Revision history:

<table>
<thead>
<tr>
<th>Revision</th>
<th>Date</th>
<th>Description</th>
</tr>
</thead>
</table>
| C.0      | 28 July 2022 | • Instructions were revised for setting up the experiment (“Set up and run the experiment” on page 13).  
• Instructions were revised for setting up plate analysis (“Set up the plate for analysis” on page 15).  
• Instructions were revised for post experiment analysis (“Post-analysis procedure” on page 16). |
| 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 reagent mix into the MAP plate” on page 8).  
• Instructions were revised for setting up plate analysis (“Set up the plate for analysis” on page 15).  
• Added images depicting duplexed samples for post experiment analysis (“Post-analysis procedure” on page 16). |
| 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.

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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.

For detailed instructions on preparing and running digital PCR (dPCR) experiments, see the QuantStudio™ Absolute Q™ Digital PCR System Installation, Use, and Maintenance Guide (Pub. No. MAN0025621).

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.

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](http://thermofisher.com). "MLS" indicates that the material is available from [fisherscientific.com](http://fisherscientific.com) or another major laboratory supplier.

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

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

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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 on preparing and running digital PCR (dPCR) experiments, see the QuantStudio™ Absolute Q™ Digital PCR System Installation, Use, and Maintenance Guide (Pub No. MAN0025621).

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, perform the run within one hour of reaction preparation.

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.

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Final Concentration</th>
<th>Volume Per Reaction (with overage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absolute Q™ DNA Digital PCR Master Mix (5X)</td>
<td>1X</td>
<td>Singleplex: 2 μL, Duplex: 2 μL</td>
</tr>
<tr>
<td>Absolute Q™ Viral Titer dPCR Assay (VIC™, 20X)</td>
<td>1X</td>
<td>Singleplex: 0.5 μL, Duplex: 0.5 μL</td>
</tr>
<tr>
<td>GOI assay (FAM™, 20X)</td>
<td>1X</td>
<td>Singleplex: —, Duplex: 0.5 μL</td>
</tr>
<tr>
<td>Purified DNA virus sample of interest</td>
<td>Variable[1]</td>
<td>Singleplex: Variable, ≤7 μL, Duplex: Variable, ≤7 μL</td>
</tr>
<tr>
<td>Nuclease-free water</td>
<td>—</td>
<td>Singleplex: Fill to 10 μL, Duplex: Fill to 10 μL</td>
</tr>
<tr>
<td>Total</td>
<td>—</td>
<td>Singleplex: 10 μL, Duplex: 10 μL</td>
</tr>
</tbody>
</table>

[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 \( \times g \) or the highest speed available for 1 minute.

7. Perform the run within one hour of reaction preparation.

Load the reagent 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 run at a time. Columns cannot be reused, but a MAP plate with unused columns can be used for subsequent experiments. When the experiment is complete, if the MAP plate has unused columns, place it back into its pouch for storage.

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

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

Chapter 2 Prepare and run an experiment

Load the reagent mix into the MAP plate
3. Using a new pipette tip for each well, at a 45° angle, load 9 μL of the dPCR reagent mix to the bottom of the well. Pipette the mixture only to the first stop to prevent bubble formation.

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

![Diagram of microfluidic channel to the microreaction chamber array and reagent remaining in the well until the instrument pushes it into the microreaction chamber array during the run.](image)

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 reagent mix. Carefully overlay the buffer on top of the reagent mix to prevent mixing or bubble formation. Pipette only to the first stop. The isolation buffer sits on top of the reagent, 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 on the 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](image)

**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.

## Set up and run the experiment

1. In the left pane of the QuantStudio™ Absolute Q™ Digital PCR Software, select ▶ to access the Instrument page.

2. In the PROTOCOL area, select ⬇️ EDIT PROTOCOL.

3. 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.
4. In the **Channels** area, select the optical channels based on the assay reporter dyes used.

<table>
<thead>
<tr>
<th>Option</th>
<th>Channel(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Singleplex</td>
<td><strong>VIC™</strong></td>
</tr>
<tr>
<td>Duplex</td>
<td><strong>VIC™</strong></td>
</tr>
<tr>
<td></td>
<td><strong>FAM™[1]</strong></td>
</tr>
</tbody>
</table>

[1] We recommend you use FAM™ 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.

**Note:** ROX™ is selected by default for the QC channel.

5. Click **SAVE** to save the protocol settings.

6. Make sure that only the columns with samples are selected before beginning the experiment.

7. To load the MAP plate, click **Open** (next to the instrument icon) to open the plate tray.
   - The instrument door opens to receive the loaded MAP plate.
   
   **IMPORTANT!** Confirm that gaskets are placed on all columns of the MAP plate, including unused columns. Failure to do so can produce poor results.

8. Click the **START** button under the instrument icon.

9. When prompted, verify that gaskets have been placed on all wells and on the column X posts on the far right as shown on the screen.

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

10. Carefully load the MAP plate in the plate nest in the plate tray.

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

11. Select **CLOSE DOOR**.

    The door closes and the MAP plate bar code is scanned and is displayed in the **Run name** dialog box.
12. When prompted, enter a Run name.

13. Click RUN.
   - The run status displays in the left sidebar.
   - While processing the run, the instrument lights slowly pulse blue.
   - When the run is complete, the instrument lights are a steady blue.
   - Data populates the ANALYSIS tab on the Runs page as it becomes available.

14. When the Run complete dialog displays, select the run name to view the final data in the Runs page.
    For more information on analyzing experiment results, see Chapter 3, “Analyze data”.

Note: If the instrument cannot scan the barcode, it can be manually added in the Plate Barcode field of Run name dialog box.
Set up the plate for analysis

1. In the QuantStudio™ Absolute Q™ Digital PCR Software, when the **Run complete** dialog displays, select the run name to view the final data from the **Runs** page.

2. From the **SETUP** page in the upper-right corner of the sample plate area, click ![EDIT SETUP] to enable editing.

3. Below the sample plate area, select **EDIT GROUPS** to open the groups dialog box. For information on managing groups, see the *QuantStudio™ Absolute Q™ Digital PCR System Installation, Use, and Maintenance Guide* (Pub No. MAN0025621).

4. Select **NEW GROUP** to create a new group for an assay or a set of conditions for a group of samples, based on your experiment setup.

5. In the **Group name** field, enter a name.

6. In the **Analysis** column, select **Signal** for the optical channels for reactions as indicated below:

<table>
<thead>
<tr>
<th>Option</th>
<th>Channel(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Singleplex</td>
<td>VIC™</td>
</tr>
<tr>
<td>Duplex</td>
<td>VIC™ FAM™</td>
</tr>
</tbody>
</table>

[1] We recommend you use FAM™ 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.

7. For all other channels in the **Analysis** column, select **Not Used**.

8. Select **SAVE** to save your changes and close the **EDIT GROUPS** dialog box.

9. Select **SAVE** in the upper-right corner of the sample plate area to save your changes and exit the editing mode.

10. On the **Runs** page, select the **ANALYSIS** tab and verify that the total number of analyzed microreaction chambers in the array is >20,000. Consider omitting or rerunning arrays with fewer than 20,000 analyzed microreaction chambers.
Post-analysis procedure

Adjust the threshold

When the run has completed, confirm that the automatic threshold is above the negative population. If the threshold is not automatically adjusted above the negative population, manually adjust the threshold by selecting and dragging the threshold bar to the desired position.

Figure 4 Observe automatic thresholds for an array (Singleplex)

Figure 5 Observe 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).

Figure 6 Observe group thresholds (Singleplex)
Figure 7  Observe group thresholds (Duplex)

1. In the left pane, click to access the Runs page.

2. Select the run, then select the ANALYSIS page.

3. Select one of the following options:
   - Select SAMPLE to set the threshold on an individual sample.
   - Select GROUP to set the thresholds for the samples contained within a group.

   **Note:** For samples that have been grouped, dragging a channel threshold changes the threshold value for all samples in the group to the same value.

4. To enable editing in the plot area, click .

5. Manually adjust the thresholds by performing the following actions:
   a. Hover over the threshold line until the threshold value appears.
   b. Click on the threshold value, then drag the threshold bar up or down to adjust the value.

      **Note:** Clicking on the threshold line results in a zoom action.

   c. Click SAVE.
Related documentation

<table>
<thead>
<tr>
<th>Document</th>
<th>Publication number</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>QuantStudio™ Absolute Q™ Digital PCR System Installation, Use, and Maintenance Guide</td>
<td>MAN0025621</td>
<td>Detailed instructions for using the QuantStudio™ Absolute Q™ Digital PCR System to prepare and run digital PCR experiments, and analyze results.</td>
</tr>
</tbody>
</table>

Note: For additional documentation, see “Customer and technical support” on page 18.

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.