Vericheck QX700™ ddPCR™ CHO Residual DNA Quantification Kit

User Guide

Catalog #	Description
12026312	**Vericheck QX700 ddPCR CHO Residual DNA Quantification Kit**, 96 reactions, includes assay and supermix

Product Description

The Vericheck QX700 ddPCR CHO Residual DNA Quantification Kit is designed for the quantification of residual CHO (Chinese hamster ovary) host cell DNA (HCD). HCD carried over during the manufacturing of therapeutic proteins and vaccines poses safety concerns and must not exceed levels established by regulatory agencies such as the World Health Organization.

Kit Contents

See Table 1 for kit contents. The kit contains reagents for 96 reactions. Store kit components at -20°C.

Table 1. Kit contents.

Product	Product Cap Color	Tubes/ Kit	Volume/ Tube, µL	Concentration of Tube
QX700 ddPCR RDQ Supermix (5x)	Gray	2	100	5x
QX700 ddPCR CHO Residual DNA Assay (20x)	Black	1	55	20x

Required Equipment, Reagents, and Consumables

See Table 2 for required materials that are not included in the kit.

Table 2. Additional required materials.

Droplet Digital™ PCR (ddPCR) System	Consumables and Reagents
• QX700 E Droplet Digital PCR System* (Bio-Rad Laboratories, Inc., #17011036), or • QX700 S Droplet Digital PCR System* (Bio-Rad, #17010638), or • QX700 HT Droplet Digital PCR System* (Bio-Rad, #17010628)	• RDG16 Cartridges (Bio-Rad, #12025252) • RDG16 Cartridge Holder (Bio-Rad, #12025262) • Staticide SW12 Anti-Static Wipes (ACL, Inc.) • Kimtech Science Precision Wipes (Kimberly Clark, #05511) • Optional: Dithiothreitol (DTT) 2 mL (Bio-Rad, #12012171) • Optional: CHO Positive Control DNA (Cygnus, #D552-1)

*For Research Use Only. Not for use in diagnostic procedures.

Recommendations for Optimal Results

- Use low-DNA-binding tubes for all sample dilutions and reaction setup
- Suggested DNA input is 3 fg–3 pg per 5 µL reaction
- Run a cell-line-specific standard curve to determine the conversion factor to convert target copy number to mass concentration. Once the conversion factor has been determined, subsequent runs with samples derived from the same cell line do not require standard curves. Ensure that the test samples and samples used to generate the standard curve are handled under the same conditions
- Run several no template control (NTC) wells

Sample Preparation

One of two strategies may be used to prepare the DNA samples: direct addition of samples to the ddPCR reaction, or DNA extraction prior to Droplet Digital PCR analysis.

Direct Addition of Samples to ddPCR Reaction

- The sample can be added directly to the ddPCR reaction if the sample is sufficiently diluted (≥100-fold) before adding to the ddPCR reaction
- Up to 0.2 mg/mL IgG can be added directly to the final reaction mix
- Add DTT to the reaction if the final reaction contains more than 0.05 mg/mL IgG
- Include DTT in the standard curve if using DTT in test samples to ensure proper calibration

DNA Extraction Prior to Droplet Digital PCR Analysis

- Perform DNA extraction for samples with high salt content (≥1.0 M salt), low pH (buffer ≤3.0 pH), or high protein content (≥1 mg/mL) before preparing the ddPCR reaction mix

Reaction Setup

1. Determine the plate layout prior to setting up the reaction mix. It is recommended to include at least two replicate wells for all controls and samples. Four replicates will maximize sensitivity. Reactions are set up in pairs of wells to avoid pipetting small volumes (Figure 1).

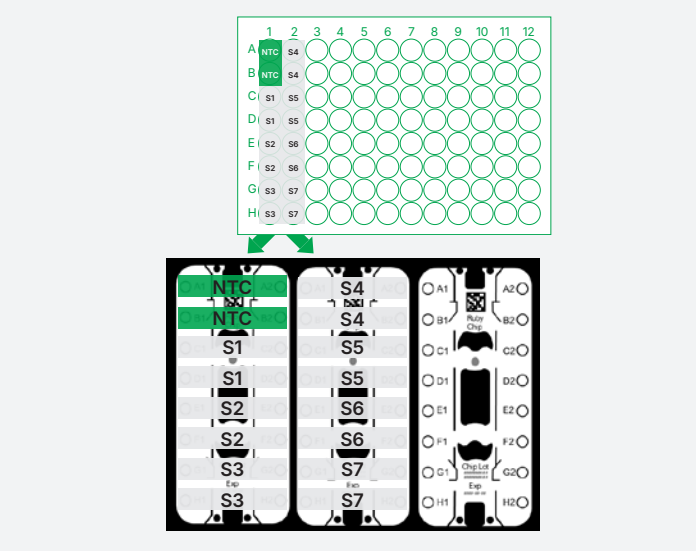


Fig. 1. Protocol schematic. NTC, no template control; S1–S7, samples 1–7.

2. Thaw all kit components to room temperature. Mix all tubes thoroughly by vortexing at maximum speed for 15 sec to ensure homogeneity. Centrifuge briefly to collect contents at the bottom of each tube.

   Tip: Thorough vortexing (maximum speed for 15 sec) of each component at this step is essential for success.

3. Prepare samples at the desired concentration before setting up the reaction mix.

4. Assemble a master mix containing all components, except the DNA sample or control, according to the master mix setup guidelines in Table 3.

Table 3. Master mix and reaction mix volumes.

Component	1x Reaction, µL	1 Pair of Wells + Overage, µL	Master Mix Setup for 1 Cartridge (16 Wells) + Overage, µL
QX700 RDQ Supermix (5x)	1	2.8	28
ddPCR CHO Assay (20x)	0.25	0.7	7
DTT (optional)	0.08	0.22	2.2
DNA sample or control	Up to 3.75 (add later)	Up to 10.5 (add later)	Up to 105 (add later)
Total Volume*	**5**	**14**	**140**

* The final 1x QX700 ddPCR reactions have a total volume of 5 µL. The reactions are prepared with overage to account for liquid loss during pipetting steps.

5. **Important:** Mix the master mix thoroughly by vortexing the tube at maximum speed for 15 sec. Ensure there are no precipitates.

6. Reactions are set up in pairs of wells to avoid pipetting small volumes.

7. Prepare one reaction mix for each set of two replicates in the wells of a ddPCR 96-Well plate or strip tube as follows:
   - If DTT was added to the master mix, dispense 3.72 µL master mix into each well. Add up to 10.28 µL of sample to the corresponding well. If less than 10.28 µL is added, bring the volume to 10.28 µL with nuclease-free water. For NTCs, add 10.28 µL of nuclease-free water

     Or

   - If DTT was not added to the master mix, dispense 3.5 µL master mix into each well. Add up to 10.5 µL of sample to the corresponding well. If less than 10.5 µL is added, bring the volume to 10.5 µL with nuclease-free water. For NTCs, add 10.5 µL of nuclease-free water

8. Seal the ddPCR 96-Well plate or tube, vortex at maximum speed for 15 sec, and centrifuge at 1,000 rcf for 1 min. Visually verify that all the liquid is at the bottom of the well. If preparing reaction mixes in strip tubes, vortex each tube at maximum speed for 15 sec and centrifuge briefly to collect the contents at the bottom of each tube.

9. Once the reaction mixes are ready, proceed to the QX700 plate setup.

QX700 Plate Setup

For detailed instructions and best practices for successful cartridge setup, refer to the QX700 Droplet Digital PCR System Instrument Guide (10000171493).

Anti-Static Treatment

1. Wrap a prewetted anti-static wipe around a finger and thoroughly wipe the underside and all edges of the cartridge with the anti-static wipe.

2. Gently place and drag the cartridge across the Kimtech precision wipe to wipe off any excess anti-static product and return it to the cartridge holder.

   Note: The same anti-static wipe can be used for up to three cartridges.

Cartridge Piercing

1. Hold the piercing tool in one hand and hold down the edges of the cartridge with the other hand.

2. Position the piercing tool above one column of wells and in contact with the foil.

3. Press the piercing tool down to pierce the foil.
4. Repeat for both columns of all cartridges.

Manually Loading Reaction Mix

Tip: Depending on the plate layout, reactions can be loaded using single-channel pipetting to load one well at a time or multi-channel pipetting to load an entire column at one time.

1. Load 5 µL of the reaction mix into the pipet tip(s).
2. Hold down the edges of the cartridge with the other hand to keep it in place while pipetting.
3. Holding the pipet vertically, insert the tip inside the inlet well until it lightly touches the bottom of the well.
4. Gently lift the pipet tip slightly off the bottom of the inlet well—approximately 1 mm above the bottom—such that the pipet tip remains inside the oil.
5. Continuing to hold the pipet at a 90° angle, eject the reaction mix by pressing the pipet to the first stop in a continuous and rapid motion. Do not lift the pipet out of the inlet well while ejecting the reaction mix.

   Important: To prevent bubbles, do not press the pipet down to the second stop.
6. Repeat for all wells of all cartridges.

Experiment Setup and Data Acquisition

The Vericheck QX700 ddPCR CHO Residual DNA Quantification Kit is compatible with the QX700 ddPCR System Control Software v1.5 and above.

Experiment setup for each QX700 run requires three files: a protocol file, an assay file, and an experiment file. A premade protocol file and assay file for the Vericheck QX700 ddPCR CHO Residual DNA Quantification Kit are available for download from the product page. These two files contain the thermal cycling and acquisition settings specific to the kit (summarized in the following sections). Users must create a new experiment file for each run. If using downloaded protocol and assay files, skip to the Experiment File Setup and Starting a Run section. For in-depth instructions on experiment setup, refer to the QX700 Droplet Digital PCR System Instrument Guide (10000171493).

Protocol File Setup

1. Select **QX700 ddPCR Supermix for RDQ** from the Mix Type dropdown menu.
2. Apply the thermal cycling conditions from Table 4.

Table 4. Thermal cycling conditions for the QX700 ddPCR System.

Step	Step Number	Temperature, °C	Time, sec	Number of Cycles	Ramp Rate, °C/sec
Partition for Ruby Chip	Step 1	-	-	-	-
Enzyme activation	Step 2	95	600	1	1
Begin loop for 5 iterations	Step 3	-	-	5	-
Denaturation	Step 3.1	95	30		1
Annealing/extension	Step 3.2	53	60		1
Begin loop for 40 iterations	Step 4	-	-	40	-
Denaturation	Step 4.1	95	30		1
Annealing/extension	Step 4.2	70	60		1
Release for Ruby Chip	Step 5	-	-	-	-

3. Under the Programs section, select **2. Reading** and define the Reading Program according to Table 5. Ensure FAM (blue), ROX (green), and Cy5.5 (infra-red) are toggled to the right, into the **ON** position. All other channels can be toggled **OFF**.

Table 5. Vericheck QX700 ddPCR CHO Residual DNA Quantification Kit channel exposure configuration for the QX700 ddPCR System.

Dye Name	Channel	Exposure, ms
FAM	Blue	85
ROX	Green	365
Cy5.5	Infra-Red	470

Assay File Setup

1. Under the Assay Structure section, ensure that the three channels listed in Table 6 are toggled to the right, into the **ON** position. The kit's target is detected in the FAM (blue) channel, as shown in Table 6.

Table 6. Assay structure details.

Dye Name	Channel	Associated Target
FAM	Blue	CHO
ROX	Green	Not used
Cy5.5	Infra-Red	Not used

2. Under the **Spill-over compensation matrix** section, select **Load compensation matrix from file (.ncm)** to load a previously created compensation matrix. Alternatively, spillover compensation can be adjusted in the result file after the data have been collected.

Experiment File Setup and Starting Run

1. Under the DIGITAL EXPERIMENTS tab, click **New**, then **Start from a blank experiment**.
2. Disable any unused cartridge or unused wells by first clicking **Enable/disable elements**, then clicking the disable button next to each unused cartridge or well.
3. If desired, add cartridge labels or sample names. This can be done manually or by using the tools under Sample Settings in the Details section to load sample information from a CSV file or apply Automatic Sample naming.
4. Under the Files section, add the previously created or downloaded protocol and assay files.
5. Apply the protocol by dragging it to each cartridge.
6. Apply the assay file by selecting all wells and dragging the file to all wells. An example plate setup is shown in Figure 2.

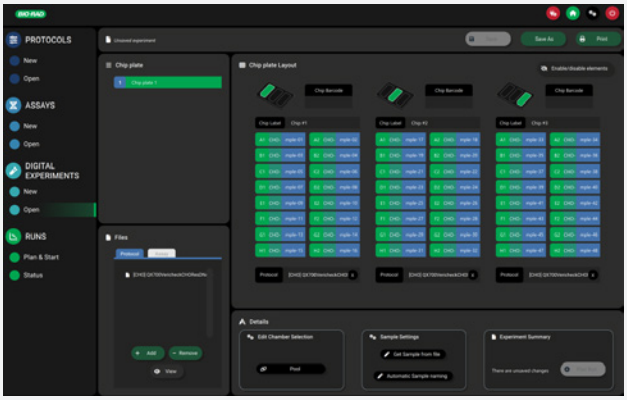


Fig. 2. Example plate setup. Apply a protocol file to all chips and an assay file to all wells.

7. At the top, click **Save As** to save the experiment file.
8. Click **Plan Run** in the bottom right Experiment Summary section to proceed to chip loading.
9. When prompted by the software, gently load the plate into the instrument until the status light begins blinking.
10. Click **Start Run**.
11. Check the Status tab to see the run progress and status. When the run is complete, click on the run and download the result file.

    Note: The user must click **Download Result File** to save the data.

Data Analysis and Result Interpretation

The CHO signal is detected in the FAM (blue) channel. The ROX (green) channel and the Cy5.5 (infra-red) channel contain no probes. The concentration reported is copies/µL of the final 1x ddPCR reaction. An overview of the data analysis process for the Vericheck QX700 ddPCR CHO Residual DNA Quantification Kit is provided below. For more detailed instructions on data analysis, refer to the QX700 ddPCR System Analysis Software User Guide (10000171494).

Quality Check

1. Open the data file and navigate to the Quality Control tab. Review the quality flags, chamber images, and droplet counts and exclude problem wells from analysis. Refer to the QX700 ddPCR System Analysis Software User Guide (10000171494) for explanations of quality flags and detailed instructions on performing quality control.
2. Navigate to the Plots & Populations section under the Analyze Data tab. Examine the FAM 1D plot replicates for uniformity and review any outliers for potential issues. Exclude wells with identified issues from the final analysis. See Figure 3 for example positive and NTC wells. The ROX and Cy5.5 channels are not used but will have a background fluorescence signal as seen in Figure 3.

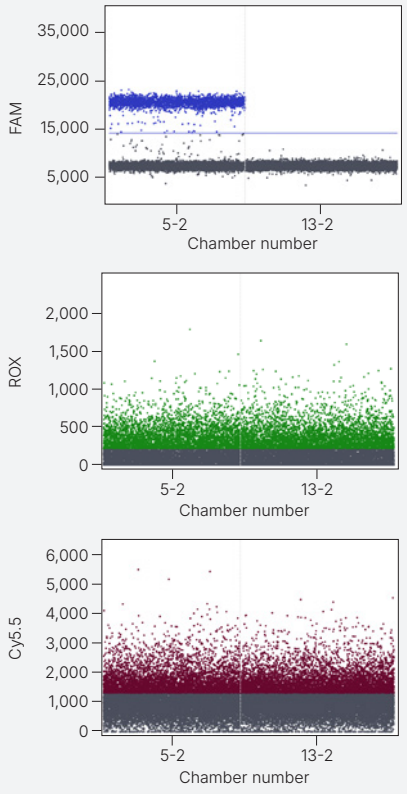


Fig. 3. Example 1D plots for FAM (top), ROX (middle), and Cy5.5 (bottom) channels showing expected fluorescence for each channel. Each set of 1D plots includes a CHO-positive well (left) and a no template control (NTC) well (right) that has been auto-thresholded.

3. Examine the automatically applied FAM threshold lines in all wells for accuracy. If any clusters are cut off by the thresholds, the threshold lines should be manually adjusted. It is recommended to use the Lines thresholding tool and to apply common thresholds for all control and sample wells.

   Tip: If any wells have significant tilt or if droplets are cut off by the y-axis, the spillover compensation can be adjusted to correct this issue. Go to the Compensate Spillover section of the Setup tab and adjust the Spillover Compensation values for FAM. The resulting compensation matrix file can be saved and applied to future runs, if desired. See the QX700 ddPCR System Analysis Software User Guide (10000171494) for detailed instructions.

Analysis and Result Interpretation

1. For added sensitivity and ease of analysis, multiple replicate wells may be pooled in the software to form a single aggregated well. Navigate to the Setup tab. Under the Edit Experiment heading, pool replicate wells together by selecting multiple wells with CTRL + click and clicking the Pool button. Do not include wells failing quality checks.
2. Concentrations are shown in the View Results tab under the Result Table heading. To export the data as a CSV, navigate to the Export tab and select the desired file types to export.
3. To convert from copies/µL to pg/µL in an unknown sample, the following equation is used:
   U = C/10,373

Where:
U = DNA concentration in the unknown sample, pg/µL
C = ddPCR concentration reported for the unknown, copies/µL

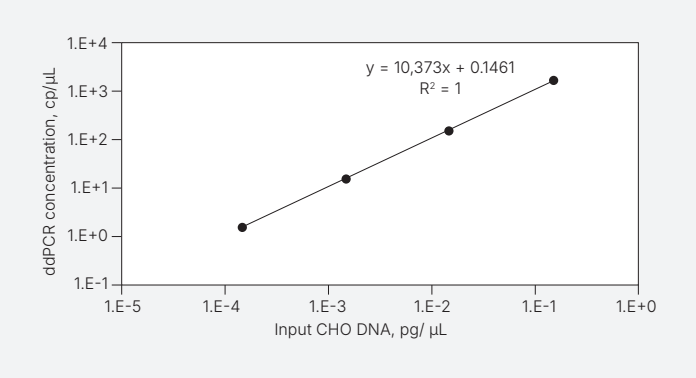


Fig. 4. Example conversion factor calculation.

The conversion factor above was determined against a cell line at Bio-Rad and may differ for other cell lines. To determine the conversion factor for a different cell line:

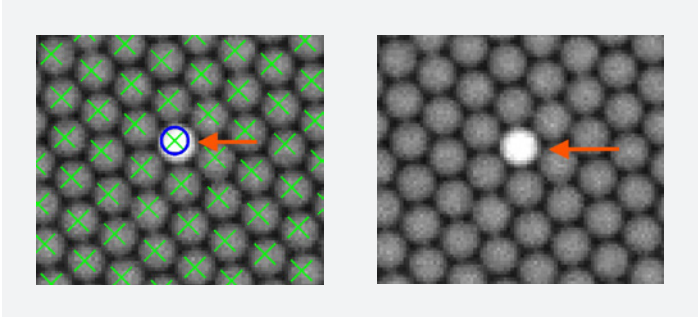
- Plot ddPCR concentration data versus a known DNA input
- Perform a least squares linear regression of the data to determine the slope of the regression line. This slope is the conversion factor. An example standard curve is shown in Figure 4, which yielded a conversion factor of 10,373

See Appendix A–C for additional result interpretation and troubleshooting guidance.

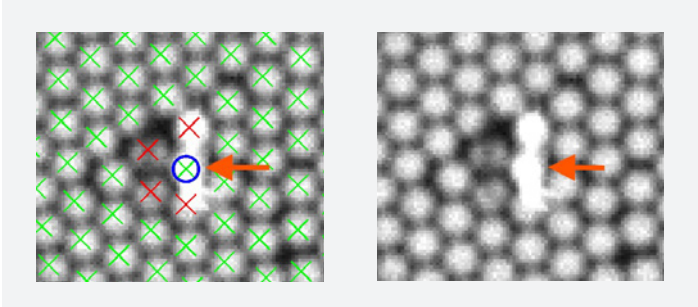
Appendix A. Evaluating Low-Concentration Positive Wells and Droplet Exclusion

For low-concentration FAM-positive wells, evaluate the chamber image to confirm that droplets classified as positive represent true positive droplets and not artifacts such as dust or lint.

1. In QX700 ddPCR System Analysis Software, navigate to the Quality Control tab and select the well with a low number of positives.
2. Identify the location of each FAM-positive droplet and zoom in to evaluate the droplet. Use the legend to toggle droplet status indicators on or off to improve visualization. Appendix Figures A1 and A2 show an example of a true positive droplet and a droplet classified as positive due to an overlapping artifact.



Appendix Fig. A1. True positive droplet with all indicators turned on (left) and all indicators turned off (right).



Appendix Fig. A2. A droplet that was incorrectly called positive due to a nearby artifact with all indicators turned on (left), and all indicators turned off (right). This droplet should be excluded from analysis.

3. Manually exclude any positive droplets determined to be artifacts. For detailed instructions on manual droplet exclusion, refer to the Performing Quality Control section of the QX700 ddPCR System Analysis Software User Guide (10000171494).

Appendix B. Verifying Sample Matrix Compatibility

The kit is compatible with cell culture media and cell lysate. Other matrices may be compatible, but their compatibility requires verification by the customer. The maximum allowable concentrations of common inhibitors are summarized in Appendix Table B1.

Appendix Table B1. Inhibitor tolerance. Maximum allowable concentrations of inhibitor in the final ddPCR reaction.

Inhibitor	Allowable Concentration in Final Reaction
EMEM complete media	10%
DMSO	5%
EDTA	2 mM
SDS	0.01%
Cell lysate	0.001%

DMSO, dimethyl sulfoxide; EDTA, ethylenediaminetetraacetic acid, EMEM, Eagle's Minimum Essential Medium; SDS, sodium dodecyl sulfate.

Appendix C. Troubleshooting

High FAM Signal in NTC

Problem: High FAM concentration (an average >0.5 cp/µL) in the NTC.

Possible cause: Environmental contamination. Contamination from the laboratory during ddPCR preparation may cause a high FAM signal in the NTC.

Resolution: Wipe down all surfaces with 10% bleach prior to starting experiments. Change gloves often, especially when handling concentrated positive samples. Work in a PCR hood if possible.

Possible cause: Artifacts. Excessive debris or damage to the RDG16 chip may cause inconsistent false positives. Inspect wells with low numbers of positive droplets.

Resolution: Minimize dust in the environment while preparing chips.

Low Droplet Counts

Problem: Consistent wells with less than 10,000 droplets.

Possible cause: Inhibitors in the sample. In the Quality Control view, numerous large, malformed droplets may be visible, as shown in Appendix Figure C1.

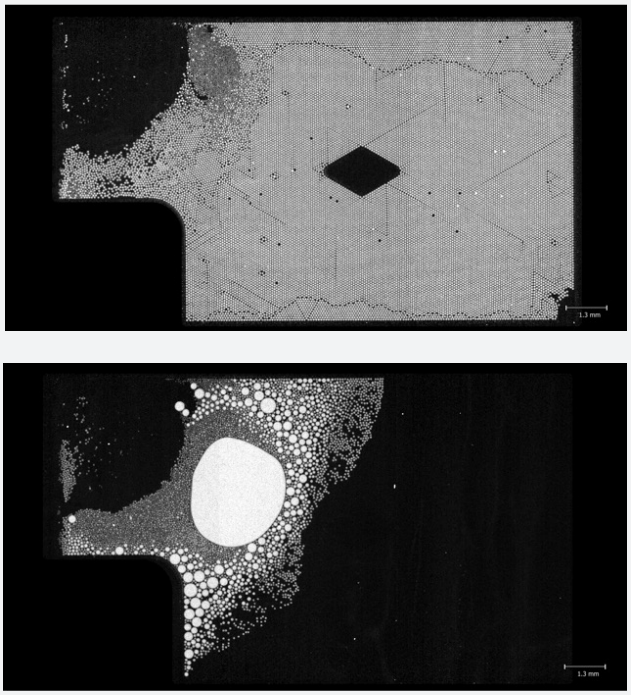
Resolution: Consult Appendix Table B1. Dilute the sample further before testing.

Possible cause: Improper sample loading. In the Quality Control view, the well may show some well-formed droplets near the injection point, but the chamber is not fully filled.

Resolution: Ensure that the full 5 µL is dispensed into each well. Dispense samples approximately 1 mm above the bottom of the well.

Possible cause: Damaged chip. In the Quality Control view, cracks may be visible. Damage may also be visible when inspecting the bottom of the chip.

Resolution: Discard the chip.



Appendix Fig. C1. Example high-quality chamber image (top) and poor-quality chamber image with bubbles caused by excess detergent (bottom).

Poorly Formed ddPCR Clusters

Problem: ddPCR positive droplets cannot be easily separated from negative droplets, as shown in Appendix Figure C2.

Possible cause: Poor mixing.

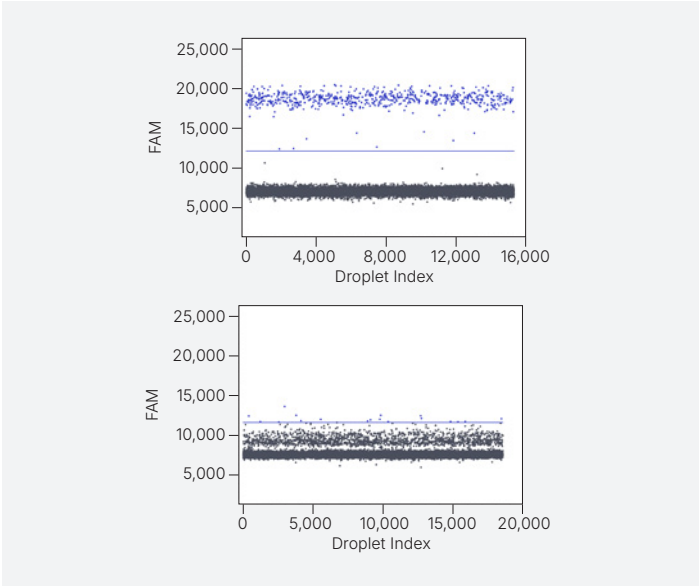
Resolution: Proper mixing is critical for the Droplet Digital PCR workflow. Vortex at all steps for at least 15 seconds.

Possible cause: Inhibitors in the sample.

Resolution: Consult Appendix Table B1. Dilute the sample further before testing.

Possible cause: Incorrect thermal cycling or acquisition settings.

Resolution: Check protocol settings. If thermal cycling conditions were incorrect, repeat the experiment. If acquisition settings were incorrect, the chip may be reimaged with correct settings.



Appendix Fig. C2. Expected 1D phenotype (top) compared to inhibited sample 1D phenotype (bottom).

No Negative Droplets

Problem: Sample well has no negative droplets and only high-amplitude clusters are present in the 1D and 2D plots. A concentration value cannot be calculated for the sample and result table reports the concentration as “inf”.

Possible cause: The sample is too concentrated and is outside the dynamic range.

Resolution: Dilute the sample further before testing.

Possible cause: Inhibitors in the sample.

Resolution: Consult Appendix Table B1. Dilute the sample further before testing.

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Life Science Group

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