applied biosystems USER GUIDE

Absolute Q™ 1-Step RT-dPCR Master Mix (4X)

Catalog Number A55146

Pub. No. MAN0026596 Rev. B.0

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

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 Applied Biosystems™ Absolute Q™ 1-Step RT-dPCR Master Mix (4X) is optimized for the amplification of RNA targets when used with the QuantStudio™ Absolute Q™ Digital PCR System.

This master mix performs reverse transcription (RT) of RNA to cDNA, followed by digital PCR (dPCR) amplification in a single, 1-step reaction. This master mix can be used for up to four target amplifications per dPCR reaction and is supplied at a 4X concentration.

The QuantStudio™ Absolute Q™ Digital PCR Instrument using software version 6.3 or later supports lab automation with the use of a robot. This document provides information for an implementation that is not using automation.

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

Contents and storage

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

Table 1 Absolute Q™ 1-Step RT-dPCR Master Mix (4X) (Cat. No. A55146)

Amount	Number of 9–µL reactions	Storage
450 µL	200	–25°C to –15°C Protect from light.

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

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

Item	Source
Other consumables	
QuantStudio™ Absolute Q™ MAP16 Plate Kit, includes the following components: • 12 QuantStudio™ Absolute Q™ MAP16 Digital PCR Plates • 60 QuantStudio™ Absolute Q™ MAP plate gasket strips • 3 mL QuantStudio™ Absolute Q™ Isolation Buffer	A52865
Low bind microcentrifuge tubes	MLS
Microcentrifuge tube rack	MLS
Nuclease-Free Water	MLS

Recommendations for RNA

For RNA isolation kits, go to **thermofisher.com/rnaisolation**.

The recommended concentration of RNA for the dPCR reactions is 1–4,000 copies/µL.

A lower or higher concentration of RNA can be used for the dPCR reactions. The use of a higher or lower concentration depends on the application. It is recommended to test a lower or higher concentration of RNA with the application.

Replicates are recommended for lower or higher concentrations of RNA.

RNA must be free of inhibitors of reverse transcription and PCR. RNA must be free of RNase activity.

Methods

Prepare the digital PCR reactions

IMPORTANT!
- Throughout this procedure, protect reagents from light when not in use.
- Store prepared reactions on ice or at 4°C.

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.

Thaw all reagents on ice or at 4°C.

1. Pulse vortex the Absolute Q™ 1-Step RT-dPCR Master Mix (4X) and the assay at high speed for 10 seconds.

2. Combine the following reagents in the order listed.

Table 2 dPCR reaction with a 20X assay

Reagent	Final concentration	Volume per reaction (with 10% overage)	Volume for 4 reactions (with 10% overage)
Absolute Q™ 1-Step RT-dPCR Master Mix (4X)	1X	2.5 µL	10.0 µL
Digital PCR assay (20X)[1]	1X	0.5 µL	2.0 µL
RNA sample	1–4,000 copies/µL	Variable	Variable
Nuclease-free water	—	Fill to 10 µL	Fill to 40 µL
Total reaction volume	**—**	**10 µL**	**40 µL**

[1] Adjust the volume if the assay is a custom assay at a different concentration. Adjust the volume of water to achieve the total reaction volume.

3. Mix the dPCR reagents well by performing one of the following actions:
   - Pipette mix 10–20 times without creating bubbles.
   - Pulse vortex 3–5 times for 1 second each.

4. Centrifuge for 1 minute with a benchtop centrifuge to collect the contents at the bottom of the tube.

5. Perform the run within 1 hour of reaction preparation.

   Store on ice or at 4°C if the run is not started immediately.

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.

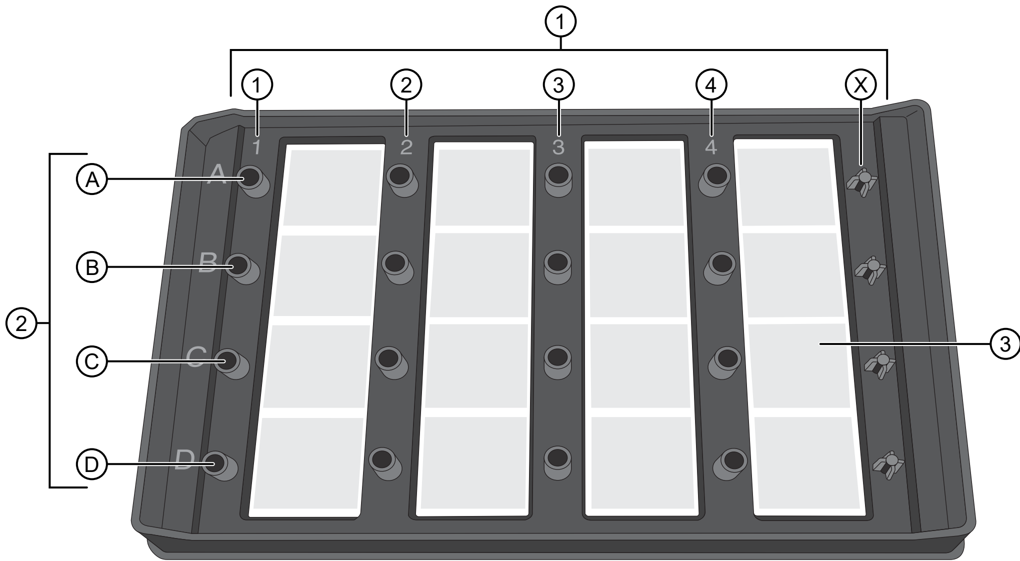


Figure 1 MAP plate without MAP plate gasket strips

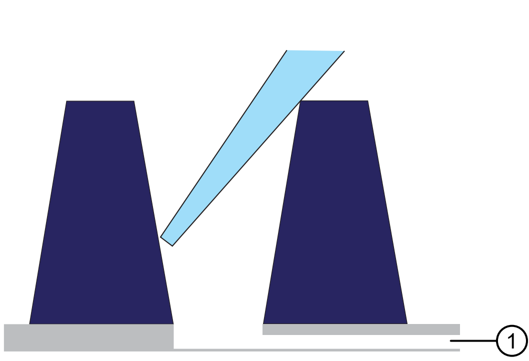
① Columns 1–4 and column X
② A–D represent wells A1–D1 associated with column 1
③ Array associated with well 4C

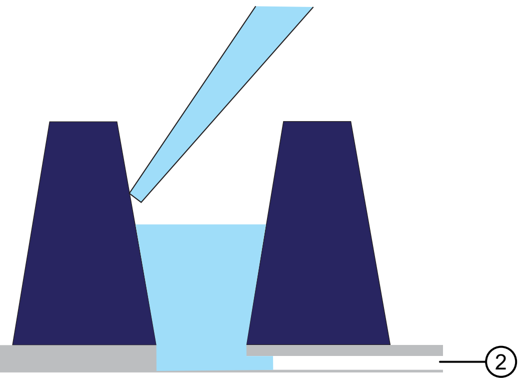
2. Place the MAP plate on a level, dust-free, dry surface.

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. Pipette 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 pipette 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.



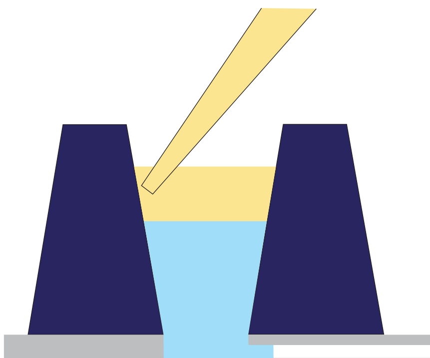


① Microfluidic channel to the microchamber array

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. Pipette 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

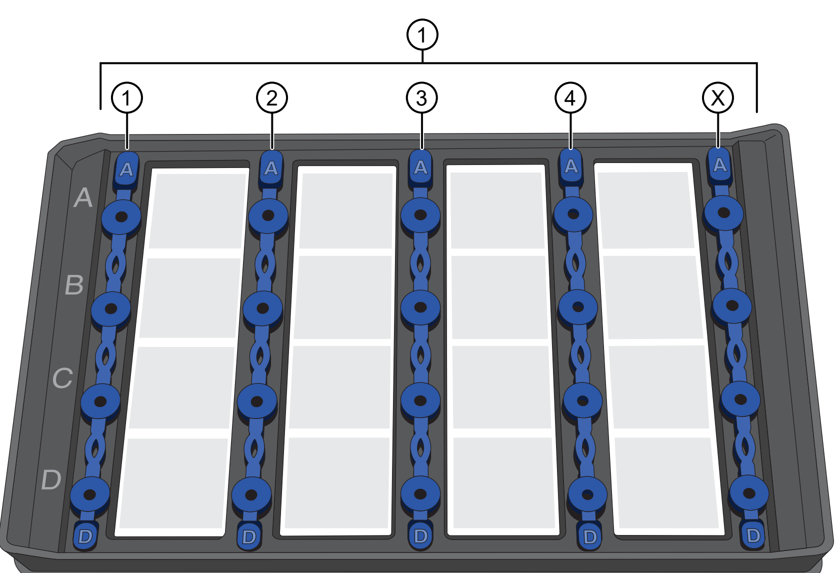


Figure 3 MAP plate with MAP plate gasket strips in place

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.

Set up the experiment

For specific instructions to set up the QuantStudio™ Absolute Q™ Digital PCR System, see the *QuantStudio™ Absolute Q™ Digital PCR System Installation, Use, and Maintenance Guide* (Pub. No. MAN0028562).

The plate nest must be cleaned before each run.

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 6.

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. Select **RNA-RT**, then set it to ***55°C*** for ***10 minutes***.
   b. Set the **Preheat** setting to ***96°C*** for ***10 minutes***.
   c. Set step one to ***96°C*** for ***5 seconds***.
   d. Change step two to ***60°C*** for ***10 seconds***.
   e. Set the **Cycles** to *40*.
   f. Ensure that **Preheat** is selected.
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.
   - **FAM**
   - **VIC**
   - **ABY**
   - **JUN**
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 6.

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 6.

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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 6.

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 7.

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. In the **Plate barcode** field, add the barcode number of the MAP plate.

4. 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.

5. 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**.

Data analysis

See the user documentation for the assay and the instrument for information about data analysis.

Limited product warranty

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. MAN0026596 B.0

Revision	Date	Description
B.0	12 October 2023	Updated to reflect changes needed for use with v6.3 or later of the QuantStudio™ Absolute Q™ Digital PCR Software
A.0	24 June 2022	New document for the Absolute Q™ 1-Step RT-dPCR Master Mix.

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

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