Vericheck QX700™ ddPCR™ Mycoplasma Detection Kit

User Guide

Catalog #	Description
12025924	**Vericheck QX700 ddPCR Mycoplasma Detection Kit**, 96 reactions, includes assay, supermix, positive control, negative control, and internal control

Product Description

The Vericheck QX700 ddPCR Mycoplasma Detection Kit is a probe-based solution used to detect common *Mycoplasma* species found in cell culture or cell media during biopharmaceutical manufacturing processes. This kit uses Droplet Digital™ PCR (ddPCR) technology, which provides quantitative results without the use of standard curves.

Kit Contents

See Table 1 for kit contents. The kit contains reagents for 96 x 5 µL 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 Mycoplasma Assay (20x)	Green	1	55	20x
ddPCR Internal Control	Pink	1	900	20,000 cp/µL ± 20%
QX700 ddPCR Mycoplasma Positive Control	Blue	1	40	1,111 cp/µL ± 20% *Mycoplasma* 2,222 cp/µL ± 20% Internal Control
Nuclease-free water	Clear	1	1,500	-

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 (ddPCR) PCR System	Consumables and Reagents
• QX700 E Droplet Digital PCR System*, or • QX700 S Droplet Digital PCR System*, or • QX700 HT Droplet Digital PCR System*	• RDG16 Cartridges (Bio-Rad Laboratories, Inc., catalog #12025252) • RDG16 Cartridge Holder (Bio-Rad, #12025262) • Staticide SW12 Anti-Static Wipes (ACL, Inc.) • Kimtech Science Precision Wipes (Kimberly Clark, #05511) • Extraction-free workflow: Droplet Digital PCR Enhancer Reagent A (Bio-Rad, #12013425)

Optional: The Vericheck QX700 ddPCR Mycoplasma Detection Kit is compatible with HindIII restriction enzyme.

Overview of Sample and Control Types

A QX700 plate consists of up to three 16-well cartridges for 48 total wells. Positive Control, Negative Control, Negative Workflow Control, and sample(s) should be included on every plate. A brief description of each reaction type is provided below. Positive Control and Negative Control should be tested in duplicate. Samples and the Negative Workflow Control can be tested using 2–4 replicates. For unknown samples, it is recommended to use four replicates to maximize sensitivity.

- **Positive Control:** Positive Control is provided as a component of the kit. It is added directly to the ddPCR reaction mix. This reaction should be positive for *Mycoplasma* (FAM) and positive for Internal Control (HEX)
- **Negative Control:** Nuclease-free water and Internal Control are mixed to prepare a Negative Control sample. This Negative Control sample is added directly to the ddPCR reaction. This reaction should be negative for *Mycoplasma* (FAM) and positive for Internal Control (HEX)

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- **Negative Workflow Control (NWC):** The NWC is a nuclease-free water sample that has been processed with the same workflow (either extracted or extraction-free) as the samples. Internal Control is added to the NWC at the start of the sample preparation workflow, and the NWC is then added directly to the ddPCR reaction mix. This reaction should be negative for *Mycoplasma* (FAM) and positive for Internal Control (HEX)
- **Sample:** Internal Control is added to each sample at the start of the sample preparation workflow. The sample is added directly to the ddPCR reaction. This reaction should be positive for Internal Control (HEX)

Sample Preparation

One of two strategies may be used to prepare the DNA samples: DNA extraction prior to Droplet Digital PCR analysis or extraction-free preparation for direct addition of samples to the ddPCR reaction. For optimal sensitivity, perform DNA extraction.

Option 1: Extraction-Based Sample Preparation

Perform DNA extraction for each sample with the QIAGEN QIAamp DNA Mini Kit following the Isolation of Genomic DNA from Bacterial Suspension Cultures protocol or using a user-preferred extraction method. For optimal performance, elute extracted samples into a total volume of 100 µL.

Internal Control can be spiked into the sample prior to extraction and used to estimate the percent recovery of extracted samples. **To use:** Ensure the Internal Control tube is fully thawed to room temperature, then vortex at maximum speed for 15 sec. For a 100 µL elution volume, add 6.7 µL of Internal Control to the sample directly after any initial sample concentration or pelleting steps.

It is recommended to run an NWC with each extraction round to ensure no contamination is introduced during the extraction process. **To use:** Begin with nuclease-free water instead of sample, add 6.7 µL of Internal Control, and carry it through the entire extraction workflow, eluting in 100 µL.

Note: Most extracted samples will not require dilution and can be added directly to the ddPCR reaction.

Calculating Internal Control Amount for Alternate Elution Volumes

The volume of Internal Control added to the sample is variable and is based on the final elution volume of the extracted sample. For elution volumes other than 100 µL, use the following equation to calculate the volume of Internal Control to add to the sample:

U = E/15
Where:
U = volume of Internal Control to spike into the sample prior to extraction, µL
E = elution volume, µL

Option 2: Extraction-Free Sample Preparation

1. Dilute ddPCR Enhancer Reagent A 1:16 in sterile water. Diluted enhancer can be stored at 4°C.
2. Aliquot 84.8 µL of each unextracted sample into the wells of a plate or a tube.
3. Add 8.5 µL of diluted ddPCR Enhancer Reagent A and 6.7 µL of Internal Control to each unextracted sample aliquot.
4. Seal the plate or tube and vortex to mix. Spin down at 1,150 rcf for 1 min to collect liquid.
5. Incubate according to Table 3. If necessary, samples can be stored at –20°C after incubation.

Table 3. Extraction-free incubation protocol.

Step	Step Number	Temperature, °C	Time, min
Incubation	Step 1	37	30
Heat lysis	Step 2	95	10
Hold	Step 3	4	hold

Ramp rate: 2°C/sec

Note: It is recommended to run an NWC with each round of sample preparation to ensure that no contamination is introduced during the sample preparation process. For the NWC, begin with nuclease-free water and carry it through each step of the extraction-free workflow.

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).

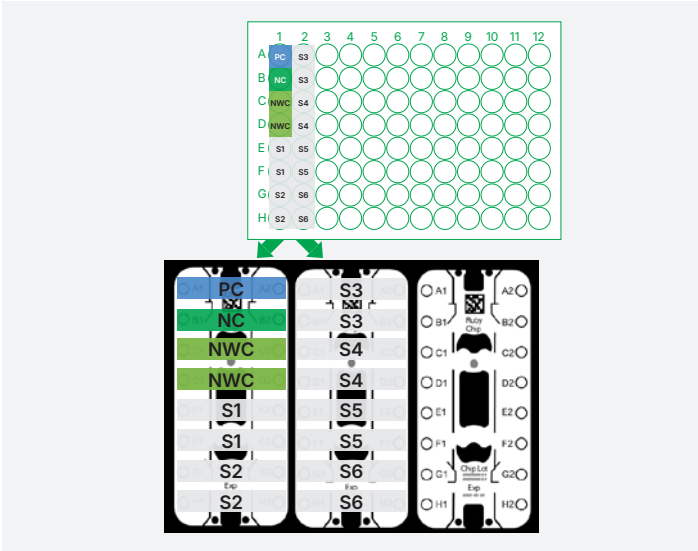


Fig. 1. Protocol schematic. NC, Negative Control; NWC, Negative Workflow Control; PC, Positive Control; S1–S6, samples 1–6.

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. To prepare the Negative Control, add 6.7 µL Internal Control to 93.3 µL water and vortex to mix thoroughly. This mixture can be added directly to the ddPCR reaction as the Negative Control in step 6B.

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

Table 4. Reaction volumes and master mix recipe.

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 Mycoplasma Assay (20x)	0.25	0.7	7
DNA sample or control	3.75	10.5 (add later)	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.

6. Prepare one reaction mix for each pair of two wells in the columns of a ddPCR 96-Well Plate or PCR strip tubes as follows:

   a. Dispense 3.5 µL of the master mix into each well.

   b. Add 10.5 µL Positive Control, Negative Control (prepared in Step 3), NWC, or DNA sample into the corresponding well.

7. Seal the ddPCR 96-Well Plate, 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.

8. Once reaction mixes are ready, proceed to the QX700 Plate Setup section.

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 a Kimtech Precision Wipe to blot 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 QX700 Run

The Vericheck QX700 ddPCR Mycoplasma Detection Kit is compatible with the QX700 ddPCR System Control Software v1.5 and above.

Experiment setup for each QX700 run requires the use of three files: a protocol file, an assay file, and an experiment file. A premade protocol file and assay file for the kit are available for download from the product page. These two files contain the thermal cycling and acquisition settings specific to the Mycoplasma Kit (summarized below). Users must create a new experiment file for each run. If using downloaded protocol and assay files, skip to the Experiment File Setup and Run Start 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 5.

Table 5. 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 40 iterations	Step 3	-	-	40	-
Denaturation	Step 3.1	94	10		1
Annealing/ extension	Step 3.2	60	60		1
Release for Ruby Chip	Step 4	-	-	-	-

3. Under the Programs section, select **2. Reading** and define the reading program according to Table 6. Ensure FAM (blue), HEX (teal), and Cy5 (red) are toggled to the right, into the **ON** position. All other channels can be toggled **OFF.**

Table 6. Vericheck QX700 ddPCR Mycoplasma Detection Kit exposure configuration for the QX700 ddPCR System.

Dye Name	Channel	Exposure, ms
FAM	Blue	85
HEX	Teal	273
Cy5	Red	51

Assay File Setup

1. Under the Assay Structure section, ensure that the three channels listed in Table 7 are toggled to the right, into the **ON** position. The kit targets are detected in the FAM (blue) and HEX (teal) channels, as shown in Table 7.

Table 7. Assay structure details. The dyes used in the assay file should match the dyes used in the protocol file.

Dye Name	Channel	Associated Target
FAM	Blue	*Mycoplasma*
HEX	Teal	Internal Control
Cy5	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 Run Start

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 file by dragging it to each cartridge (Figure 2).

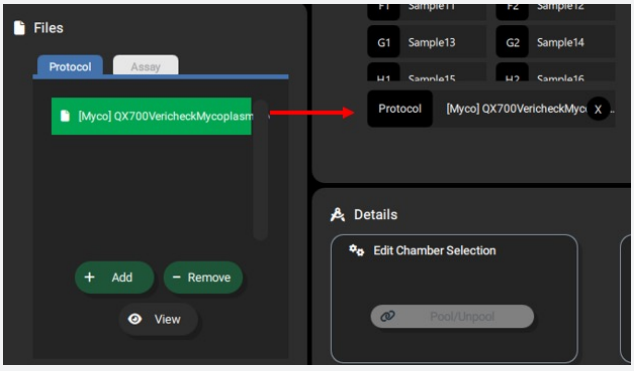


Fig. 2. Applying the protocol file. Drag the desired protocol from the Files section to the protocol box under each cartridge (red arrow).

6. Apply the assay file by selecting all wells and dragging the file to all wells (Figure 3).

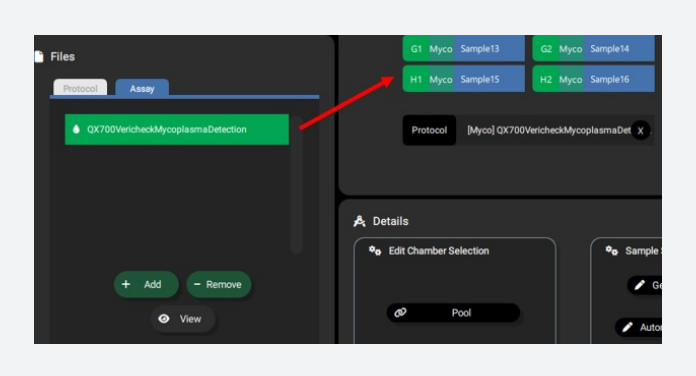


Fig. 3. Applying the assay file. Drag the desired assay from the Files section to the desired well(s) (red arrow). The assay will be assigned to all selected wells.

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 *Mycoplasma* signal is detected in the FAM channel (blue) and the Internal Control signal is detected in the HEX channel (teal). The Cy5 channel (red) contains 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 Mycoplasma Detection Kit is provided below. For more detailed instructions on data analysis, refer to the QX700 ddPCR System Analysis Software User Guide (10000171494).

Quality Check and Thresholding Adjustment

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 heading under the Analyze Data tab. Examine the control well FAM/HEX 1D and 2D plots for uniformity. See Figure 4 for example Positive Control and Negative Control FAM/HEX 2D plots. The Cy5 channel is not used but will show background fluorescence.

   Tip: Click the gears icon to adjust the 2D plots so the FAM channel is on the vertical axis and the HEX channel is on the horizontal axis.

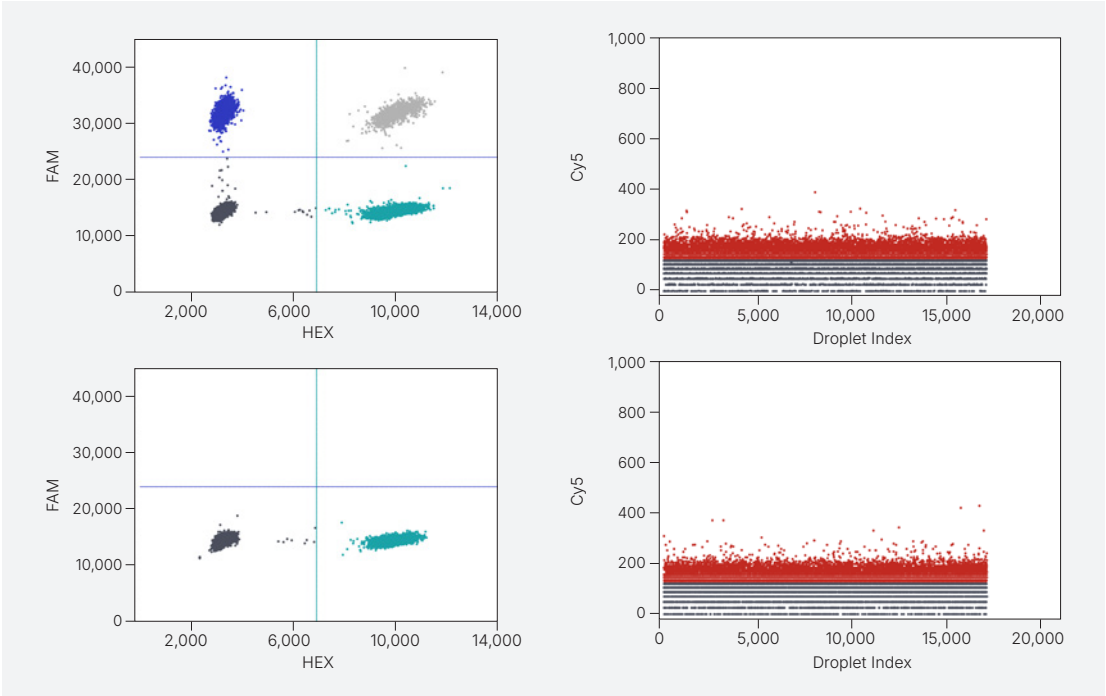


Fig. 4. Example FAM/HEX 2D plots (left) for Positive Control wells (top) and Negative Control wells (bottom). Corresponding 1D plots for the Cy5 channel (right) show expected background fluorescence and auto-thresholding. Negative Workflow Control wells are expected to have a similar phenotype to the Negative Control shown above.

3. Examine the replicate sample well plots for uniformity and examine any outliers for problems. Do not include any problem wells in the final analysis.
4. Examine the automatically applied FAM and HEX 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 and/or HEX. 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. Navigate to the View Results tab. Under the Result Table heading, check the concentration of the Positive Control and Negative Control wells. The expected FAM (blue) and HEX (teal) concentrations for the controls are summarized in Table 8.

Table 8. Expected Positive Control and Negative Control concentrations based on reaction type.

Well Type	Expected FAM Range	Expected HEX Range
Positive Control	333–3,333 cp/µL	333–3,333 cp/µL
Negative Control	<2 positive droplets	700–1,300 cp/µL

Expected concentration ranges are based on a sample input of 3.75 µL per 5 µL well.

3. Examine each sample's FAM (blue) concentration to determine if the sample is *Mycoplasma* positive. Table 9 can be used as a guide to interpret results. See Appendix B for samples with FAM concentrations close to the *Mycoplasma* Positive cutoff.

Table 9. Unknown sample result interpretation guide.

Status	FAM Concentration	Expected HEX Concentration	Interpretation
Negative	<2 positive droplets	>0 copies/µL	*Mycoplasma* not detected
Positive	≥2 positive droplets	>0 copies/µL	*Mycoplasma* detected

4. Examine the HEX (teal) concentration for each sample and the Negative Control. The following formula can be used to determine the percent recovery of each extracted sample or the percentage expected for each extraction-free sample:

   R = (S/C) x 100%
   Where:
   R = percent recovery
   S = sample well HEX concentration, copies/µL
   C = Negative Control well HEX concentration, copies/µL

   The equation assumes that both Negative Control and sample are added at the same volume, 3.75 µL per 5 µL well. If less sample was used, the equation should be adjusted accordingly.
5. To export the data as a csv, navigate to the Export tab and select the desired file types to export.

See Appendices A–C for additional performance information, result interpretation, and troubleshooting guidance.

Appendix A. Kit Performance

Sensitivity

The limit of detection (LOD) was ≤3 CFU/mL for extracted samples from nine *Mycoplasma* species. The LOD in genome copies (GC)/well was ≤5. LOD was defined as the lowest concentration sample detected in ≥95% of tests.

Specificity

The Vericheck QX700 ddPCR Mycoplasma Detection Kit detects 74 *Mycoplasma* species. The kit does not detect *Clostridium sporogenes*, *Lactobacillus acidophilus*, or *Streptococcus bovis* with ≥99.9% specificity (bacterial species recommended by the European Pharmacopoeia as assay specificity examples). The kit does not detect *E. coli*, Chinese hamster ovary (CHO), and HEK293 genomic DNA with ≥99.9% specificity.

Sample Extraction

The QIAGEN QIAamp DNA Mini Kit was used during development of the Vericheck QX700 ddPCR Mycoplasma Detection Kit. Other sample extraction methods may be compatible but require verification by the customer.

Inhibitor Tolerance

Maximum allowable concentrations of common inhibitors are summarized in Appendix Table A1.

Appendix Table A1. 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	0.01%

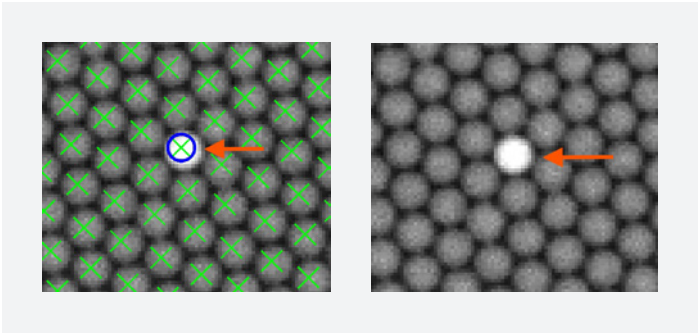
Sample Matrix

The kit is compatible with cell culture media and cell lysate. Other matrices may be compatible, such as transformed cell culture, but compatibility with the Vericheck QX700 ddPCR Mycoplasma Detection Kit requires verification by the customer.

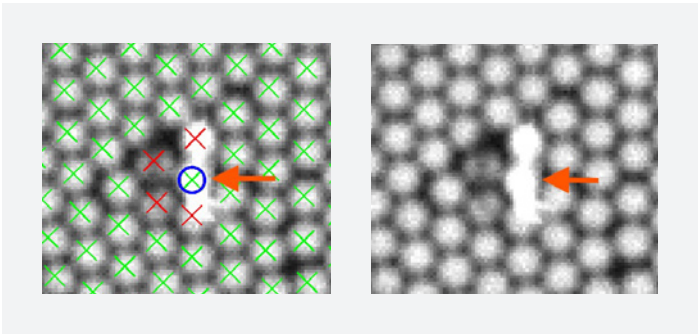
Appendix B. Evaluating Low-Concentration Positive Wells and Droplet Exclusion

For low-concentration FAM-positive wells, evaluate the chamber image to confirm that the droplets counted as positive are 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 a 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 B1 and B2 provide an example of a true positive droplet and a droplet classified as positive due to an overlapping artifact.
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 Fig. B1. A true positive droplet with all indicators turned on (left) and all indicators turned off (right).



Appendix Fig. B2. 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.

Appendix C. Troubleshooting

Examples of common problems are provided below. For additional examples and suggested troubleshooting steps, see the Experiment Troubleshooting section in Appendix A of the QX700 Droplet Digital PCR System Instrument Guide (10000171493).

High FAM Signal in Negative Controls

Problem: Multiple FAM-positive droplets (≥2) in the Negative Control or Negative Workflow Control.

Possible cause: Contamination during extraction. Contamination during extraction may cause a high FAM signal only in the Negative Workflow Control; the Negative Control will be clean.

Resolution: Ensure that clean reagents are used throughout the extraction process. Use high-quality validated extraction kits.

Possible cause: Environmental contamination. Contamination from the laboratory during ddPCR prep may cause a high FAM signal in both the Negative Control and Negative Workflow Control.

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.

Resolution: Inspect low-positive wells as described in Appendix B. Minimize dust in the environment when preparing chips.

Positive Control out of Expected Range

Problem: Positive Control is out of the expected 333–3,333 copies/µL range.

Possible cause: Poorly formed ddPCR clusters. Positive Control cannot be easily thresholded.

Resolution: Check thermal cycling and acquisition settings. Ensure reagents have been stored correctly and are not expired.

Possible cause: Pipetting inaccuracy.

Resolution: Check the master mix recipe. All vortex steps should be done for at least 15 sec.

Internal Control out of Expected Range

Problem: Internal Control is out of the expected 700–1,300 copies/µL range, or not detected for unknown samples.

Possible cause: Poorly formed ddPCR clusters.

Resolution: Check thermal cycling and acquisition settings. Ensure reagents have been stored correctly and are not expired.

Possible cause: Inefficient extraction. No HEX copies are detected for an unknown sample; the control was lost during extraction.

Resolution: Use validated extraction workflows.

Possible cause: Pipetting inaccuracy.

Resolution: Ensure Internal Control was added to samples if needed. All vortex steps should be done for at least 15 sec.

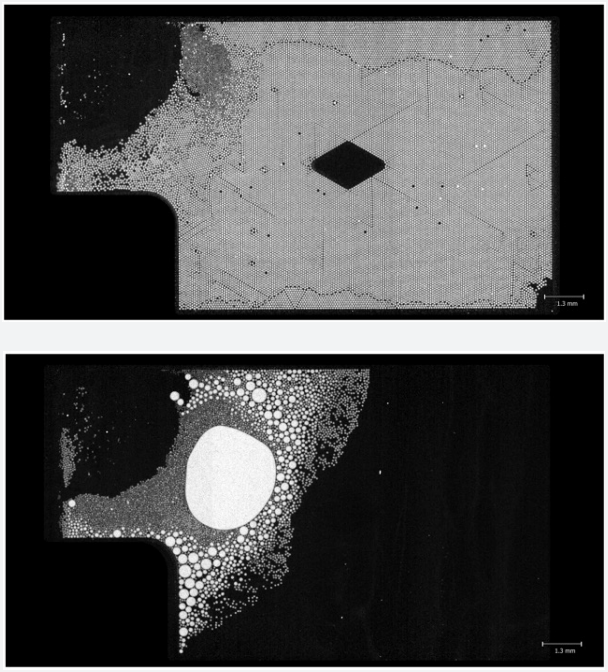
Low Droplet Counts

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 the inhibitor tolerance Appendix Table A1. 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.



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

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.

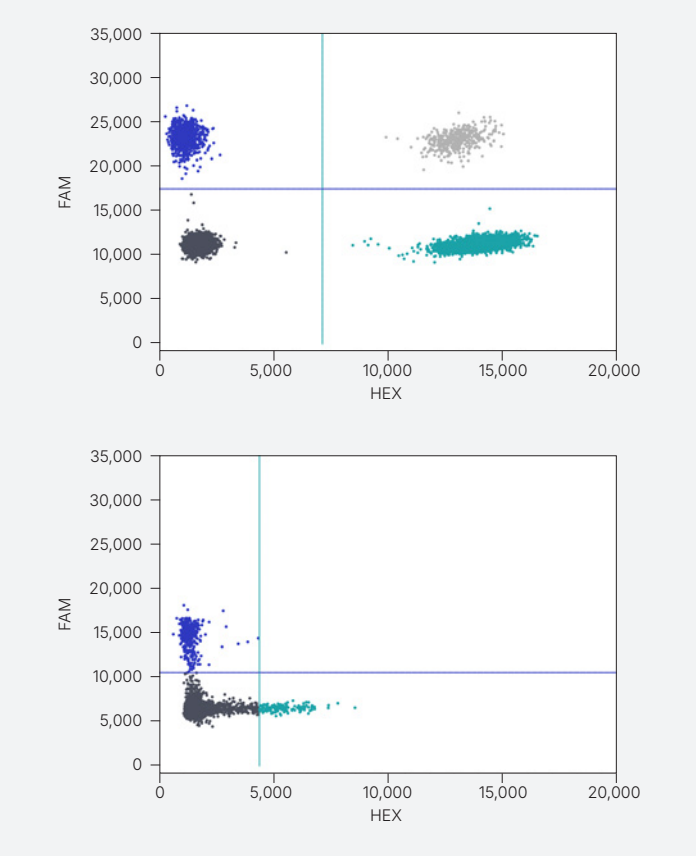
Poorly Formed ddPCR Clusters

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 sec.

Possible cause: Inhibitors in the sample.



Appendix Fig. C2. Expected 2D phenotype (top) and inhibited sample 2D phenotype (bottom).

Resolution: Consult inhibitor tolerance Appendix Table A1. 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.

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 A1. Dilute the sample further before testing.

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