Absolute Q™ Viral Titer dPCR Assays

USER GUIDE

for use with QuantStudio™ Absolute Q™ Digital PCR Software v6.3 or later

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Revision D.0

ThermoFisher SCIENTIFIC

Life Technologies Corporation | 6055 Sunol Blvd | Pleasanton, California 94566 USA
For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: MAN0025691 D.0 (English)

Revision	Date	Description
D.0	1 November 2023	Updated to reflect changes needed for use with v6.3 or later of the QuantStudio™ Absolute Q™ Digital PCR Software.
C.0	28 July 2022	• Instructions were revised for setting up the experiment ("Set up the experiment" on page 13). • Instructions were revised for setting up plate analysis ("Set up the plate for analysis" on page 17). • Instructions were revised for post experiment analysis ("Post-analysis procedure" on page 18).
B.0	26 July 2022	• The Absolute Q™ Viral Titer dPCR Assays product description was revised ("Product description" on page 4). • Removed references to individual assays and directed the customer to the website for a complete list of assays ("Assay ordering and specifications" on page 4). • Added consumables for compatible surfactants ("Required materials not supplied" on page 5). • Instructions were revised for preparing the dPCR reaction mix ("Prepare the dPCR reaction mix" on page 6). • Instructions were revised for loading the MAP plate ("Load the reaction mix into the MAP plate" on page 8). • Instructions were revised for setting up plate analysis ("Set up the plate for analysis" on page 17). • Added images depicting duplexed samples for post experiment analysis ("Post-analysis procedure" on page 18).
A.0	13 September 2021	New publication documenting the use of the Absolute Q™ Viral Titer dPCR Assays with the QuantStudio™ Absolute Q™ Digital PCR System.

The information in this guide is subject to change without notice.

Contents

1 Product Information

Note: For safety and biohazard guidelines, refer to the "Safety" section in the *QuantStudio™ Absolute Q™ Digital PCR System Installation, Use, and Maintenance Guide* (Pub. No. MAN0028562).

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

The Absolute Q™ Viral Titer dPCR Assays enable accurate, absolute quantification of viral vectors used in gene therapy research. The pre-designed and validated vector backbone assays are part of a streamlined workflow, formulated to work seamlessly with the QuantStudio™ Absolute Q™ Digital PCR System. The assays are designed to be run individually or duplexed with a custom assay that targets your unique gene of interest (GOI) to help determine copies per µL and evaluate quality for biopharma research.

Assay ordering and specifications

For a complete list of available Absolute Q™ Viral Titer dPCR Assays, contact your local sales representative or go to **http://www.thermofisher.com/dpcr-viraltiter**.

The list that follows provides Absolute Q™ Viral Titer dPCR Assay specifications.

- 1 tube at 20X concentration
- Uses a VIC™ fluorophore that enables duplexing with an appropriate custom assay
- Store at –20°C

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Catalog numbers that appear as links open the web pages for those products.

Item	Source
Digital PCR System	
QuantStudio™ Absolute Q™ Digital PCR System	A52864
Equipment	
Centrifuge, table top	MLS
Vortex mixer	MLS
Pipette, P20	MLS
Filter pipette tips, P20	MLS
Other consumables	
QuantStudio™ Absolute Q™ MAP16 Plate Kit • 12 QuantStudio™ Absolute Q™ MAP16 Digital PCR Plates • 60 QuantStudio™ Absolute Q™ MAP plate gasket strips • 3 mL QuantStudio™ Absolute Q™ Isolation Buffer	A52865
Absolute Q™ DNA Digital PCR Master Mix (5X)	A52490
Custom Gene of Interest assay (GOI) with FAM™ probe (20x)	**http://www.thermofisher.com/dpcr-viraltiter**
Low bind microcentrifuge tubes	MLS
Microcentrifuge tube rack	MLS
Nuclease-free water	MLS
DNA suspension buffer	MLS
Tween 80	MLS
Pluronic™ F68	MLS

2 Prepare an experiment

This section provides a procedure for preparing a dPCR reaction mix with an Absolute Q™ Viral Titer dPCR Assay to quantify the concentration of a vector backbone.

For detailed instructions about preparing and running digital PCR (dPCR) experiments for implementations with or without automation, see the *QuantStudio™ Absolute Q™ Digital PCR System Installation, Use, and Maintenance Guide* (Pub. No. MAN0028562).

Singleplex versus duplex experiments

The viral titer assay can be duplexed with a custom assay that targets your gene of interest (GOI) to provide further information on vector quality and concentration. The volume of water used for the dPCR reaction can be adjusted accordingly, dependent on experiment requirements.

We recommend that you use FAM™ as the fluorophore for your custom GOI assay when duplexing with the VIC™-labeled Absolute Q™ Viral Titer dPCR Assay. For information on ordering a custom assay, contact your local sales representative or visit **http://www.thermofisher.com**.

Note: Duplexing assays may require optimization of the dPCR reaction mix and reaction conditions.

Prepare the dPCR reaction mix

Gather the following materials:

- P10 or P20 pipette and filter pipette tips
- DNA suspension buffer
- Surfactant
- Absolute Q™ DNA Digital PCR Master Mix (5X)
- Absolute Q™ Viral Titer dPCR Assay
- GOI assay, if applicable for your assay
- Nuclease-free water

The volume of the dPCR reaction can be adjusted depending on experimental requirements. Scale the components proportionally according to the number of reactions and include 10% overage.

IMPORTANT!

- Throughout this procedure, protect reagents from light when not in use.
- For best results, use Applied Biosystems™ TaqMan™ Assays or Absolute Q™ digital PCR assays with the Absolute Q™ DNA Digital PCR Master Mix (5X). Applied Biosystems™ reagents have been tested for in-plate stability at ambient temperature for up to 96 hours, to support automation.

1. Dilute the viral sample in buffer containing a PCR compatible surfactant, for example:
   - 0.05% Tween 80
   - 0.01% Pluronic™ F68

   Note: We recommend testing 2–3 10-fold dilutions to find an optimal input range.

2. Thaw and equilibrate all reagents to room temperature before use.

   Note:
   - Store reagents on ice when not in use.
   - Limit number of reagent freeze/thaw cycles.

3. Pulse vortex the following reagents at high speed for 10 seconds:
   - Absolute Q™ DNA Digital PCR Master Mix (5X)
   - Absolute Q™ Viral Titer dPCR Assay
   - *(Optional)* GOI assay (FAM™ labeled), if applicable for your assay

4. Combine the following reagents in the order listed.

Reagent	Final concentration	Volume per reaction (with overage)	
		Singleplex	Duplex
Absolute Q™ DNA Digital PCR Master Mix (5X)	1X	2 µL	2 µL
Absolute Q™ Viral Titer dPCR Assay (VIC™, 20X)	1X	0.5 µL	0.5 µL
GOI assay (FAM™, 20X)	1X	—	0.5 µL
Purified DNA virus sample of interest	Variable[1]	Variable, ≤7 µL	Variable, ≤7 µL
Nuclease-free water	—	Fill to 10 µL	Fill to 10 µL
Total reaction volume	—	**10 µL**	**10 µL**

[1] We recommend testing 2–3 10-fold dilutions to find an optimal concentration.

5. Mix well by performing one of the following actions:
   - Pipette mix 10–20 times, or
   - Pulse vortex 3–5 times for 1 second each.
6. Using a benchtop, centrifuge at 10,000 × *g* or the highest speed available for 1 minute.

Load the reaction mix into the MAP plate

At a clean lab bench, gather the following materials.

- P10 or P20 pipette and filter pipette tips
- Prepared dPCR reaction mix
- QuantStudio™ Absolute Q™ Isolation Buffer
- MAP plate with sufficient unused columns for the experiment
- MAP plate gasket strips (unused)

IMPORTANT! At least 1 column of the MAP plate must be used for each run and all wells in the column must contain a sample (or water plus isolation buffer if there is insufficient sample to fill all wells). Columns cannot be reused, but a MAP plate with unused columns can be used for subsequent experiments. If the MAP plate has unused columns, when the experiment is complete, place it back into its pouch for storage.

Note: The MAP plate follows SBS standard plate format, allowing for use with an automated liquid handling workflow.

1. Just prior to use, remove the MAP plate from its package.

   Note:
   - Leave the MAP plate in the package until ready to load sample.
   - Be careful to handle the MAP plate by its frame.
   - Place the MAP plate back into the package when not in use.

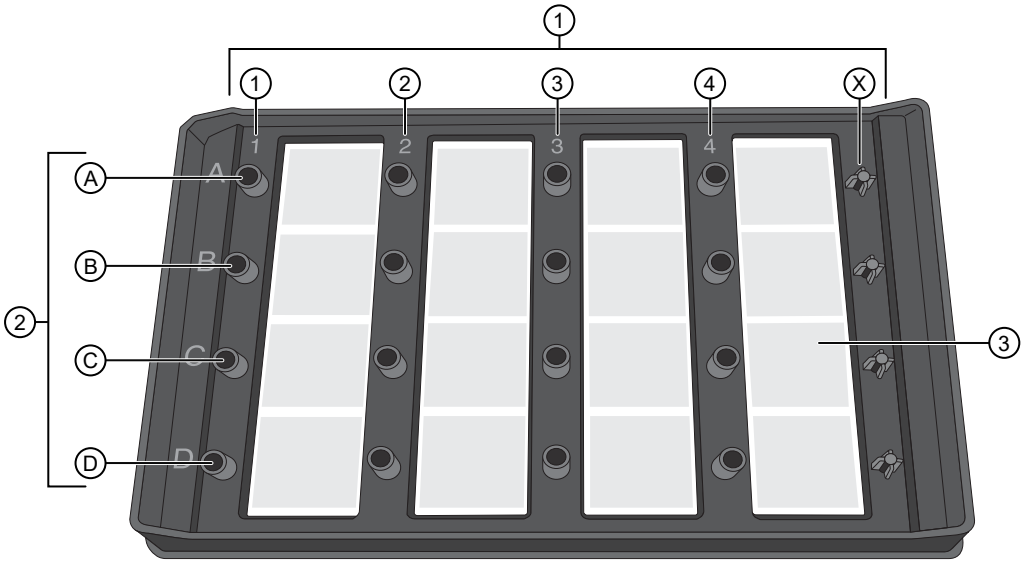


Figure 1 MAP plate without MAP plate gasket strips

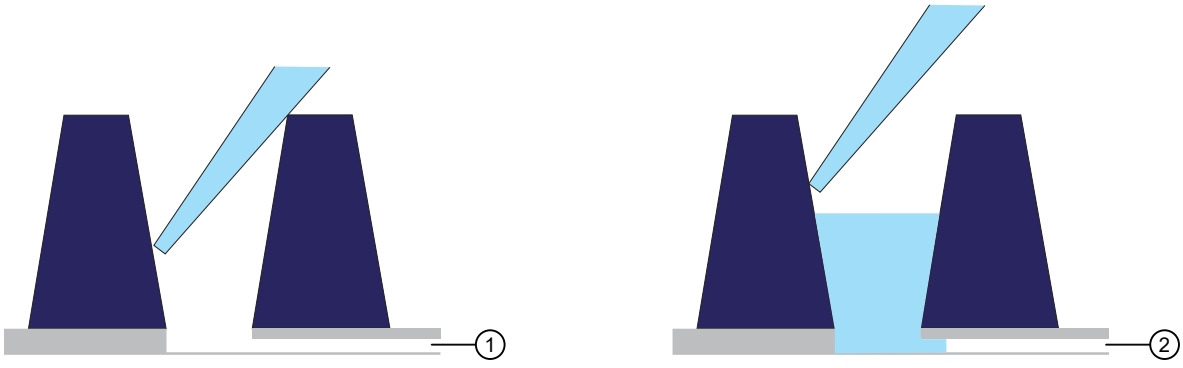
① Columns 1–4 and column X
② A–D represent wells A1–D1 associated with column 1
③ Array associated with well 4C

2. Place the MAP plate on a level, dust-free, dry surface.

3. Using a new pipette tip for each well, holding the pipette at a 45° angle, load 9 µL of the dPCR reaction mix to the bottom of the well. Pipet the mixture only to the first stop to prevent bubble formation.

IMPORTANT! To avoid the transfer of contents from the bottom of the centrifuged dPCR reaction mix tube, do not pipet from the bottom of the tube.

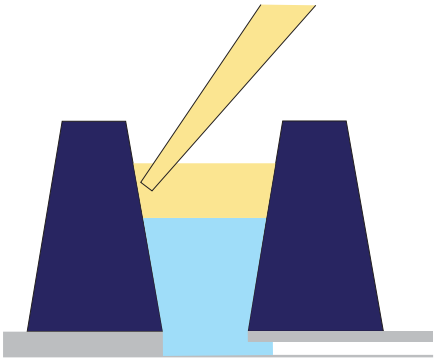
IMPORTANT! Do not contact bottom of well with the pipette tip or puncture the thin film at the bottom of the well.



① Microfluidic channel to the microchamber array
② Reaction mix remains in the well until the instrument pushes it into the microchamber array during the run

4. Using a new pipette tip for each well, at a 45° angle, load 15 µL of the Absolute Q™ Isolation Buffer on the side of the well above the top of the reaction mix. Carefully overlay the buffer on top of the reaction mix to prevent mixing or bubble formation. Pipet only to the first stop.

   The isolation buffer sits on top of the reaction mix, preventing contamination and evaporation.



5. Place a total of 5 MAP plate gasket strips on all 4 columns of wells and the X-shaped posts of column X on the right side of the plate. Orient the MAP plate gasket strip so that the side labeled A–D aligns with rows A–D marked on the plate. Be sure to cover the columns completely and press the MAP plate gasket strips firmly into place.

   IMPORTANT! MAP plate gasket strips must be placed on all columns, including unused columns. Failure to do so can produce poor results.

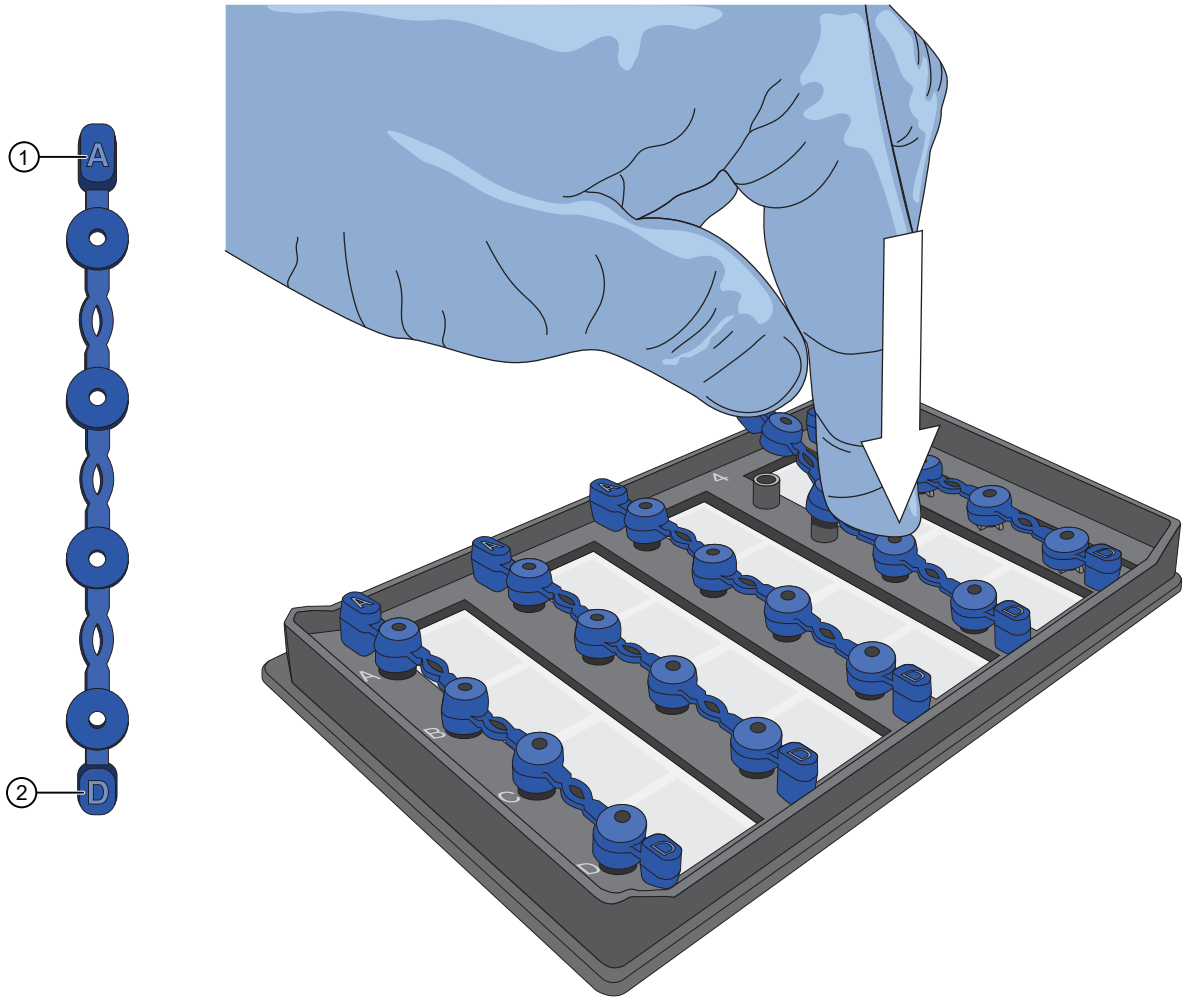


Figure 2 Place the MAP plate gasket strips firmly into place

① Place this end of the MAP plate gasket strip on row A
② Place this end of the MAP plate gasket strip on row D

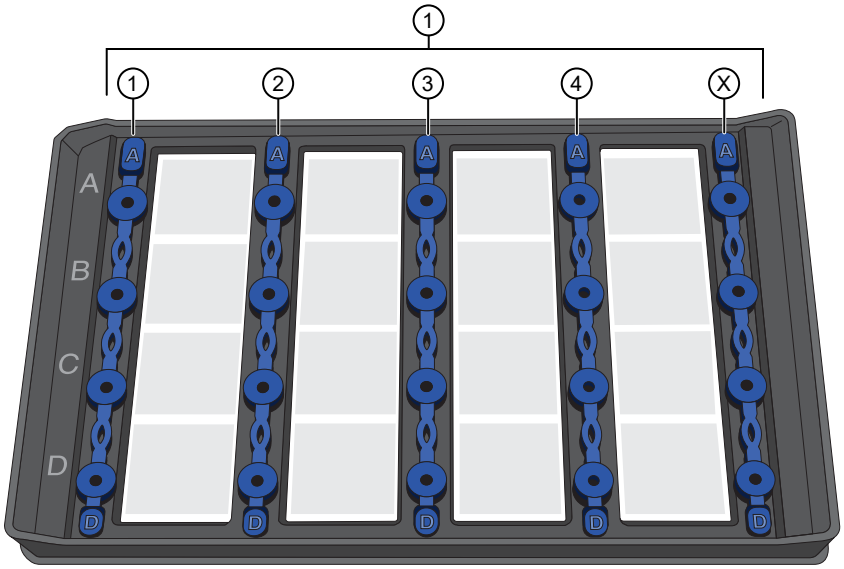


Figure 3 MAP plate with MAP plate gasket strips in place

① MAP plate gasket strips on columns 1–4 and column X

6. Move the MAP plate to the instrument.

IMPORTANT! Do not tip, invert, or shake the filled MAP plate.

3 Run an experiment

This section provides information for running experiments on the QuantStudio™ Absolute Q™ Digital PCR Instrument in a non-automated implementation.

For detailed instructions about preparing and running digital PCR (dPCR) experiments for implementations with or without automation, see the *QuantStudio™ Absolute Q™ Digital PCR System Installation, Use, and Maintenance Guide* (Pub. No. MAN0028562).

Set up the experiment

This section provides information about setting protocol parameters and plate setup configurations by creating a template.

Note: If you have an existing template for the assay with the required protocol and plate setup configuration, you can proceed to create a run from that template. See “Create a run” on page 15.

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1. From the left pane of the QuantStudio™ Absolute Q™ Digital PCR Software click to open the **Templates** list page.
2. Select **CREATE TEMPLATE** from upper-right corner of the **Templates** list page.
3. When prompted, enter a template name, then click **CREATE TEMPLATE**.

   The new template opens in the **PROTOCOL** tab.
4. In the thermal protocol settings area, enter the following settings.
   a. Set the **Preheat** setting to ***96°C*** for ***10 minutes***.
   b. Set step one to ***96°C*** for ***5 seconds***.
   c. Set step two to ***60°C*** for ***15 seconds***.
   d. Set the **Cycles** to ***40***.

e. Ensure that **Preheat** is selected.

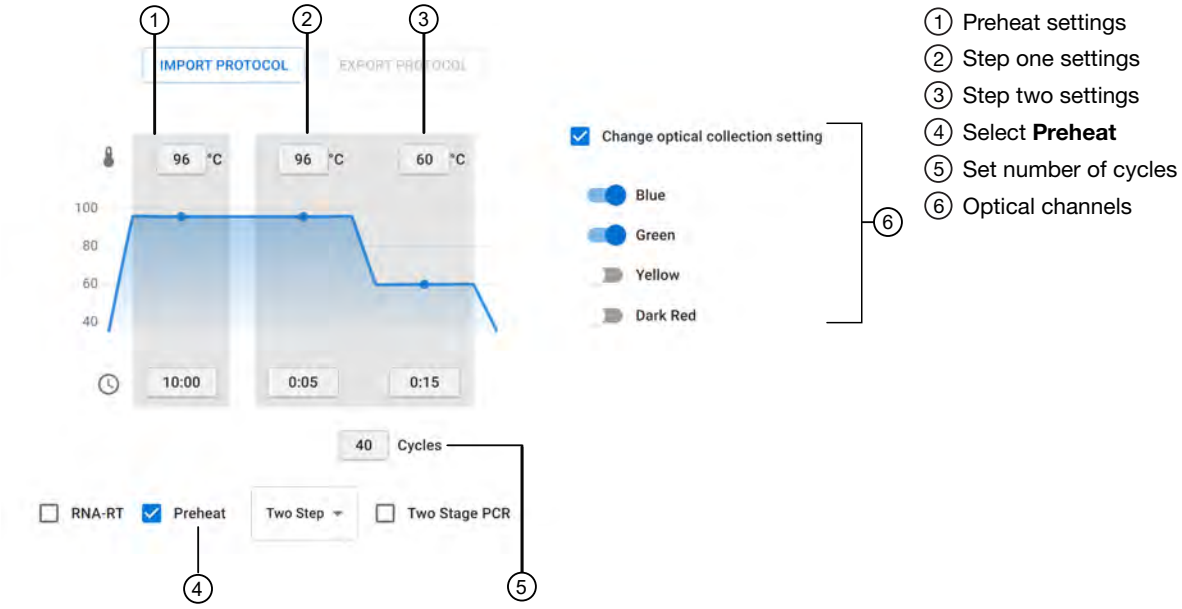

Figure 4 Thermal protocol

5. Select the **SETUP** tab to modify the plate configuration.
6. In the **Sample Groups** area, click ✎ in the group to be edited to select the appropriate optical channels for the assay.
7. Toggle the optical channels on or off based on the assay reporter dyes used.

Option	Channel(s)
Singleplex	**VIC**
Duplex	**VIC** **FAM**[1]

[1] We recommend you use FAM™ dye as the fluorophore for your custom GOI assay when duplexing with the VIC™-labeled Absolute Q™ Viral Titer dPCR Assay. Depending on your assay, other channels can also be used.

8. Modify the **Target**, **Analysis**, and **Default threshold** options if needed.
9. Ensure that the correct grouping option is selected, then click **CONTINUE**.
10. On the **SETUP** page, modify the plate settings if needed, then click **SAVE**.
11. Proceed to "Create a run" on page 15.

Create a run

This section provides information about creating a run from the **Templates** or **Runs** pages.

IMPORTANT! A template for the assay must be set up before creating a run for the assay. See "Set up the experiment" on page 13.

1. Use one of the following options to create a run.

Option	Actions
Create a run from the **Templates** page.	1. From the left pane, click to open the **Templates** list page. 2. From the template list, select the template for this assay. 3. In the **Select action** dialog, select **Create run from template**. 4. When prompted, enter a name for the run, then click **CREATE RUN**.
Create a run from the **Runs** page.	1. From the left pane, click to open the **Runs** list page. 2. In the upper-right corner of **Runs** list page, click **CREATE RUN**. 3. When prompted, enter the following information, then click **CREATE RUN**. • **Run name**: Enter a name for the run. • **Template**: From the dropdown list, select the template for this assay.

2. On the **PROTOCOL** page, ensure that the settings are correct. See "Set up the experiment" on page 13.

3. Select the **SETUP** tab to modify the plate configuration if needed, then click **SAVE**.

   IMPORTANT! Ensure that only the columns with samples are selected before beginning the experiment.

4. Proceed to "Run the experiment" on page 15.

Run the experiment

1. From the left pane click to open the **Runs** list page.

2. From the **RUNS**, **DRAFT** page, select the run, then click **START RUN**.

   The **Start run** dialog box opens and the instrument door opens to receive the loaded MAP plate.

   IMPORTANT! Ensure that gaskets are placed on all columns of the MAP plate, including unused columns. Ensure that gaskets have been placed on all wells and on the column X posts on the far right as shown on the screen. Failure to do so can produce poor results.

   Note: See callout 5 in the following figure for the location of column X.

3. Carefully load the MAP plate in the plate nest.

IMPORTANT! Be sure to load the MAP plate gently to avoid damage to the plate nest.

4. Select **CLOSE DOOR**, then **START RUN**.

   The door closes and the MAP plate barcode is scanned.

   Note: If the barcode number does not match the number entered, or the instrument cannot scan the barcode, you are prompted to add it in the **Plate barcode** field of the **Start Run** dialog box.

   When the run has successfully started, the **Runs** page returns to the **DRAFT** tab and the status of the selected run displays **IN PROGRESS**.

4 Analyze data

For detailed information about performing analysis, see the *QuantStudio™ Absolute Q™ Digital PCR System Installation, Use, and Maintenance Guide* (Pub No. MAN0028562).

Set up the plate for analysis

1. In the QuantStudio™ Absolute Q™ Digital PCR Software, in the left pane click to open the **Runs** list page.
2. On the **COMPLETED** tab, use the search field to find a run or select a run from the list.
   The run opens on the **SETUP** tab.
3. In the **Sample groups** area, click **+ ADD GROUP**, then in the **Group name** field, enter a name for the group.
4. In the **Analysis** column, select **Signal** for the optical channels for reactions as indicated in the following table.

Option	Channel(s)
Singleplex	**VIC**
Duplex	**VIC** **FAM**[1]

[1] We recommend you use FAM™ dye as the fluorophore for your custom GOI assay when duplexing with the VIC™-labeled Absolute Q™ Viral Titer dPCR Assay. Depending on your assay, other channels can also be used.

5. For all other channels in the **Analysis** column, set the toggle to the off position to exclude them from analysis.
6. Select **CONTINUE** to return to the **SETUP** tab, then click **SAVE**.
7. On the **Runs** page, select the **RESULTS** tab and verify that the total number of analyzed microchambers in the array is >20,000.
   Consider omitting or rerunning arrays with fewer than 20,000 analyzed microchambers.

Post-analysis procedure

When the run has completed, ensure that the automatic threshold is above the negative population. If the threshold is not automatically adjusted above the negative population, manually adjust the threshold to the desired position.

For information about adjusting thresholds on samples, see “Adjust the threshold on samples” on page 19.

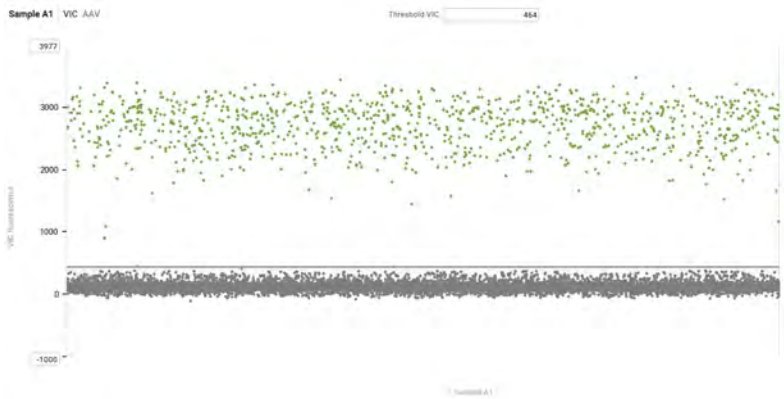

Figure 5 Automatic thresholds for an array (Singleplex)

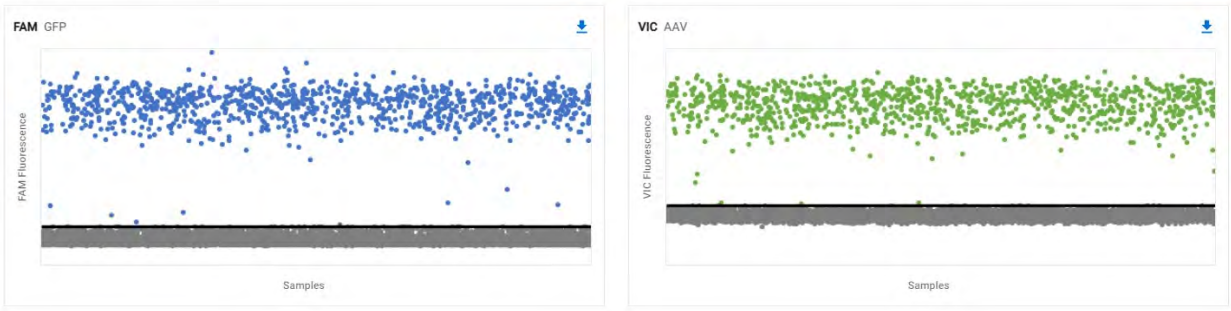

Figure 6 Automatic thresholds for an array (Duplex)

The group feature can be used to set thresholds for all samples within a group. Adjust the group threshold so that it is above the negative population (lower band).

Note: Although the threshold line is visible in duplex view, the actual values are only displayed in singleplex view. To view the values of group thresholds, you must access each channel in singleplex view.

For information about adjusting thresholds on sample groups, see “Adjust the threshold on sample groups” on page 21.

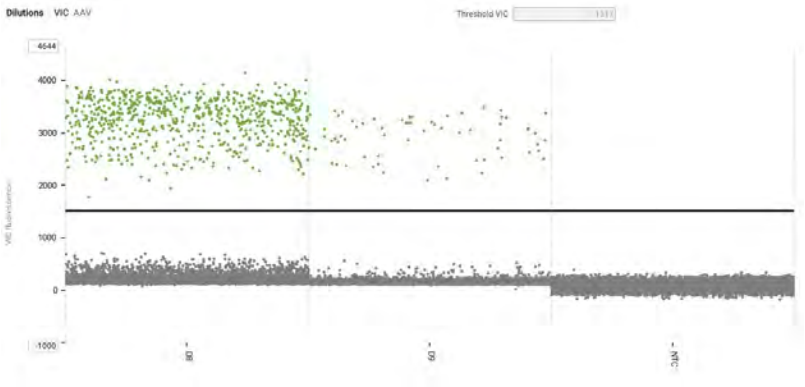

Figure 7 Group thresholds (Singleplex)

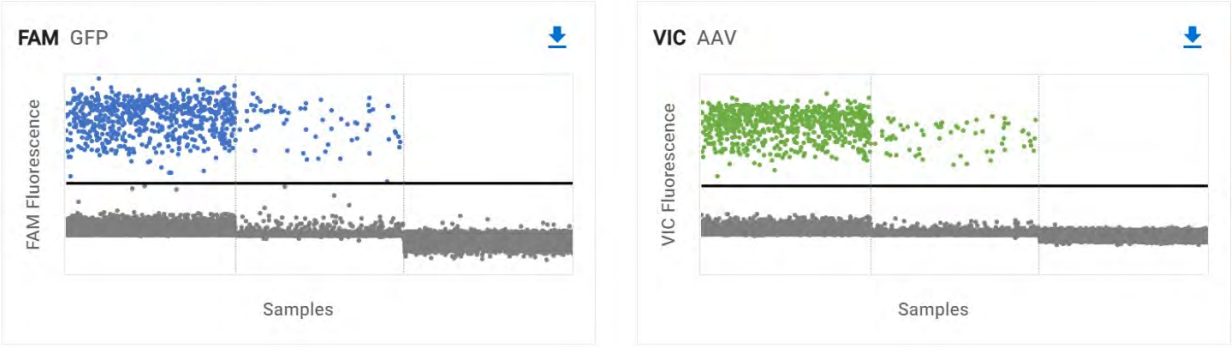

Figure 8 Group thresholds (Duplex)

Adjust the threshold on samples

1. In the QuantStudio™ Absolute Q™ Digital PCR Software, in the left pane click to open the **Runs** list page.
2. On the **COMPLETED** tab, use the search field to find a run or select a run from the list.
   The run opens on the **SETUP** tab.
3. Click the **ANALYSIS** tab to display the run data.
   By default the first sample group is selected in the **Analyze by** area and the plots are in 1D Scatter format.
4. *(Optional)* To change the plot type to 2D Scatter, click .

5. In the **Analyze by** area, use one of the following options to select a sample.

Option	Action
Sample Group view—Select a sample from the sample list.	1. *(Optional)* In the **View sample** dropdown, set the sample view to either **Ungrouped** or **Omitted**. 2. *(Optional)* Modify the sort of the information in the sample list using directional arrows in the column headings to set the sort to ascending or descending order. 3. Select a sample from the sample list. The sample opens on the **PLOTS** page in the gallery view.
Sample view—Select a sample from the sample list.	1. Click **Sample**. The sample opens on the **PLOTS** page in the gallery view. 2. *(Optional)* In the **View sample** dropdown, set the sample view to either **Ungrouped** or **Omitted**. 3. *(Optional)* Modify the sort of the information in the sample list using directional arrows in the column headings to set the sort to ascending or descending order. 4. *(Optional)* Select a different sample from the sample list. The sample opens on the **PLOTS** page in the gallery view.

6. To see the detail view of a channel, select a channel plot in the gallery.

7. To change the threshold of the sample data, use one of the following options.

Note: When changes are made to the data, the plots turn gray to indicate that the data are outdated and must be saved to recalculate the plot.

Option	Action
FROM GROUP—the software sets the threshold for this sample to the group threshold to which the sample is assigned.	1. Click **FROM GROUP**. 2. Click **SAVE**.
AUTO—the software sets the threshold for the sample and prevents it from being changed by any threshold adjustments that are made to the group to which it is assigned.	1. Click **AUTO**. 2. Click **SAVE**.
MANUAL—manually sets the threshold for the sample and prevents it from being changed by any threshold adjustments that are made to the group to which it is assigned.	1. Click **MANUAL**. 2. Use one of the following options to set the threshold. • Drag the threshold bar in the plot to the desired value. • Enter a value in the **Group threshold** field at the top of the table. 3. Click **SAVE**.

Adjust the threshold on sample groups

1. In the QuantStudio™ Absolute Q™ Digital PCR Software, in the left pane click to open the **Runs** list page.
2. On the **COMPLETED** tab, use the search field to find a run or select a run from the list.
   The run opens on the **SETUP** tab.
3. Click the **ANALYSIS** tab to display the run data.
   By default the first sample group is selected in the **Analyze by** area and the plots are in 1D Scatter format.
4. *(Optional)* To change the plot type to 2D Scatter, click .
5. In the **Analyze by** area, click **Sample Group**, then select the group to be analyzed.
6. To see the detail view of a channel, select a channel plot in the gallery.
7. To change the threshold of the sample data, use one of the following options.

Note: When changes are made to the data, the plots turn gray to indicate that the data are outdated and must be saved to recalculate the plot.

Option	Action
AUTO GROUP—The software sets the threshold for the channel group.	1. Click **AUTO GROUP**. 2. Click **SAVE**.
AUTO SAMPLE—The software sets the threshold for each sample in the channel group.	1. Click **AUTO SAMPLE**. 2. Click **SAVE**.
MANUAL—Manually set the threshold for the channel group.	1. Click **MANUAL**. 2. Use one of the following options to set the threshold. • Drag the threshold bar in the plot to the desired value. • Enter a value in the **Group threshold** field at the top of the table. 3. Click **SAVE**.

A Documentation and support

Related documentation

Document	Publication number	Description
QuantStudio™ Absolute Q™ Digital PCR System Installation, Use, and Maintenance Guide	MAN0028562	Detailed instructions for using the QuantStudio™ Absolute Q™ Digital PCR System to prepare and run digital PCR experiments, and analyze results.

Note: For additional documentation, see “Customer and technical support” on page 22.

Customer and technical support

Visit **thermofisher.com/support** for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

  Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.