maintenance on the instrument.

4.2.2 Replacing Droplet Reader Oil and Removing Waste

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

- Use each empty oil supply bottle as a new waste bottle only once, then discard. Add 50 ml 10% bleach to the waste bottle to prevent microbial growth and place a label on the waste bottle to identify it as waste

> ❗ Discard the waste bottle after one time use. Reuse may lead to microbial growth, instrument damage, or run failures.

Correct   Incorrect
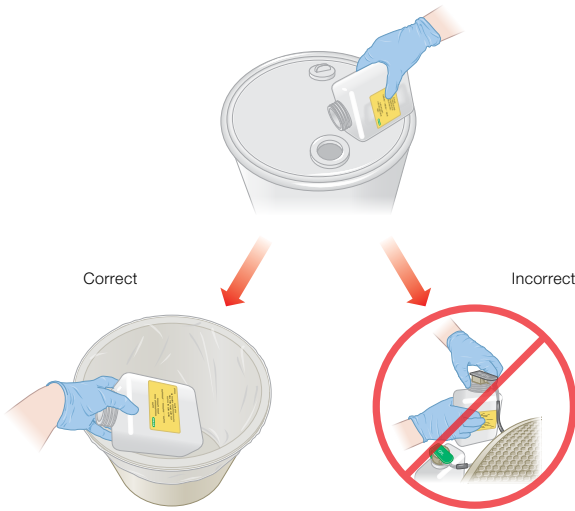
Fig. 25. Discarding the waste bottle after one time use.

> (!) The reader waste oil contains PCR products, which will serve as a substrate for microbial growth (mold and fungus). To avoid instrument damage, always be sure to add 50 ml of 10% bleach to the empty waste bottle to prevent microbial growth.

- 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

Instrument Routines

Flush System

Tip Clean

Move to Waste

Prime

Color Calibration

Custom...
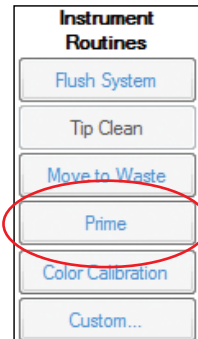
Fig. 26. Replacing droplet reader oil and removing waste. Slide out the carriage containing the droplet reader oil and waste receptacles to remove and replace the bottles (left). After replacing oil, click **Prime** to fill the lines; other instrument maintenance routines are for use by field specialists only.

4.2.3 Error Codes

Error code 201: This error means that the needle had trouble puncturing the foil. Check first that the foil isn't doubled, and that the plate holder lid is flat and secure. Also check the silver, donut-shaped oil-rinse reservoir that sits behind the plate holder. If it contains oil or debris, it's possible that the waste oil pump needs service. You should contact Technical Support.

All other error codes: Since the computer directly controls the instrument's fluidics, problems with computer performance can trigger instrument errors. The first step in troubleshooting is to restart the entire system, including restarting Windows on the host computer. If the error persists, contact Technical Support.

> **Tips:**
> The computer sees the QX200 Droplet Reader as an attached "removable disk" drive. Sometimes Windows updates will stall when Windows tries to query the reader; this problem can easily be solved by making sure you have the instrument powered off or disconnected when running Windows updates on the computer.

Appendix A
Ordering Information

Droplet Digital PCR (ddPCR) Systems

QX200 Droplet Digital PCR Systems

Catalog #	Description
1864001	**QX200 Droplet Digital PCR System**, includes droplet generator, droplet reader, laptop computer, software, associated component consumables
1864002	**QX200 Droplet Generator**, includes droplet generator, 1 box of 24 cartridges, 1 pkg of 24 gaskets, 2 cartridge holders, 1 power cord
1864003	**QX200 Droplet Reader**, includes droplet reader, 2 plate holders, 1 USB cable, 1 power cord

Droplet Digital PCR Reagents

ddPCR Supermix for Probes (No dUTP)

1863023	**ddPCR Supermix for Probes (No dUTP)**, 2 ml (2 x 1 ml), 2x supermix, for use in nucleic acid sample preparation with the QX200 Droplet Generator
1863024	**ddPCR Supermix for Probes (No dUTP)**, 5 ml (5 x 1 ml), 2x supermix, for use in nucleic acid sample preparation with the QX200 Droplet Generator
1863025	**ddPCR Supermix for Probes (No dUTP)**, 25 ml (5 x 5 ml), 2x supermix, for use in nucleic acid sample preparation with the QX200 Droplet Generator

ddPCR Supermix for Probes

1863026	**ddPCR Supermix for Probes**, 2 ml (2 x 1 ml), 2x supermix, for use in sample preparation with the QX200 Droplet Generator
1863010	**ddPCR Supermix for Probes**, 5 ml (5 x 1 ml), 2x supermix, for use in sample preparation with the QX200 Droplet Generator
1863027	**ddPCR Supermix for Probes**, 25 ml (5 x 5 ml), 2x supermix, for use in sample preparation with the QX200 Droplet Generator
1863028	**ddPCR Supermix for Probes**, 50 ml (10 x 5 ml), 2x supermix, for use in sample preparation with the QX200 Droplet Generator

ddPCR Supermix for Residual DNA Quantification

Catalog #	Description
1864037	**ddPCR Supermix for Residual DNA Quantification**, 2 ml (2 x 1 ml), 2x supermix, for use in residual DNA detection with the QX200 Droplet Generator
1864038	**ddPCR Supermix for Residual DNA Quantification**, 5 ml (5 x 1 ml), 2x supermix, for use in residual DNA detection with the QX200 Droplet Generator
1864039	**ddPCR Supermix for Residual DNA Quantification**, 25 ml (5 x 5 ml), 2x supermix, for use in residual DNA detection with the QX200 Droplet Generator
1864040	**ddPCR Supermix for Residual DNA Quantification**, 50 ml (10 x 5 ml), 2x supermix, for use in residual DNA detection with the QX200 Droplet Generator

One-Step RT-ddPCR Advanced Kit for Probes

1864021	**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
1864022	**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

QX200 ddPCR EvaGreen® Supermix

1864033	**QX200 ddPCR EvaGreen® Supermix**, 2 ml (2 x 1 ml), for use in nucleic acid sample preparation with the QX200 Droplet Generator
1864034	**QX200 ddPCR EvaGreen® Supermix**, 5 ml (5 x 1 ml), for use in nucleic acid sample preparation with the QX200 Droplet Generator
1864035	**QX200 ddPCR EvaGreen® Supermix**, 25 ml (5 x 5 ml), for use in nucleic acid sample preparation with the QX200 Droplet Generator
1864036	**QX200 ddPCR EvaGreen® Supermix**, 50 ml (10 x 5 ml), for use in nucleic acid sample preparation with the QX200 Droplet Generator

ddPCR Mutation Screening Kits

1863506	**ddPCR *KRAS G12/G13* Screening Kit**, includes 20x multiplex assay and 2x ddPCR Supermix for Probes (No dUTP)
12001626	**ddPCR *KRAS Q61* Screening Kit**, includes 20x multiplex assay and 2x ddPCR Supermix for Probes (No dUTP)
12001037	**ddPCR *BRAF V600* Screening Kit**, includes 20x multiplex assay and 2x ddPCR Supermix for Probes (No dUTP)
12001006	**ddPCR *NRAS Q61* Screening Kit**, includes 20x multiplex assay and 2x ddPCR Supermix for Probes (No dUTP)
12001094	**ddPCR *NRAS G12* Screening Kit**, includes 20x multiplex assay and 2x ddPCR Supermix for Probes (No dUTP)
12001627	**ddPCR *NRAS G12/G13* Screening Kit**, includes 20x multiplex assay and 2x ddPCR Supermix for Probes (No dUTP)
12002392	**ddPCR *EGFR* Exon 19 Deletions Screening Kit**, includes 20x multiplex assay and 2x ddPCR Supermix for Probes (No dUTP)

ddPCR Residual DNA Quantification Kits

17000031	**ddPCR CHO Residual DNA Quantification Kit**, 200 x 20 µl reactions, includes 20x CHO RDQ assay and 2x ddPCR Supermix for Residual DNA Quantification
17000032	**ddPCR *E. coli* Residual DNA Quantification Kit**, 200 x 20 µl reactions, includes 20x *E. coli* RDQ assay and 2x ddPCR Supermix for Residual DNA Quantification

ddPCR Copy Number Determination Kits

Catalog #	Description
1863500	**ddPCR *SMN1* Copy Number Determination Kit**, 200 x 20 µl reactions, includes assay at 20x concentration, 2x ddPCR Supermix for Probes (No dUTP), and positive controls
1863503	**ddPCR *SMN2* Copy Number Determination Kit**, 200 x 20 µl reactions, includes assay at 20x concentration, 2x ddPCR Supermix for Probes (No dUTP), and positive controls

ddPCR Library Quantification Kits

1863040	**ddPCR Library Quantification Kit for Illumina TruSeq®**, 200 x 20 µl reactions, includes 1 vial of primers and probes at 20x concentration, 2x ddPCR Supermix for Probes (No dUTP)

ddPCR Genome Edit Detection Assays

12002312	**ddPCR HDR Gene Edit Assay**, 100 rxns
12002313	**ddPCR HDR Gene Edit Assay**, 500 rxns
12003796	**ddPCR HDR Gene Edit Pkg**, 1,000 rxns
12003805	**ddPCR HDR Ref Assay, Predesigned**, 100 rxns
12003806	**ddPCR HDR Ref Assay, Predesigned**, 500 rxns
12003793	**ddPCR HDR Ref Pkg, Predesigned**, 1,000 rxns
12002314	**ddPCR NHEJ Gene Edit Assay**, 100 rxns
12002315	**ddPCR NHEJ Gene Edit Assay**, 500 rxns
12003794	**ddPCR NHEJ Gene Edit Pkg**, 1,000 rxns

Droplet Digital PCR Consumables and Accessories

Consumables and Accessories for the QX200 ddPCR System

Catalog #	Description
1863004	**ddPCR Droplet Reader Oil**, 2 L (2 x 1 L)
1863005	**Droplet Generation Oil for Probes**, 70 ml (10 x 7 ml)
1864008	**DG8 Cartridges**, 1 pkg of 24 cartridges
1863009	**DG8 Gaskets**, 1 pkg of 24 gaskets
1864007	**DG8 Cartridges and Gaskets**, 5 pkg of 24 cartridges, 5 pkg of 24 gaskets
1863051	**DG8 Cartridge Holder**
12001925	**ddPCR 96-Well Plates**, pkg of 25, clear shell, clear well, semi-skirted plates, for use with the QX200 and the QX200 AutoDG Droplet Digital PCR Systems

Consumables and Accessories for QX200 ddPCR System Only

1864005	**Droplet Generation Oil for EvaGreen®**, 14 ml (2 x 7 ml)
1864006	**Droplet Generation Oil for EvaGreen®**, 70 ml (10 x 7 ml)

QX200 Droplet Reader System Maintenance Log

Droplet Reader S/N: _____

Last Annual Maintainence Date: _____

Month: _____ Year: _____

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
As Necessary																															
Replace Droplet Reader Oil																															
Wipe Droplet Reader plate holder if it becomes wet																															
Discard the waste bottle after one time use																															
Use each empty oil supply bottle as a new waste bottle																															
Add 50 ml of 10% bleach to the new waste bottle																															
Signature:																															
Daily																															
Clean Work Area (prior & post use)																															
Discard used consumables																															
Weekly (at start of week)																															
Check expiration date of Droplet Reader Oil																															
Reboot the Droplet Reader																															
Restart the PC & QuantaSoft Software																															
Monthly																															
Clean Droplet Reader PCR plate holder metal surface in the instrument with 80% ethanol or isopropanol																															
Prime Instrument																															
Quarterly																															
Flush Droplet Reader Oil																															
Backup Database																															
Annual Maintenance (call Bio-Rad)																															

Refer to QX200 Droplet Reader System Instrument Manual maintenance section for detailed instructions

BIO-RAD

**Bio-Rad
Laboratories, Inc.**

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*Life Science
Group*