QX ONE™ and QX200™ ddPCR™ Quantification Standard vKit User Guide

Catalog #	Description
12024820	QX ONE and QX200 Quantification Standard Kit

For research purposes only.

Introduction

The QX ONE and QX200 ddPCR Quantification Standard Kit can be used for validating system precision and accuracy. The kit contains reference DNA plasmid material at a known concentration, certified by the Australian National Measurement Institute (NMI), as well as the necessary reagents for quantification on Droplet Digital™ PCR (ddPCR) Systems. The standard's concentration and 95% confidence interval are listed on the tube label. The reference material is identical to that used in the QX600™ ddPCR Quantification Standard Kit. When a full plate of this kit is run on a properly functioning system, it should meet the specifications shown in Table 5 in the Data Interpretation section. Partial plates may also be run to diagnose specific issues.

Kit Contents

The contents of the kit are shown in Table 1. The kit contains sufficient reagents for 96 ddPCR reactions. Store contents at –20°C.
Note: Avoid excessive freeze thaw. The NMI Standard concentration may be affected if not properly mixed following freeze-thaw.

Table 1. Reagent kit contents.

Reagent	1x Volume	Kit Volume
ddPCR Supermix for Probes (No dUTP)	12.5 µL	700 µL x 2
ddPCR Four-Color System Check Assay	1.25 µL	140 µL
NMI Std Six-Color System Check	1.25 µL	150 µL
Nuclease-free H_2O	10 µL	1.5 mL

Required Equipment, Reagents, and Consumables

Table 2 lists the additional materials required to run the assay. Use the specified items to maintain consistency and data quality.

Table 2. Additional required materials.

Instrument	Bio-Rad Catalog #
QX200 Droplet Generator or Automated Droplet Generator	1864002 1864101
QX200 Droplet Reader	1864003
C1000 Touch Thermal Cycler with 96–Deep Well Reaction Module or PTC Tempo Deepwell Thermal Cycler	1851197 12015392
PX1 PCR Plate Sealer	1814000
QX ONE Droplet Digital PCR System	12006536
Reagents and Consumables	
Droplet Generation Oil for Probes or Automated Droplet Generation Oil for Probes	1863005 1864110
ddPCR Droplet Reader Oil or ddPCR Droplet Reader Oil EcoTank for Probes	1863004 12019215
ddPCR 96-Well Plates	12001925
DG8 Cartridges for QX200/QX100 Droplet Generator or DG32 Automated Droplet Generator Cartridges	1864008 1864108, 1864109
ddPCR Buffer Control for Probes	1863052
PCR Plate Heat Seal, foil, pierceable	1814040
QX ONE Droplet Generation Oil for Probes	12006058
GCR96 Cartridges	12006859
GCR96 Foil Heat Seal	12006843
Microseal 'B' Film	MSB1001

Table 3. ddPCR master mix recipe.

Component	Volume for 1 well, µL	96x (+ overage), µL
ddPCR Supermix for Probes (No dUTP)	12.5	1,320
ddPCR Four-Color System Check Assay	1.25	132
NMI Std Six-Color System Check	1.25	132
Nuclease-free H_2O	10	1,056
Total	**25**	**2,640**

ddPCR Reaction Setup

1. Bring ddPCR reagents to room temperature. Mix vigorously by vortexing the tubes at maximum speed (3,200–3,500 rpm) for 15 sec. This process will ensure its homogeneity and help avoid the formation of a concentration gradient.
2. Assemble the ddPCR master mix as shown in Table 3. Pulse vortex thoroughly to mix, then centrifuge briefly.
3. Dispense 25 µL of the ddPCR master mix in each well of a 96-well plate.
4. If necessary, add 25 µL 1x ddPCR Buffer Control for Probes to any empty wells of the plate. A 1:1 mixture of ddPCR Multiplex Supermix and nuclease-free water can be used if no Buffer Control is available.
   - **For QX200 Droplet Generator and Automated Droplet Generator Instruments:** Droplet generation occurs in columns. Add 25 µL Buffer Control to any unused column wells from which droplets will be generated. No Buffer Control is required in empty wells if an entire column is unused
   - **For QX ONE Droplet Digital PCR System:** Droplet generation occurs in sets of two columns. (That is, droplets for columns 1 and 2 are generated simultaneously.) If necessary, add 25 µL of Buffer Control to any unused wells in each set of columns from which droplets will be generated
5. Seal the plate. For the Automated Droplet Generator Instrument, heat seal the plate using the PX1 PCR Plater Sealer at 180°C for 5 sec. Allow the foil seal to briefly cool before the next step. For manual droplet generation or the QX ONE Droplet Digital PCR system, microseal B may be used instead. Press the seal securely against the plate and proceed to the next step.
6. Vortex briefly, then centrifuge at 1,150 rcf for 1 min.
7. Droplet generation steps differ slightly depending on the system in use. Transfer the reaction mix from the tubes or plate prepared in step 6 to the appropriate Droplet Generation Cartridge or ddPCR 96-Well Plate and generate droplets as follows:
   - **For manual droplet generation (QX200 Droplet Digital PCR System):** Remove the plate seal. Load 20 µL of each reaction mix into the sample wells of a DG8 Cartridge. Then load 70 µL of Droplet Generation Oil for Probes into the oil wells. Refer to the QX200 Droplet Generator Instruction Manual (10031907) for detailed instructions
   - **For automated droplet generation (QX200 AutoDG™ ddPCR System):** Place the sealed plate in the Automated Droplet Generator and follow instructions in the Automated Droplet Generator Instruction Manual (10043138)
   - **For the QX ONE ddPCR System:** Remove the plate seal. Load 20 µL of each reaction mix into the wells of a GCR96 Cartridge. Follow subsequent instructions for heat sealing, centrifuging, and loading the plate specified in the QX ONE Droplet Digital PCR System and QX ONE Software Instrument Guide (10000116512). Use the thermal cycling conditions as specified in Table 4
8. **Important:** When setting up the plate template, first select the wells to include/exclude under the Exclude tab. Then move to the Edit tab and designate the following:
   - **Experiment Type:** Direct Quantification (DQ)
   - **Sample Descriptions:** Determined by user
   - **Sample Type:** Unknown
   - **Supermix:** ddPCR Supermix for Probes (No dUTP)
   - **Assay Type:** Single Target per Channel
   - **Target Name(s):** Determined by user
   - **Target Type:** Unkn
   - **Signal Ch1:** FAM
   - **Signal Ch2:** HEX
   - **Signal Ch3:** Cy5
   - **Signal Ch4:** Cy5.5
9. Press **Apply** and then save the template. Press **Start Run**.

Table 4. Cycling conditions.*

Cycling Step		Temperature, °C	Time	Number of Cycles	Ramp Rate
Hold (QX ONE ddPCR System only)		25	3 min	1	2°C/sec
Initial denaturation		95	10 min	1	
Denaturation		94	30 sec	40	
Annealing/ extension		58	1 min	40	
Enzyme deactivation		98	10 min	1	
Hold	QX200 ddPCR System	4	∞	1	
	QX ONE ddPCR System	25	1 min	1	

* For the C1000 Touch or PTC Tempo Thermal Cyclers, use a heated lid set to 105°C and set the sample volume to 40 µL.

Thermal Cycling

Follow the instructions for thermal cycling based on the system in use:

- **For QX200 Droplet Generator:** After droplet generation, carefully transfer each column of the droplet emulsions into a clean ddPCR 96-Well Plate using a P50 multichannel pipettor. Seal the plate using the PX1 PCR Plate Sealer at 180°C for 5 sec. Proceed to thermal cycling with the conditions specified in Table 4
- **For Automated Droplet Generator:** Remove the droplet plate containing ddPCR droplets from the Automated Droplet Generator. Seal the plate using the PX1 PCR Plate Sealer at 180°C for 5 sec. Proceed to thermal cycling with the conditions specified in Table 4
- **For QX ONE ddPCR System:** Thermal cycling is integrated into and sequentially performed by the system itself. Hence, no additional equipment or sample handling is required for this step

Data Acquisition

Follow the instructions for data acquisition based on the system in use:

For QX200 Droplet Reader:

1. The kit is compatible with QX Manager Software version 1.2 or higher (Standard, Regulatory, and Premium Editions). Refer to the QX Manager Software Standard Edition (10000107223), Regulatory Edition (10000107224), or Premium Edition (1000153878) User Guides for detailed information on the QX200 instrument and plate setup.
2. Place the sealed 96-well plate in the Droplet Reader.
3. **Important:** When setting up the plate template, first select the wells to include/exclude under the Exclude tab. Then move to the Edit tab and designate the following:
   - **Experiment Type:** Direct Quantification (DQ)
   - **Sample Descriptions:** Determined by user
   - **Sample Type:** Unknown
   - **Supermix:** ddPCR Supermix for Probes (No dUTP)
   - **Assay Type:** Single Target per Channel
   - **Target Name(s):** Determined by user
   - **Target Type:** Unkn
   - **Signal Ch1:** FAM
   - **Signal Ch2:** HEX
4. Press **Apply** and then save the template. Press **Start Run**.

For QX ONE ddPCR System:

1. The kit is compatible with QX ONE Software version 1.2 or higher (Standard and Regulatory Editions). Data acquisition is integrated into and sequentially performed by the system itself. Hence, no additional equipment or sample handling is required for this step.

Data Analysis

The kit is compatible with QX Manager Software and QX ONE Software version 1.2 or higher (all editions). All compatible editions of software offer combined-well autothresholding, which generates thresholds for each channel based on the combined appearance of all wells. Refer to the user guide of the software in use for detailed instructions about data analysis.

1. Navigate to the 2D Amplitude tab. Select all wells.
2. Click **Auto with Tilt**, select **Combined Wells** at the bottom of the dropdown menu, then click **OK**. These steps apply the same autothreshold to all selected wells based on their combined fluorescence. See Figure 1 for details.
3. To view the autothreshold lines that have been applied to the wells, click the crosshair tool.
4. To export data, navigate to the Data Table tab.
5. Select **Individual**.
6. Click the Table Menu icon on the far right and click **Export to CSV** from the dropdown menu (Figure 2).

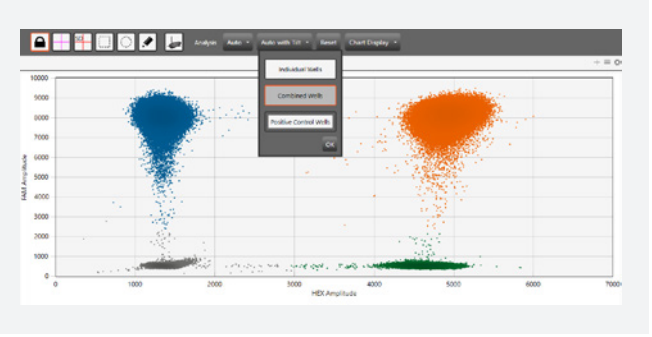
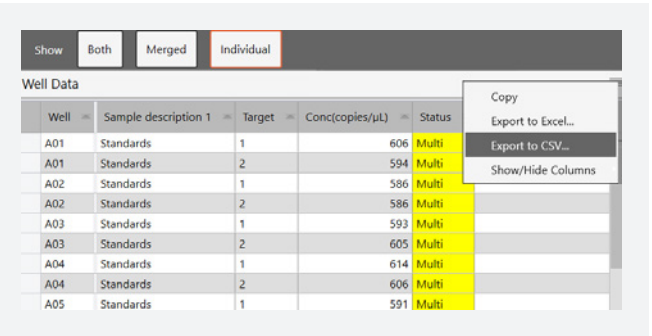


Fig. 1. Autothresholding using combined wells. On the 2D Amplitude tab, select all wells and choose **Combined Wells** from the Auto with Tilt dropdown menu.



Well	Sample description 1	Target	Conc(copies/µL)	Status
A01	Standards	1	606	Multi
A01	Standards	2	594	Multi
A02	Standards	1	586	Multi
A02	Standards	2	586	Multi
A03	Standards	1	593	Multi
A03	Standards	2	605	Multi
A04	Standards	1	614	Multi
A04	Standards	2	606	Multi
A05	Standards	1	591	Multi

Fig. 2. Exporting data for analysis. On the Data Table tab, select **Individual** and choose **Export to CSV** from the dropdown menu.

Data Interpretation

The expected 2-D plots for each system are shown in Figure 3. All droplet clusters should be distinctly separated. A full-plate system check should meet the specifications shown in Table 5. Consult the Troubleshooting section if any of these are not met. If issues persist, contact Technical Support.

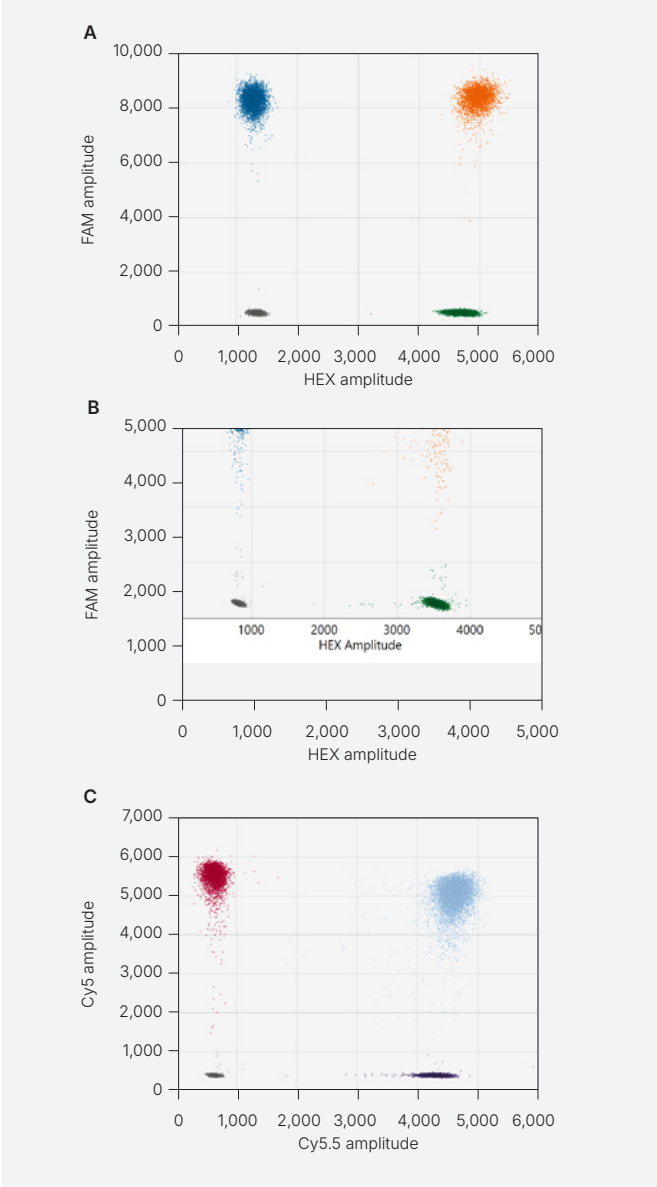


Fig. 3. Expected 2-D phenotype. A, QX200 ddPCR System; **B**, QX ONE ddPCR System, FAM/HEX; **C**, QX ONE ddPCR System, Cy5/Cy5.5.

Table 5. System check specifications.

Specification	QX200 ddPCR System	QX ONE ddPCR System
Droplet count minimum	≤2 wells below 10,000	≤2 wells below 10,000
Droplet count average	Plate average ≥15,000	Plate average ≥15,000
Concentration precision	≤10% concentration %CV for each of the 2 channels	≤10% concentration %CV for each of the 4 channels
Concentration accuracy	±20% mean concentration from outer bounds of NMI COA specification for each of the 2 channels	±20% mean concentration from outer bounds of NMI COA specification for each of the 4 channels

CV, coefficient of variation; NMI COA, Certificate of Analysis from the National Measurement Institute.

Data Interpretation Using the ddPCR Quantification Analysis Worksheet

The ddPCR Quantification Analysis Worksheet is available to automatically calculate pass/fail status on the specifications listed in Table 5. To use it:

1. Export to .csv as shown in Figure 2.
2. Open the .csv file with the exported data and ddPCR Quantification Analysis Worksheet.
3. Select all data (Ctrl+A) in the .csv file, then copy and paste the data into cell A1 of the workbook Input tab (Figure 4).
4. Enter the NMI concentration and 95% confidence interval listed on the tube in the corresponding cell in the Results tab.
5. Calculations are performed on the workbook Helper tab. The pass/fail status will automatically populate in the Results tab.

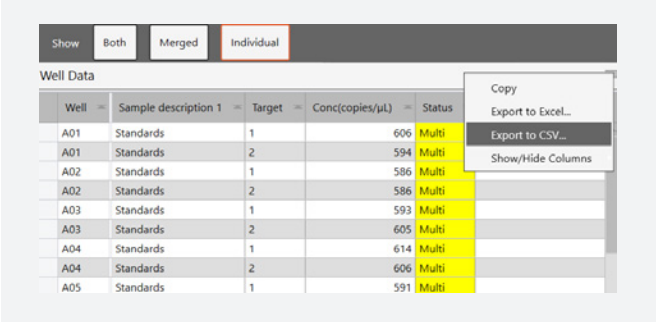


Fig. 4. Importing data into the ddPCR Quantification Analysis Worksheet. Copy data from the exported .csv file. Paste the data into cell A1 of workbook Input tab.

Appendix A. Troubleshooting

This section lists some common failure modes with their phenotypes, descriptions, and suggested resolutions. For a complete list of failure modes, refer to the Droplet Digital PCR Applications Guide (bulletin 6407) and the instrument instruction manual.

No or Low Total Droplet Counts

Problem: More than two wells have droplet counts <10,000. Plate droplet average <15,000.

Several causes are possible:

Possible Cause: Droplet Shredding

Shredded droplets appear on the diagonal through the negative cluster.

Possible Resolutions:

- Use only approved pipet tips for droplet generation and droplet transfer. (Rainin and tips are approved)
- When possible, avoid wearing latex gloves and touching the ddPCR Plate wells to avoid static electricity
- Work at room temperature, 18–30°C
- Use only approved plates (Bio-Rad ddPCR 96-Well Plates) and approved Bio-Rad PCR Plate Heat Seal foil
- If transferring droplets manually, use a manual P50 pipet with a normal bore P200 tip (not a wide or narrow bore) to transfer droplets. Angle the P200 tip in the well to prevent droplets from having to squeeze between the pipet tip and well bottom. (Angle the tip position such that it is not vertical in the well.) Slowly draw 40 µL of droplets into the pipet tip over approximately 5 seconds. Typically, 5 µL of air will be pulled into the tip, which helps prevent the oil from leaking out. Position the pipet tip (containing the droplets) near the bottom of the well and dispense the sample, leaving enough space between the well and pipet tip to prevent droplet shearing upon dispensing

Possible Cause: Evaporation

Low volume after thermocycling, particularly along edges. Droplet clusters are poorly formed and dispersed.

Possible Resolutions: Properly seal the 96-well plate. Under- or over-sealed plates result in oil evaporation during thermal cycling and compromise droplet data quality. If using the Bio-Rad PX1 PCR Plate Sealer, seal plates at 180°C for 5 sec. Do not use the PX1 sealing protocol twice on the same plate, as this often disrupts the original seal. Do not roll the plate after sealing.

Possible Cause: Insufficient Volume

Low volume before and after thermocycling. Droplet clusters appear normal.

Possible Resolutions:

- Ensure that the full 20 µL of sample is transferred to the DG8 Cartridge. If less than 20 µL of sample is loaded, fewer droplets will be generated
- Before droplet generation, centrifuge the plate at 1,000 rcf for 1 min and verify that no bubbles remain at the bottom of the wells

Low Concentration Precision

Problem: Concentration %CV is greater than 10% in one or more channels.

Possible Cause: Insufficient Mixing

Possible Resolution: When creating technical replicates, thoroughly mix the reaction mixture (master mix, sample, and assay) by pipetting it up and down ten times using 90% volume strokes. Alternatively, pulse vortex the reaction mixture 15 sec, then spin the sample down. Do not assemble or mix reaction mixtures in the DG8 Cartridge.

Inaccurate Concentration

Problem: Concentration not within listed specification.

Possible Cause: Pipetting Inaccuracy

Possible Resolution: Ensure the correct volumes are transferred to the reaction mix. Mix reaction mixture thoroughly as described above.

Possible Cause: Insufficient Mixing

Possible Resolution: Before adding the standard, pulse vortex 15 sec, then spin the tube down. Avoid excessive freeze-thaw cycles.

Visit bio-rad.com/ddPCR_QXONE_QX200_QuantStandKit for more information.