Vericheck QX700™ ddPCR™ Empty-Full Capsid Kit

User Guide

Catalog #	Description
17011024	**Vericheck QX700 ddPCR Empty-Full Capsid AAV2 Kit**
17011114	**Vericheck QX700 ddPCR Empty-Full Capsid AAV5 Kit**
17011221	**Vericheck QX700 ddPCR Empty-Full Capsid AAV8 Kit**
17011222	**Vericheck QX700 ddPCR Empty-Full Capsid AAV9 Kit**

Introduction

Adeno-associated virus (AAV) vectors are used in cell and gene therapy as vehicles to deliver therapeutic genes to patients. Producing AAV vectors is a complex process that requires rigorous testing and verification to meet robust safety standards and ensure high efficacy. One critical test in this process is assessing the empty-to-full capsid ratio, which is essential for preventing potential immunotoxicity. AAV vectors consist of a capsid filled with genome cargo. During manufacturing, empty capsids lacking the intended genome cargo may be produced. The Vericheck QX700 ddPCR Empty-Full Capsid Kit can be used to simultaneously quantify AAV genomes and capsids to produce a highly precise measurement of the percentage of filled capsids. The kit is compatible with both purified and unpurified samples.

Protocol Overview

The protocol is executed in four distinct stages, shown in Figure 1.

1. **Sample prep:** AAV samples, Positive Control, and Negative Control are treated with a nuclease to remove unencapsulated DNA. Samples are diluted within the dynamic range.
2. **Binding:** AAV samples, Positive Control, and Negative Control are bound with antibodies.
3. **Ligation:** A new template is created by a ligation reaction, which is then stopped by transferring the reaction to new wells containing Stop Solution.
4. **Droplet Digital™ PCR (ddPCR) analysis:** Each stopped ligation reaction is tested in four ddPCR wells.

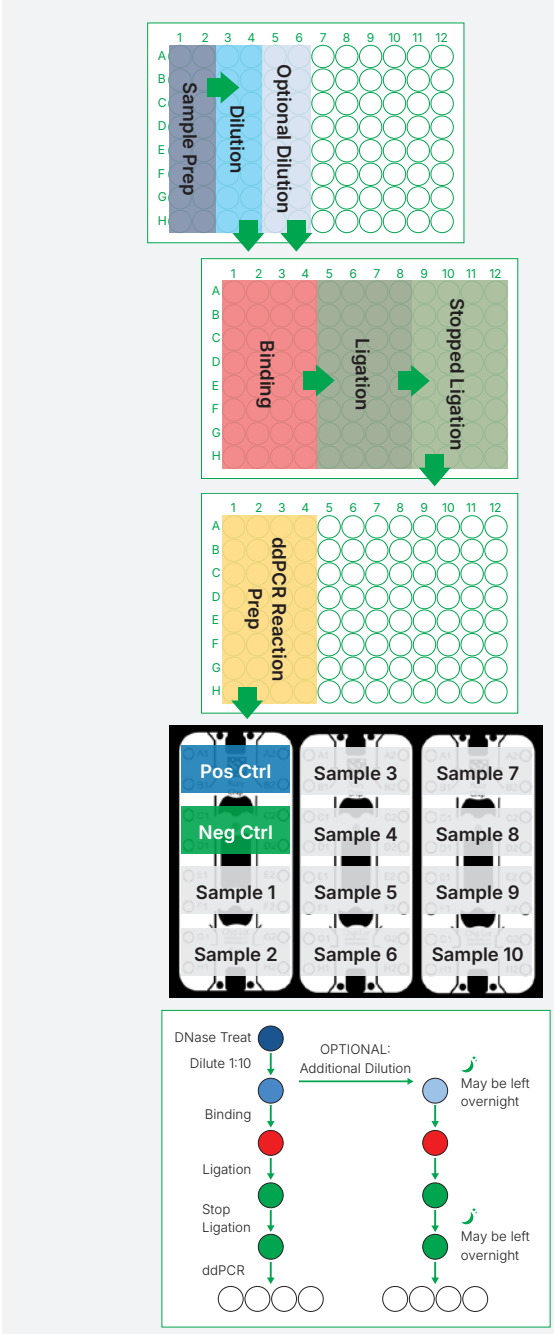

Fig. 1. Protocol schematic. ddPCR, Droplet Digital PCR; Neg Ctrl, Negative Control; Pos Ctrl, Positive Control.

Kit Contents

The kit is provided in two boxes. Contents are listed in Tables 1 and 2. The kit contains four reagent subsets corresponding to each stage of the protocol and includes sufficient reagents for 18 sample prep reactions, 32 binding reactions, 32 ligation reactions, and 128 ddPCR reactions. There is an excess of ddPCR reactions to enable partial capsid testing. See Appendix A for more details. Store both boxes at –20°C.

Table 1. Reagent kit contents.

Reagent	1x Volume, µL	Kit Volume, µL	Stage
DNase	2	50	Sample prep
10x DNase Buffer	2	50	Sample prep
Dilution Buffer	60	1,500	Sample prep
20x Ligase	1	40	Ligation
10x Ligase Buffer	2	80	Ligation
10x Splint	2	80	Ligation
Stop Solution	18	700	Ligation
QX700 ddPCR RDQ Supermix (5x)	1	3 x 100	ddPCR
20x ddPCR Capsid Detection Assay (FAM)	0.25	120	ddPCR
20x ddPCR ITR2 Assay (HEX)	0.25	120	ddPCR

Table 2. Serotype-specific kit contents.

Reagent	1x Volume, µL	Kit Volume, µL	Stage
AAV Positive Control*	2	30	Sample prep
AAV Antibody 1	2.5	90	Binding
AAV Antibody 2	2.5	90	Binding

*Biological source material. Treat as potentially infectious. The Positive Control contained in this kit is a replication-incompetent adeno-associated virus (AAV) that is not known to cause illness in healthy adults. Standard laboratory precautions, including engineering controls and appropriate personal protective equipment (PPE), are recommended.

Required Equipment, Reagents, and Consumables

See Table 3 for required materials that are not included in the kit.

Table 3. 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, pack of 12 (Bio-Rad, #12025252) • RDG16 Cartridge Holder (Bio-Rad, #12025262) • ddPCR 96-Well Plates (Bio-Rad, #12001925) • Staticide SW12 Anti-Static Wipes (ACL, Inc.) • Kimtech Science Precision Wipes (Kimberly Clark, #05511)

Sample Preparation

Note: AAV samples must be DNase-treated prior to the binding workflow. This kit includes DNase I, but other nucleases may also be suitable.

Note: Alternative nucleases should be validated before use. When using alternative nucleases, dilute the samples 1:10 in AAV Dilution Buffer after digestion, then proceed to the binding protocol. Heat inactivation of nucleases is not recommended.

1. The dynamic range of the test is ≈ 1 x 10^9 – 1 x 10^{12}, with 1 x 10^{11} optimal for both genome copies (GC)/mL and viral particles (VP)/mL. More concentrated samples may be treated with DNase and diluted further in step 10.
2. Thaw and briefly vortex the AAV sample(s), Positive Control, and sample prep reagents.
3. Assemble reaction (Table 4).

Table 4. Sample preparation reaction recipe.

Component	1x Reaction, µL	16x Reaction + Overage, µL
10x DNase Buffer	2	35.2
DNase	2	35.2
Nuclease-free water	14	246.4
Total	18	316.8

4. Mix the reaction mix thoroughly by vortexing.
5. Aliquot 18 µL reaction mix into the wells of a 96-well plate.

   Note: PCR strip tubes may be used instead of a 96-well plate for this step and all subsequent steps calling for a 96-well plate.

6. Add 2 µL of each AAV or Positive Control sample to each well. To a Negative Control well, add 2 µL of Dilution Buffer.
7. Seal the plate or cap tubes, pulse mix, and centrifuge briefly.
8. Incubate in a thermal cycler for 30 min at 37°C, then cool to 4°C.
9. Dilute DNase-digested AAV samples and controls 1:10 by adding 2 µL of the sample prep reaction to 18 µL Dilution Buffer.
10. **Optional:** To expand the dynamic range of the test, perform additional 1:10 serial dilutions in Dilution Buffer, resulting in the ranges specified in Table 5. Sufficient reagents are included to test two dilutions per sample.

    Note: It is not necessary to perform additional dilutions of the Negative or Positive Controls.

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

Table 5. Dilution guidance. For unknown samples, select two dilutions.

Dilution	Dynamic Range	
1:10	1 x 10^9 – 1 x 10^{12}	Required in step 8
1:100	1 x 10^{10} – 1 x 10^{13}	Optional
1:1000	1 x 10^{11} – 1 x 10^{14}	Optional
1:10000	1 x 10^{12} – 1 x 10^{15}	Optional

Binding

1. Bring DNase-digested samples, controls, and antibody probes to room temperature. Pulse vortex tubes to mix, then centrifuge briefly.
2. Assemble the binding reaction (Table 6).

Table 6. Binding reaction mix recipe.

Component	1x Reaction, µL	32x Reaction + Overage, µL
Antibody 1	2.5	88
Antibody 2	2.5	88
Dilution Buffer	3	105.6
Total	8	281.6

3. Mix the binding reaction mix thoroughly by vortexing.
4. For each sample and control to be tested, dispense 8 µL binding reaction mix per well in a 96-well plate.
5. Add 2 µL DNase-digested and diluted AAV sample, Positive Control, or Negative Control from the sample preparation reaction to each binding reaction.
6. Seal the plate, vortex it briefly, then centrifuge at 1,150 rcf for 1 min.
7. Incubate in a C1000 Touch Thermal Cycler with 96-Deep Well Reaction Module according to the conditions in Table 7 per serotype.

Table 7. Incubation conditions by AAV serotype.

AAV2	60 min at 30°C, then cool to 4°C
AAV5	60 min at 37°C, then cool to 4°C
AAV8	60 min at 30°C, then cool to 4°C
AAV9	60 min at 37°C, then cool to 4°C

Ligation

1. Bring the ligation reagents to room temperature. Pulse vortex to mix and centrifuge briefly.
2. Assemble the ligation reaction (Table 8).

Table 8. Ligation reaction mix recipe.

Component	1x Reaction, µL	32x Reaction + Overage, µL
10x Ligase Buffer	2	70.4
20x Ligase	1	35.2
10x Splint	2	70.4
Nuclease-free water	11	387.2
Total	16	563.2

3. Mix the ligation reaction mix thoroughly by vortexing.
4. Remove and discard the plate seal and dispense 16 µL ligation reaction mix into a clean well in the plate for each binding reaction.
5. Add 4 µL of binding reaction to ligation mix wells.
6. Seal the plate, vortex it briefly, then centrifuge at 1,150 rcf for 1 min.
7. Incubate in a C1000 Touch Thermal Cycler with 96-Deep Well Reaction Module for 10 min at 37°C, then cool to 4°C.
8. Remove seal and add 18 µL of Stop Solution to a different clean well in the plate for each ligation reaction.
9. Transfer 2 µL of the ligation reaction to wells containing Stop Solution to stop the ligation reaction.

   Note: The ligation reaction should be transferred promptly to the Stop Solution after incubation.
10. Seal the plate, vortex it briefly, then centrifuge at 1,150 rcf for 1 min.

    Note: Stopped ligation reactions may be stored at 4°C overnight before testing in a ddPCR reaction.

Reaction Setup

1. Bring ddPCR reagents to room temperature. Pulse vortex to mix and centrifuge briefly.
2. Assemble the ddPCR mix (Table 9). Reactions are prepared in pairs. It is recommended to run two pairs for maximum precision.

   Note: Excess supermix, assay, and stopped ligation reaction are included in the kit and may be used to test targets other than ITR2 to assess genome integrity. See Appendix A for details.

Table 9. ddPCR master mix recipe.

Component	1x Reaction, µL	16x Reaction (1 RDG16 Cartridge) + Overage, µL
QX700 RDQ Supermix (5x)	1	28
20x ddPCR Capsid Detection Assay (FAM)	0.25	7
20x ddPCR ITR2 Assay (HEX)	0.25	7
Nuclease-free water*	2.5	70
Total**	4	112

* Additional 20x AAV genomic assays may be added in other colors if desired. In that case, reduce the volume of water accordingly.

** 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.

3. Mix the ddPCR master mix thoroughly by vortexing.
4. For each sample, dispense 11.2 µL ddPCR master mix into two wells of a new 96-well plate.
5. Add 2.8 µL of stopped ligation reaction to each well.
6. Each of the two wells above will be split into two separate 5 µL reactions when loading into the QX700 ddPCR System.

   Note: AAV may settle out of solution if left sitting for an extended time. Mix samples well.
7. Seal the plate, vortex it briefly, then centrifuge at 1,150 rcf for 1 min.

   Note: It is critical for AAV lysis and thermal cycling to begin promptly after chip loading. Avoid extended wait times >4 hours in the plate hotel.
8. Proceed to the QX700 ddPCR instrument and begin cartridge 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 (one well at a time) or multi-channel pipetting (one full column at a time).

1. Load 5 µL of 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 Empty-Full Capsid Kit is compatible with 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. Premade protocol and assay files for the Vericheck QX700 ddPCR Empty-Full Capsid Kit are available for download from the product page and contain the thermal cycling and acquisition settings specific to the kit (summarized below). A new experiment file must be created for each run. If using the downloaded protocol and assay files, skip to the Experiment File Setup and Starting Run section. For detailed 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 10.

Table 10. 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	95	30		1
Annealing/ extension	Step 3.2	55	60		1
Release for Ruby Chip	Step 4	-	-	-	-

3. Under the Programs section, select **2**. **Reading** and define the Reading Program according to Table 11. Ensure all channels are toggled to the right, into the **ON** position.

   Important: To avoid data loss, it is critical that all channels remain **ON**, even if there are no assays in these channels.

Table 11. Vericheck QX700 ddPCR Empty-Full Capsid Kit channel exposure configuration for the QX700 ddPCR System.

Dye Name	Channel	Exposure, ms
FAM	Blue	85
HEX	Teal	273
ROX	Green	365
ATTO-590	Yellow	337
Cy5	Red	51
Cy5.5	Infra-Red	470
ATTO-550	Purple	110

Assay File Setup

1. Under the Assay Structure section, ensure that all channels 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 12.

   Important: To avoid data loss, it is critical that all channels remain **ON**, even if there are no assays in these channels.

Table 12. 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	Capsid
HEX	Teal	ITR2
ROX	Green	Target3*
ATTO-590	Yellow	Target4*
Cy5	Red	Target5*
Cy5.5	Infra-Red	Target6*
ATTO-550	Purple	Target7*

* These channels are not included as part of the Empty-Full Capsid Kit, so no associated target needs to be named. However, additional AAV genomic assays may be added to these channels if desired.

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 clicking **Enable/disable elements**, then clicking the disable button  next to each unused cartridge or well.

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

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 to 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.
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.
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

The capsid signal is detected in the FAM (blue) channel, and the AAV genome titer based on ITR2 is detected in the HEX (teal) channel. The concentration for the capsid channel and the percentage of filled capsids must be calculated as described below.

1. Ensure that the Positive Control wells and Negative Control wells for the assay are marked as having the sample type **Positive Control** or **Negative Control**. If necessary, navigate to the Setup tab, select **Edit Experiment**, and label the Positive and Negative Control wells. Under the Type column, use the dropdown menu to select **P** for Positive Control and **N** for Negative Control. See Figure 3 for details.

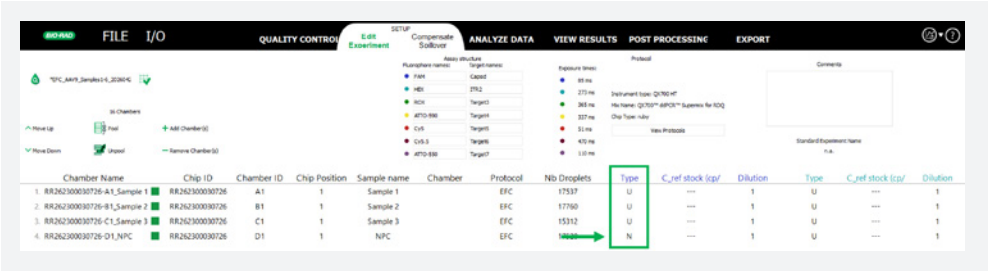

Fig. 3. Example of Negative Control well sample labeling. Double-click the current sample type (green box) for each control well to display a dropdown menu and select **P** or **N** accordingly (green arrow).

2. 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.
3. Navigate to the Plots & Populations heading under the Analyze Data tab. Examine the control well 1D and 2D plots for uniformity. Any unused channels will have a low level of background fluorescence signal. Fluorescence in unused channels can be ignored.

   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.
4. Examine the replicate sample well plots for uniformity and examine any outliers for problems. Do not include any problem wells in the final analysis.
5. Examine the automatically applied threshold lines for each used channel 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. See Figure 4 for an example plot.
6. 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.

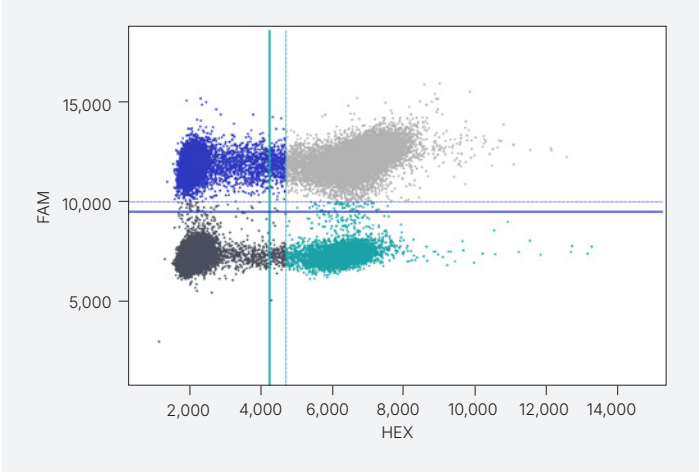

Fig. 4. Example thresholding adjustment for Positive Control.

Linkage Analysis

See Figure 5.

1. To set up linkage analysis, navigate to the Post-Processing tab and click **Setup**.
2. Under the Post-Processing Type section on the left, click **Linkage Analysis** so that a green checkmark appears in the circle.
3. Under the Settings section on the right, fill in the first two dropdown boxes with **Blue (FAM)** first and **Teal (HEX)** second to look at the linkage between the FAM and HEX channels.
4. Click **Apply** to conduct a Linkage Analysis.
5. To export data, navigate to the Export tab and click **Export** below Browse.

See Appendix B for additional troubleshooting guidance and example results.

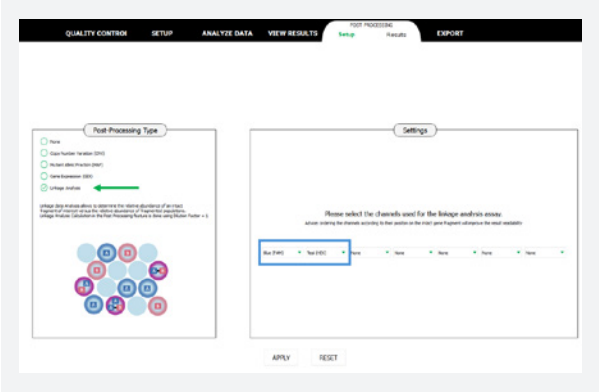

Fig. 5. Setting up a linkage analysis. Under Post-Processing tab, select **Setup**. Choose **Linkage Analysis** (green arrow) to conduct a linkage analysis. Under Settings, select **Blue (FAM)** in the first dropdown box and **Teal (HEX)** in the second dropdown box (blue box) to analyze the linkage between those two channels.

Data Interpretation Using the Vericheck QX700 ddPCR Empty-Full Capsid Analysis Worksheet

The Vericheck QX700 ddPCR Empty-Full Capsid Worksheet for the QX700 ddPCR System should be used to automatically calculate genome titer, capsid titer, and percent full metrics. Before exporting data, Negative Control wells must be specified by Sample Type in the QX700 ddPCR System Analysis Software for the workbook to function correctly.

1. Export data to .xlsx.
2. Open the .xlsx file with exported data and the Vericheck QX700 ddPCR Empty-Full Capsid Worksheet.
3. In the exported data file, click the Results tab, select all (Ctrl+A) data in the .xlsx file, then copy and paste to cell A1 of the Input - Results tab of the workbook (Figure 6).
4. In the exported data file, click the PP Result tab, select all (Ctrl+A) data in the .xlsx file, then copy and paste to cell A1 of the Input - PP tab of the workbook (Figure 7).
5. Calculations are performed on the Helper tab of the workbook. Results for genome titer, capsid titer, and percent full will automatically populate in the Results tab of the workbook.

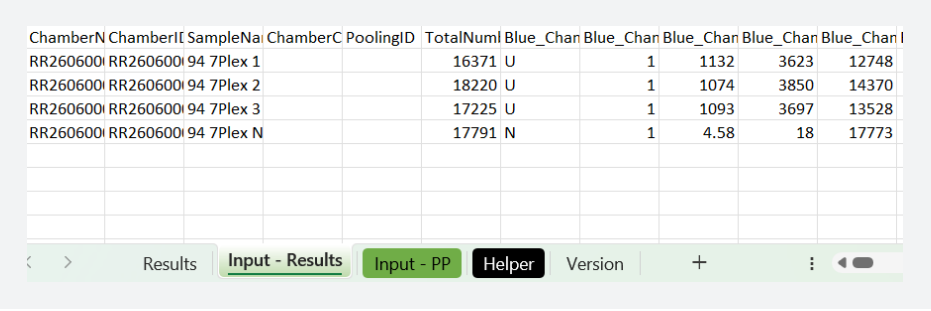

Fig. 6. Importing results into the workbook. Copy data from the exported .xlsx file under the Results tab and paste into cell A1 of the Input - Results tab in the workbook.

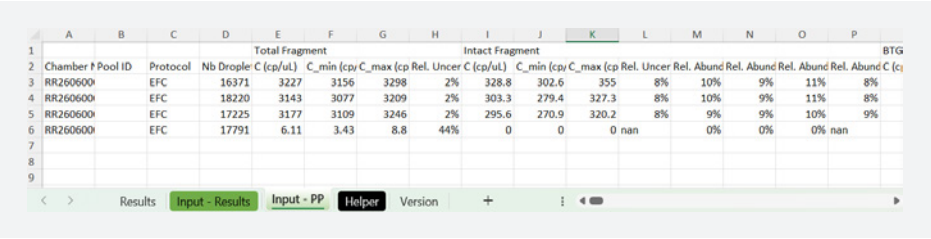

Fig. 7. Importing post-processing linkage analysis into the workbook. Copy data from the exported .xlsx file under the PP result tab and paste into cell A1 of the Input - PP tab in the workbook.

Data Interpretation

The equations in Table 13 calculate the genome titer, percent full, and capsid titer using the outputs from the Post-Processing tab and the Results tab of the software export.

Table 13. Explanation of equations.

Metric	Formula
Genome concentration	= HEX concentration
FAM_adjusted	= FAM concentration $_{Unknown}$ – FAM concentration $_{NegCtrl}$
Efficiency	$= \frac{\text{Linkage}}{\text{(HEX concentration)}}$
Percent full	$= \frac{\text{Linkage}}{\text{FAM\_adjusted}}$
Capsid concentration	$= \frac{\text{FAM\_adjusted}}{\text{Efficiency}}$
Original genome titer	= Genome concentration x 125,000 x extra dilutions
Original capsid titer	= Capsid concentration x 125,000 x extra dilutions

The Vericheck QX700 ddPCR Empty-Full Capsid Kit works by simultaneously measuring capsids and genomes in the same droplet. Genome concentration is simply the HEX concentration. As the capsids are lysed within droplets, it is not necessary to account for two copies of ITR; both copies are always contained within the same droplet.

The capsid signal in the FAM channel must be calibrated, as it is not 100% efficient and has a small background signal. First, subtract the background signal in the Negative Control from all unknown samples. Next, use the genome signal to calibrate the capsid signal based on two assumptions: 1) PCR is 100% efficient, whereas the capsid assay may not be, and 2) in nuclease-treated samples, all viral genomes are located within capsids. Droplets that contain a genome signal in HEX but no capsid signal in FAM therefore represent capsids that were not directly detected. Capsid labeling efficiency is calculated as the concentration of genomes colocalized with capsids (linkage) divided by the genome concentration. Capsid concentration is then determined by correcting the FAM signal for labeling efficiency.

The percent full is calculated as the ratio of linkage to the adjusted FAM concentration, which is mathematically equivalent to the ratio of genome concentration to capsid concentration. To ensure precision, both FAM and HEX concentrations must be <20,000 copies/µL, and the FAM concentration in test samples should be at least 3x higher than that of the Negative Control.

In addition, the genome and capsid titers of the original sample can be back-calculated. When performed as described above, the original sample is diluted 1:125,000 through a series of sequential dilution steps: a 1:10 dilution during DNase digestion, a subsequent 1:10 dilution, a 1:5 dilution during binding, a 1:5 dilution during ligation, a 1:10 dilution during ligation stopping, and a 1:5 dilution during the ddPCR reaction. If additional dilutions were performed, incorporate those changes into the calculations.

Appendix A. Alternate Genome Reference Targets

The Empty-Full Capsid Kit uses AAV2 ITR as a reference target. Additional targets may be measured to assess genome integrity. To do so, add additional 20x assays in Cy5, Cy5.5, ROX, ATTO-550, or ATTO-590 to the ddPCR reaction setup and reduce the water volume accordingly. Alternatively, sufficient reagent volume is provided to test additional targets across multiple wells.

To analyze multiple targets, each additional channel used must be selected in the dropdown option in the Post-Processing tab prior to export. The workbook is compatible with additional targets and uses the same equations described in the Data Interpretation section. The export can be added to the workbook as described in the Data Interpretation Using the Vericheck QX700 ddPCR Empty-Full Capsid Analysis Worksheet section; no changes are required. The workbook reports the percentage of capsids filled with each individual target, while genome titer is reported based on the HEX channel only. The percentage of capsids containing every target present in the reaction is reported in the Capsids with All Genome Targets column. The percentage of capsids containing some, but not all, targets is reported in the Capsids with Incomplete Genomes column. The percentage of capsids containing no detected target is reported in the Empty Capsids column.

Appendix B. Troubleshooting

This section lists common failure modes with their associated phenotypes, descriptions, and suggested resolutions. For a complete list of failure modes, refer to the QX700 Droplet Digital PCR System Instrument Guide (10000171493), Appendix A, Experiment Troubleshooting.

No or Low Total Droplet Counts

Problem: Wells have droplet counts <10,000.

Resolution: Ensure that the full 5 µL is dispensed into each well. Dispense samples approximately 1 mm above the bottom of the well. Ensure no visible cracks are present in the Quality Control view or when inspecting the bottom of the chip.

PCR Inhibition

Problem: The separation between clusters decreases and rain increases. Droplet counts may also be higher or lower than expected.

Resolution: If a concentration cannot be calculated, exclude the well from analysis. Ensure the sample was properly DNase-treated prior to antibody binding, as excessive residual DNA may inhibit amplification.

Inconsistent Concentration Results

Problem: Concentration estimates are inconsistent between technical replicates. This is likely due to insufficient mixing at one or more steps in the protocol.

Resolution: Repeat the Droplet Digital PCR workflow. When creating technical replicates, thoroughly mix the reaction mixture (master mix, sample, and assays) by pipetting up and down ten times using 90% volume strokes. Alternatively, pulse vortex the reaction mixture for 15 sec, followed by spinning the sample down. Do not assemble or mix reaction mixtures in the RDG16 Cartridge. For accurate results from the Vericheck QX700 ddPCR Empty-Full Capsid Kits, reactions at all steps must be thoroughly mixed before proceeding.

Wrong Serotype Kit Used

Problem: Low or no estimated concentration is detected in the FAM channel of sample wells, while the Positive Control shows expected clusters.

Resolution: The Vericheck QX700 ddPCR Empty-Full Capsid Kits are serotype-specific. Detection of a signal from the ITR2 Assay (HEX) but not from the Capsid Detection Assay (FAM) indicates an AAV sample not recognized by the kit antibody probes. Ensure that the tested samples correspond to the correct AAV serotype for the kit used.

Low FAM Signal

Problem: High genome concentration detected, but very low capsid assay concentration.

Resolution: Ensure sufficient antibody binding time (≥1 hr). Ensure nucleases are deactivated by dilution before antibody addition. Avoid heat inactivation of reactions containing AAV.

No Linkage

Problem: No linkage is detected despite high concentrations in the FAM and HEX channels. Percent full and capsid titer cannot be calculated.

Resolution: Check thresholding and thermal cycling conditions. Ensure the sample has not been heated or lysed after antibody binding.

No Negative Droplets

Problem: Only high-amplitude clusters are present in the sample well. The sample is too concentrated and outside the dynamic range.

Resolution: Exclude the well from analysis. Further dilute the DNase-treated sample using the provided AAV Dilution Buffer and test again.

High ITR2 (HEX) Signal in Negative Control

Problem: Presence of >10 copies/µL in the ITR2 (HEX) channel. The Negative Control should be free of AAV particles and not include ITR2 DNA.

Resolution: Ensure the correct sample was selected as the Negative Control. Follow good PCR laboratory practices to prevent contamination, including wiping down work areas and equipment with 5–10% bleach, preparing master mixes in a template-free environment, preparing samples in a low-template environment, and not reusing ddPCR consumables.

High Capsid (FAM) Signal in Negative Control

Problem: The Negative Control capsid concentration (FAM) exceeds 50 copies/µL with no or few ITR2 positive droplets.

Resolution: Exclude the plate data and repeat the protocol. Ensure the ligation reaction is promptly diluted in the provided Stop Solution after incubation.

Inconsistent Percent Full for Long Wait Time Plates

Problem: Increased variability for plates left in the plate hotel >4 hours.

Resolution: Ensure plate proceeds promptly into thermal cycling after loading.

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Life Science Group

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