

QuantStudio™ Absolute Q™ Digital PCR Starter Kit

USER GUIDE

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

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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	<ul style="list-style-type: none">• 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 7).• Instructions were revised for setting up the experiment (“Set up the experiment” on page 12).• Instructions were revised for setting up the plate for analysis (“Set up the plate for analysis” on page 16).• Instructions were revised for post experiment analysis (“Post-analysis procedure” on page 18).
B.0	4 October 2021	<ul style="list-style-type: none">• Instructions were revised for preparing the dPCR reaction mix (“Prepare the dPCR reaction mix” on page 6).• Instructions were revised for setting up an experiment (“Set up the experiment” on page 12).
A.0	13 September 2021	New publication documenting the use of the Starter Kit assay with the QuantStudio™ Absolute Q™ Digital PCR System.

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

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Contents

■	CHAPTER 1	Product Information	4
		Product description	4
		Kit contents and storage	4
		Required materials not supplied	5
■	CHAPTER 2	Prepare an experiment	6
		Prepare the dPCR reaction mix	6
		Load the reaction mix into the MAP plate	7
■	CHAPTER 3	Run an experiment	12
		Set up the experiment	12
		Create a run	14
		Run the experiment	14
■	CHAPTER 4	Analyze data	16
		Set up the plate for analysis	16
		Post-analysis procedure	18
		Adjust the threshold on sample groups	19
		Adjust the threshold on samples	20
■	APPENDIX A	Documentation and support	22
		Related documentation	22
		Customer and technical support	22
		Limited product warranty	22



Product Information

- Product description 4
- Kit contents and storage 4
- Required materials not supplied 5

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](https://www.thermofisher.com/support).

Product description

The QuantStudio™ Absolute Q™ Digital PCR Starter Kit is designed to ensure the performance of the QuantStudio™ Absolute Q™ Digital PCR System, either at the time of system installation or as part of future, customer-defined system verification procedures. The kit can also be used for training on the QuantStudio™ Absolute Q™ Digital PCR System workflow.

The kit contains the Absolute Q™ DNA Digital PCR Master Mix (5X), Absolute Q™ Starter Assay, and CEPH DNA control, providing a complete set of starter reagents and assay for use with the QuantStudio™ Absolute Q™ MAP16 Plate Kit.

Kit contents and storage

Table 1 QuantStudio™ Absolute Q™ Digital PCR Starter Kit (Cat. No. [A52732](#))

Component	Unit part number	Quantity	Storage
Absolute Q™ Starter Assay (20X)	A52856	1 tube (200 reactions)	-25°C to -15°C
CEPH DNA control (50 ng/μl)	4458703	1 tube	
Absolute Q™ DNA Digital PCR Master Mix (5X)	A52490	200 reactions	

Required materials not supplied

Unless otherwise indicated, all materials are available through [thermofisher.com](https://www.thermofisher.com). "MLS" indicates that the material is available from [fisherscientific.com](https://www.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 includes: <ul style="list-style-type: none"> • 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

2

Prepare an experiment

- Prepare the dPCR reaction mix 6
- Load the reaction mix into the MAP plate 7

This section provides a procedure for using the Absolute Q™ Starter Assay to prepare a dPCR reaction mix.

For detailed instructions on 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).

Prepare the dPCR reaction mix

Gather the following materials:

- P10 or P20 pipette and filter pipette tips
- Absolute Q™ DNA Digital PCR Master Mix (5X)
- Absolute Q™ Starter Assay (20X)
- CEPH gDNA control
- Nuclease-free water

The volume of the dPCR reaction can be adjusted depending on the requirements of the experiment. 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. 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.

-
2. Pulse vortex the Absolute Q™ DNA Digital PCR Master Mix (5X) and the Absolute Q™ Starter Assay (20X) at high speed for 10 seconds.
 3. Dilute the stock 50 ng/μL CEPH DNA control sample to 10 ng/μL using nuclease-free water. For example, add 4 μL of stock CEPH DNA control to 16 μL of nuclease-free water.

4. Combine the following reagents in the order listed.

Reagent	Final concentration	Volume per reaction (with 10% overage)	Volume for 4 reactions (with 10% overage)
Absolute Q™ DNA Digital PCR Master Mix (5X)	1X	2 µL	8 µL
Absolute Q™ Starter Assay (20X)	1X	0.5 µL	2 µL
Diluted CEPH DNA control (10 ng/µl)	1.1 ng/ µL ^[1]	1.1 µL	4.4 µL
Nuclease-free water	—	6.4 µL	Fill to 40 µL
Total reaction volume	—	10 µL	40 µL

^[1] 1.1 ng/ µL is approximately 333 copies/µL

5. Mix the dPCR reagents 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, centrifuge at 10,000 × g for 1 minute.
7. Perform the run within 1 hour of reaction preparation.

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.

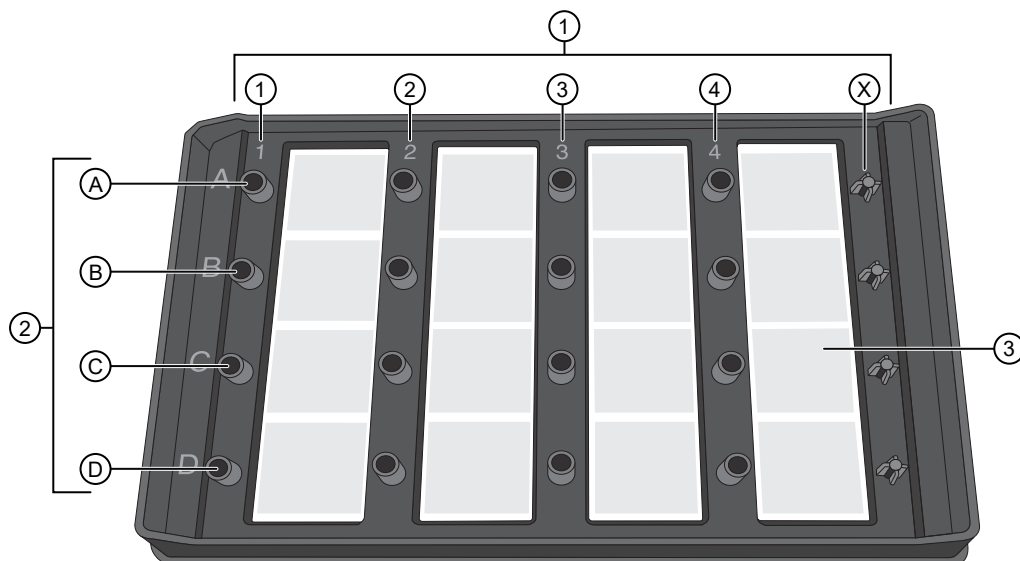


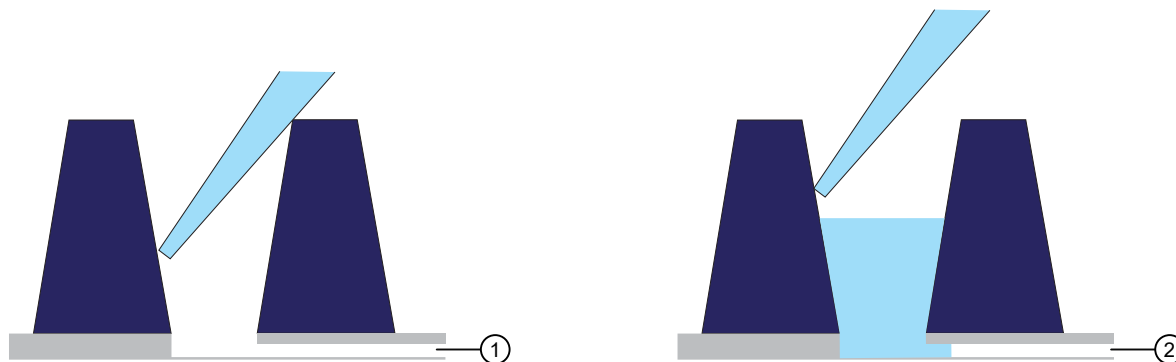
Figure 1 MAP plate without MAP plate gasket strips

- 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.

- 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.

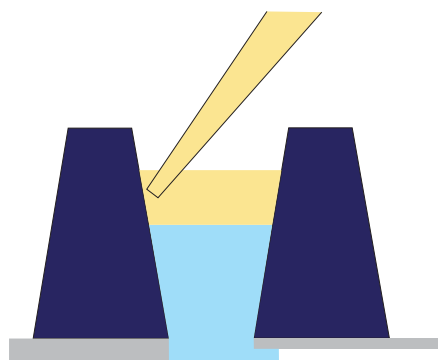
IMPORTANT! Do not contact bottom of well with the pipette tip or puncture the thin film at the bottom of the well.



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

- 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.



- 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.

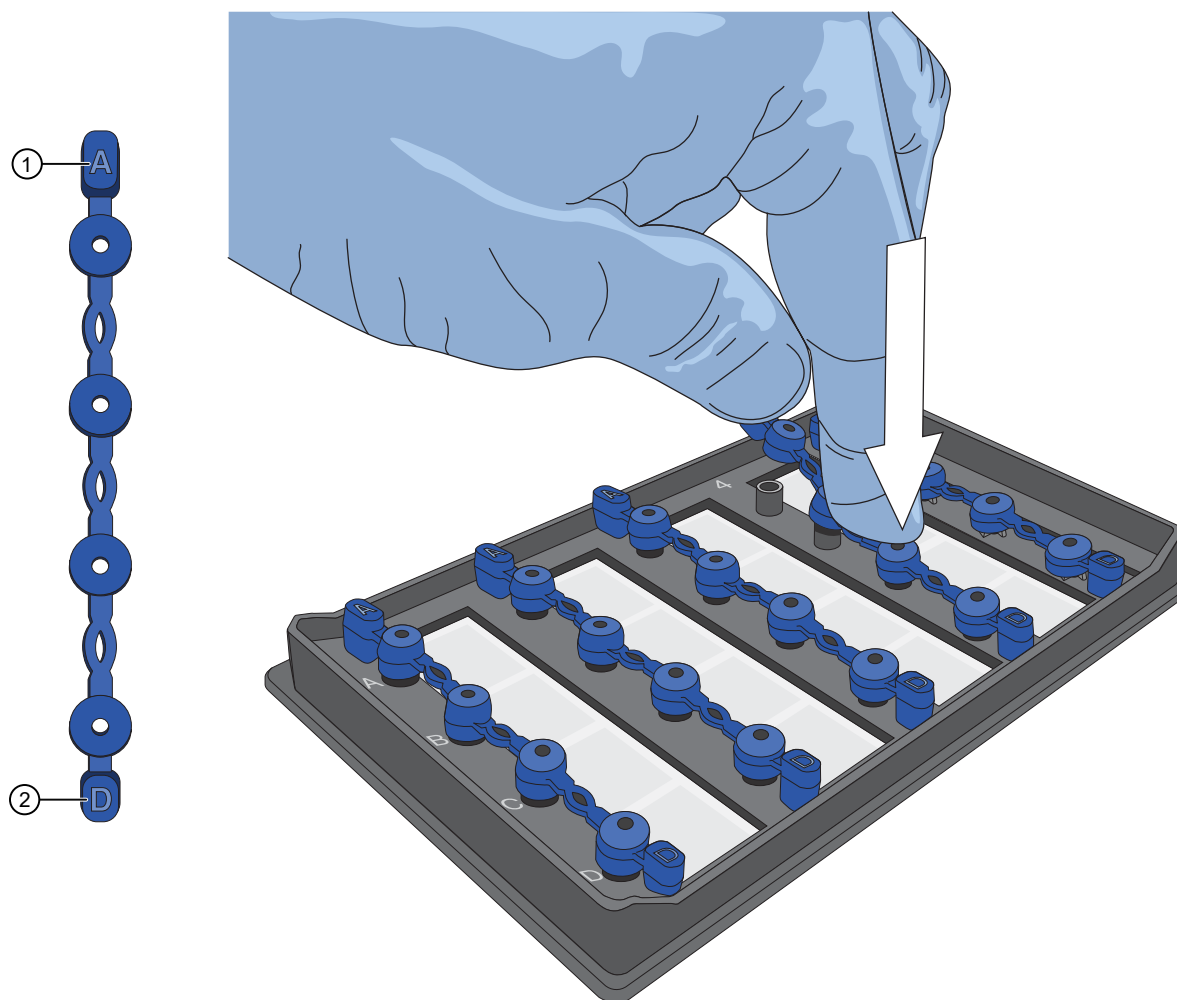


Figure 2 Place the MAP plate gasket strips firmly into place

- 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

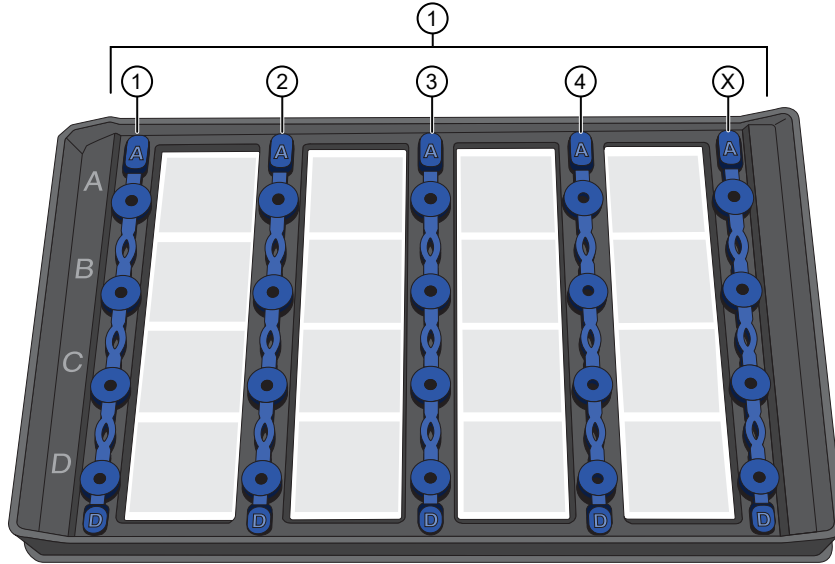


Figure 3 MAP plate with MAP plate gasket strips in place

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.



Run an experiment

- Set up the experiment 12
- Create a run 14
- Run the experiment 14


This section provides information about running an experiment using the Absolute Q™ Starter Assay on the QuantStudio™ Absolute Q™ Digital PCR Instrument in a non-automated implementation.

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.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 14.

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.

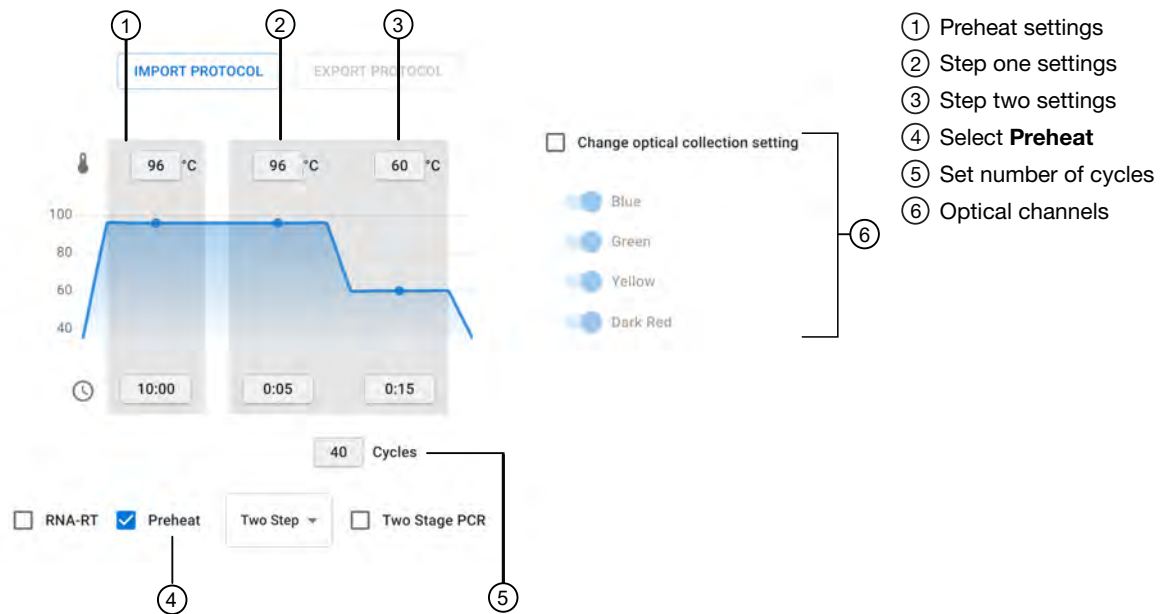



Figure 4 Thermal protocol



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 following optical channels to the on position.
 - **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 14.

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 12.

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


Option	Actions
Create a run from the Templates page.	<ol style="list-style-type: none"> 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.	<ol style="list-style-type: none"> 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. <ul style="list-style-type: none"> • 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 12.
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 14.

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

- Set up the plate for analysis 16
- Post-analysis procedure 18

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. Enter the following parameters for the optical channels.

Dye (Optical Channel)	Target DNA	Analysis	Copies
FAM™ dye	Target 1	CNV	—
VIC™ dye	Target 2	CNV	—
ABY™ dye	Target 3	CNV Ref	2
JUN™ dye	Target 4	CNV	—

Note: ROX™ dye is set to QC by default.

5. In the **Samples** area, select **Replicates**.
6. Select **CONTINUE** to return to the **SETUP** tab, then click **SAVE**.

7. On the **Runs** page, select the **RESULTS** tab and ensure that each of the targets performs within the following expectations.
 - Total analyzed microchambers should be >20,000.
Consider omitting or rerunning the sample if the total analyzed micro-chambers are <20,000.
 - FAM™ and VIC™ copy numbers should be 2 (±0.3).
 - JUN™ copy numbers should be 4 (±0.5).

Sample/Group	Name	Well	DF	QC	Total	Target	Conc. cp/uL	SD	CV%	95%CI	Precision %	Lambda(cp/Rxn)	Lambda_95%CI	Positives	Copy #	CNV_95
Group 1	Average				20141.75	Target 1	322.34	187.05	58.029	3,157,0.000				2497	1.95	0.10,0
Sample A1	A1	1/			20431	Target 1	264.23			10.810				2204	2.05	0.12,0
Sample A2	A2	1/			20447	Target 1	273.55			11.010				2279	2.07	0.12,0
Sample A3	A3	1/			20454	Target 1	274.73			11.034				2289	2.01	0.11,0
Sample A4	A4	1/			20432	Target 1	290.66			11.381				2411	2.11	0.12,0
Sample B1	B1	1/			20348	Target 1	252.02			10.559				2099	1.95	0.11,0
Sample B2	B2	1/			20406	Target 1	275.54			11.064				2290	2.13	0.12,0
Sample B3	B3	1/			20461	Target 1	280.11			11.148				2332	2.04	0.11,0
Sample B4	B4	1/			20427	Target 1	271.54			10.971				2261	2.01	0.11,0
Sample C1	C1	1/			15744	Target 1	1046.01			26.976				5724	1.67	0.06,0

Figure 5 Target performance

Post-analysis procedure

When the run has completed, observe automatic thresholds are 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 20.

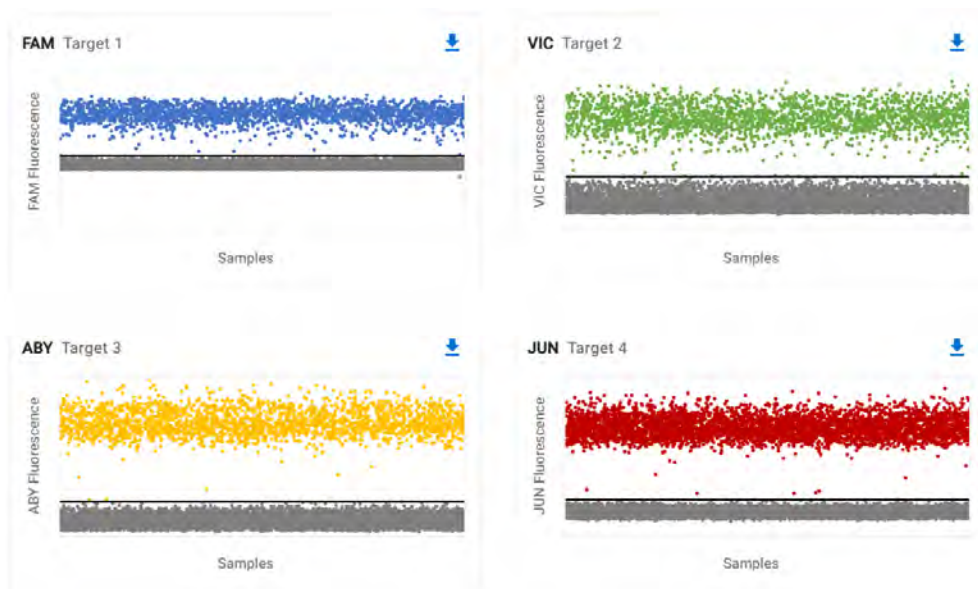


Figure 6 Automatic thresholds

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).

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

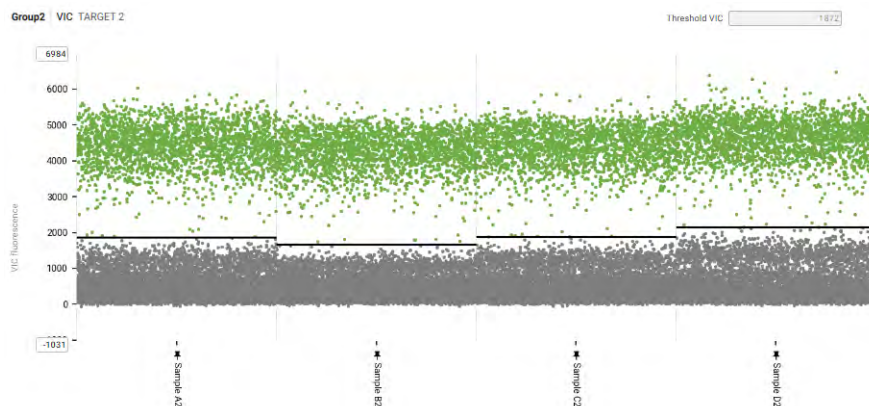




Figure 7 Group thresholds



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.	<ol style="list-style-type: none"> 1. Click AUTO GROUP. 2. Click SAVE.
AUTO SAMPLE —The software sets the threshold for each sample in the channel group.	<ol style="list-style-type: none"> 1. Click AUTO SAMPLE. 2. Click SAVE.
MANUAL —Manually set the threshold for the channel group.	<ol style="list-style-type: none"> 1. Click MANUAL. 2. Use one of the following options to set the threshold. <ul style="list-style-type: none"> • 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 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.	<ol style="list-style-type: none"> 1. <i>(Optional)</i> In the View sample dropdown, set the sample view to either Ungrouped or Omitted. 2. <i>(Optional)</i> 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.	<ol style="list-style-type: none"> 1. Click Sample. The sample opens on the PLOTS page in the gallery view. 2. <i>(Optional)</i> In the View sample dropdown, set the sample view to either Ungrouped or Omitted. 3. <i>(Optional)</i> 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. <i>(Optional)</i> 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
<p>FROM GROUP—the software sets the threshold for this sample to the group threshold to which the sample is assigned.</p>	<ol style="list-style-type: none"> 1. Click FROM GROUP. 2. Click SAVE.
<p> 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.</p>	<ol style="list-style-type: none"> 1. Click AUTO. 2. Click SAVE.
<p> 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.</p>	<ol style="list-style-type: none"> 1. Click MANUAL. 2. Use one of the following options to set the threshold. <ul style="list-style-type: none"> • 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.



Documentation and support

Related documentation

Document	Publication number	Description
<i>QuantStudio™ Absolute Q™ Digital PCR System Installation, Use, and Maintenance Guide</i>	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](https://www.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.

