



QX ONE™ Droplet Digital™ PCR (ddPCR™) System

Workflow Tips for Success

Instrument Power-Up Sequence

- Turn on the back switch, then the front switch, and wait 5 min before launching the QX ONE Software. Do not touch the instrument or screen while the instrument is initializing
- After 5 min, launch the QX ONE Software and log in. The instrument will switch from busy to ready in 2–3 minutes. Do not touch the instrument or screen while the instrument is initializing

Note: If the instrument is being power cycled, turn off the back instrument switch and wait a full 3 min before following the power-up sequence.

Starting a New Set of Experiments

- If the QX ONE instrument is already powered on and has previously been running experiments, it is recommended that the QX ONE Software be fully closed, then reopened after 3 min. This ensures a fresh instance of the software for the upcoming run

Plate Preparation

Sample Preparation

- Always refer to the supermix product insert for instructions on sample handling, preparation, reaction setup, thermal cycling conditions, and sample fragmentation, specific to the supermix being used
- For DNA applications, thaw samples and reagents completely at room temperature for 15–30 minutes
- Vortex samples and reagents well as outlined in the product insert for the supermix being used
- Ensure the supermix concentration is 1x in final reaction
- Use RAININ tips for all sample handling and reaction setup steps
- Ensure pipets are calibrated according to the manufacturer's recommendations
- Vortex the master mix on high for a minimum of 15 seconds when using the 2x supermix and 30 sec when using the 4x multiplex supermix
- Centrifuge tubes and/or plates to settle contents and eliminate bubbles

Sample Loading to the GCR96 Cartridges

- Transfer 20 μ l of the ddPCR reaction to the bottom of each well within the GCR96 Cartridge
- Do not press the pipet plunger beyond the initial stop, as it will introduce unwanted bubbles inside the well
- The GCR96 Cartridge contains 6 individual DG16 Cartridges representing 6 sets of 16 wells (2 columns per DG16 Cartridge). Each set of 16 must be filled with either a sample or 1:1 ratio of water and ddPCR Buffer Control for Probes (catalog #1863052). Do not use water or leave empty wells blank. Correct backfilling with ddPCR Buffer Control is required for successful and efficient droplet generation in all wells
- Do not vortex the GCR96 Cartridges. You should vortex the samples/reagents/master mix before loading into the GCR96 Cartridges
- Load only 20 μ l per GCR96 well. GCR96 Cartridges are not designed for more than a 20 μ l reaction volume

Plate Sealing

- Make sure the PX1 PCR Plate Sealer is set at 180°C for 0.5 sec. Confirm settings, specifically if you have a QX200™ Droplet Digital PCR (ddPCR) System nearby
- Make sure the foil seal is squarely centered on top of the GCR96 Cartridge with no overhangs. Double red stripes should be visible
- Use only a 1-foil seal. Double-foil seals will create issues assessing contents in the wells
- Before sealing the GCR96 Cartridge, ensure the support block is oriented correctly. Position the block so that the overhanging edge faces away from the user
- Seal the GCR96 Cartridge with a foil seal, rotate the cartridge 180 degrees, place back on the support block, and seal a second time

Centrifuge

- Centrifuge the GCR96 Cartridge for 30 sec at 1,150 rcf
- Do not centrifuge for more than 1 minute

Plate Layout

- For software version 1.0–1.2, when running fewer than 96 wells, fill the remaining wells in the GCR96 plate with ddPCR Buffer Control for Probes (#1863052). For software version 1.3 or later, DG16 strips not containing samples may be left empty

Protocol Setup

- Unlike the QX200 ddPCR System, a 30 min hold at 4°C is not recommended to increase droplet counts. There is little positive impact on droplet count from a 4°C hold on the QX ONE ddPCR System

Loading Plates into the System

- When loading GCR96 plates into the new plate hotel, ensure that plates click into place and the position-indicator LEDs switch from yellow to green
- When scanning the radio frequency identification (RFID) tag on a GCR96 Cartridge, the software will automatically open and close the inbox hotel door. Alternatively, users can use the Inbox/Outbox buttons in the software to manually control the hotel doors. Do not physically manipulate the hotel doors

Loading Multiple Plates into the Instrument

- When adding a plate after a run has already started and an error occurs when scanning, follow the prompts to delete the new plate row before attempting to rescan the RFID tag on the GCR96 Cartridge
- If the RFID scanner does not recognize a new GCR96 plate, reboot the QX ONE Software if the system is idle and attempt to rescan the plate. If the system is not idle, store the plate at 4°C and wait until the current plates are finished processing to power cycle the instrument

Data Analysis

- It is recommended that fully processed data files are not analyzed on the QX ONE instrument while the instrument is running workflows, particularly when the instrument is running data acquisition with the droplet reader. Data acquisition is a memory-intensive process for the computer
- It is recommended to move the .ddpcrone/.ddpcrsone files to a separate computer for data analysis if the QX ONE instrument is running workflows. There is an option to automatically transfer all files to a networked drive
- If you wish to use live analysis of a plate that is currently running, give the system time to process. Do not click multiple times if the computer is running slowly

Automatic Plate Recovery

- In a case of a failure during a run, follow the automatic plate recovery process detailed in the QX ONE System User Guide (Automatically Recovering Plates from Failed Runs section or Recovering Plates from Failed or Canceled Runs section)
- If you are using manual recovery, contact Bio-Rad Technical Support for assistance before proceeding

Oil Bottles

- Keep all droplet generation (DG) oil, droplet reader (DR) oil, and waste bottles loaded during operation to improve run outcomes and reduce errors
- When encountering more than 1 instance where the QX ONE Software misidentifies an oil bottle as empty, even though bottles are partially filled, please contact Bio-Rad Technical Support for assistance
- If running with only 1 DG oil, 1 DR oil, or 1 waste bottle, do not remove bottles during the run
- If the QX ONE System is idle for more than 1 week, please remove the waste oil bottle(s), invert, and store until needed

Future Upgrades

- All hardware, firmware, and software upgrades to your QX ONE System need to be performed by a Bio-Rad Field Service Engineer

CPU/Software Compatibility

- To ensure successful compatibility between the computer/laptop and QX ONE Software, confirm the current CPU does not have an Iris Xe Graphics Chipset. In most cases, installing the QX ONE Software on PCs containing Iris Xe Chipsets can lead to extreme performance degradation
- When installing the QX ONE Software on a laptop/PC, select English as the default language. Selecting a default language other than English can lead to unwanted errors
- To increase the speed of analysis while using all pertinent software features, tailor your focus and analysis to only the desired wells utilizing the well selector. Select only the wells that you wish to analyze

Visit [bio-rad.com/QXONE](https://www.bio-rad.com/QXONE) for more information.

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**Bio-Rad
Laboratories, Inc.**

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