applied biosystems

Absolute Q™ Liquid Biopsy dPCR Assays

USER GUIDE

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

Publication Number MAN0025690

Revision C.0

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

ThermoFisher SCIENTIFIC

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

Revision history: MAN0025690 C.0 (English)

Revision	Date	Description
C.0	1 November 2023	Updated to reflect changes needed for use with v6.3 or later of the QuantStudio™ Absolute Q™ Digital PCR Software.
B.0	27 July 2022	• Information was added regarding assay ordering and specifications ("Assay ordering and specifications" on page 4). • 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 the experiment ("Set up the experiment" on page 12). • Instructions were revised for setting up the plate for analysis ("Post-analysis procedure" on page 18).
A.0	13 September 2021	New publication documenting the use of the Absolute Q™ Liquid Biopsy 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™ Liquid Biopsy dPCR Assays enable the detection of somatic mutations with high sensitivity and specificity. For liquid biopsy analysis, dPCR assays are a precise cost-effective and rapid method for cancer research, including studying the potential of response monitoring and resistance to potential treatment. These assays are formulated to work seamlessly with the QuantStudio™ Absolute Q™ Digital PCR System.

Assay ordering and specifications

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

Absolute Q™ Liquid Biopsy dPCR Assay specifications:

- 1 tube at 20X concentration
- 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, bench top	MLS
Vortex mixer	MLS
Pipettes, P20 or P200	MLS
Filter pipette tips, P20 or P200	MLS
NanoDrop™ 2000/2000c Spectrophotometer *or* Qubit™ 4 Fluorometer	ND-2000 Q33238
Other consumables	
QuantStudio™ Absolute Q™ MAP16 Plate Kit includes: • 12 QuantStudio™ Absolute Q™ MAP16 Digital PCR Plates • 60 QuantStudio™ Absolute Q™ MAP plate gasket strips • 3 mL of QuantStudio™ Absolute Q™ Isolation Buffer	A52865
Absolute Q™ DNA Digital PCR Master Mix (5X)	A52490
Control DNA (from CEPH Individual 1347-02) (CEPH gDNA control) (used as a wild-type control)	403062
Low bind microcentrifuge tubes	MLS
Microcentrifuge tube rack	MLS
Nuclease-free water	MLS

2 Prepare an experiment

This section provides a procedure for preparing a dPCR reaction mix with an Absolute Q™ Liquid Biopsy dPCR Assay.

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).

Mutant alleles versus wild-type alleles

For liquid biopsy dPCR assays, copies of the mutant allele are positive in the FAM™ channel, while copies of wild-type alleles are positive in the VIC™ channel. For details on using a wild-type control to calculate the mutant allele frequency percentage of unknown samples, see "Normalize the background and calculate the mutant allele frequency percentage (%MAF)" on page 19.

Note: For each run, we recommend 2–3 replicates of each unknown sample plus 1 wild-type control (CEPH gDNA control; see "Required materials not supplied" on page 5).

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™ Liquid Biopsy dPCR Assay (40X)
- CEPH gDNA control, if needed for your assay
- Nuclease-free water

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

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. Isolate and prepare DNA from each liquid biopsy sample of interest. Prepare 500–2,000 copies of purified sample DNA in a volume of ≤7.75 µL per reaction.

   Note:
   - Do not freeze and reuse working DNA solution.
   - We recommend using the NanoDrop™ 2000/2000c Spectrophotometer or the Qubit™ 4 Fluorometer to determine quantity.
   - A DNA copy and dilution calculator can be found at **http://www.thermofisher.com/DNA-calculator**.

2. Prepare the CEPH gDNA control as the wild-type control. Dilute the CEPH gDNA control to the same concentration as your unknown sample.

3. 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.

4. Pulse vortex the Absolute Q™ DNA Digital PCR Master Mix (5X) and Absolute Q™ Liquid Biopsy dPCR Assay at high speed for 10 seconds.

5. Combine the following reagents in the order listed.

Reagent	Final concentration	Volume per reaction (with 10% overage)[1]
Absolute Q™ DNA Digital PCR Master Mix (5X)	1X	2 µL
Absolute Q™ Liquid Biopsy dPCR Assay (40X)	1X	0.25 µL
Liquid biopsy DNA sample (unknown sample) or CEPH gDNA control (wild-type control)	500–2,000 copies of unknown sample or same concentration of CEPH gDNA control	Variable, ≤7.75 µL
Nuclease-free water	—	Fill to 10 µL
Total reaction volume	**—**	**10 µL**

[1] After calculating the number of reactions required, prepare dPCR mix for the appropriate number of reactions and scale those components by 10% for overage. Dilute assay accordingly to avoid pipetting less than 1 µL volumes.

6. Mix well by performing one of the following actions:
   - Pipette mix 10–20 times, or
   - Pulse vortex 3–5 times for 1 second each.

7. Using a benchtop centrifuge, 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.

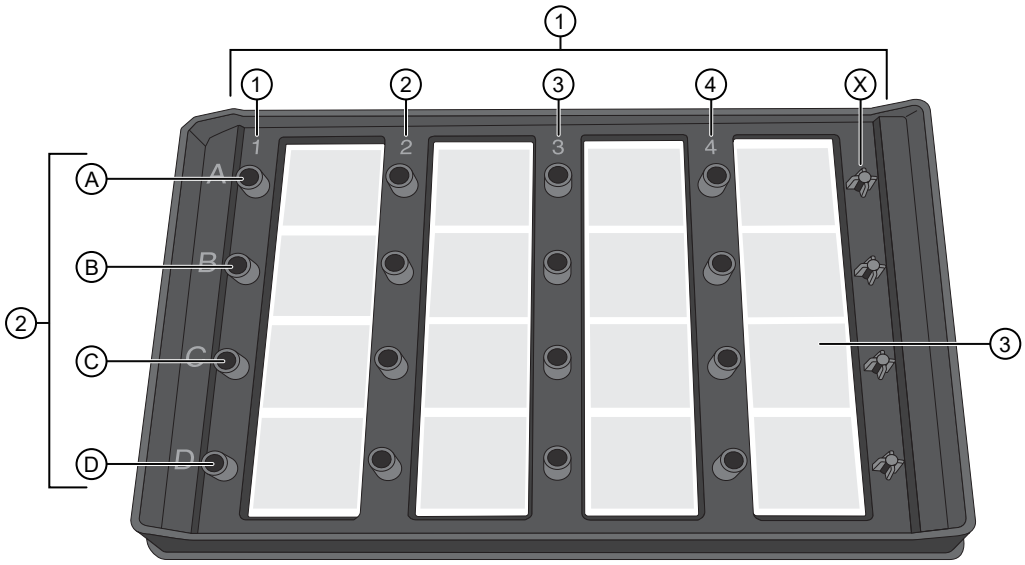


Figure 1 MAP plate without MAP plate gasket strips

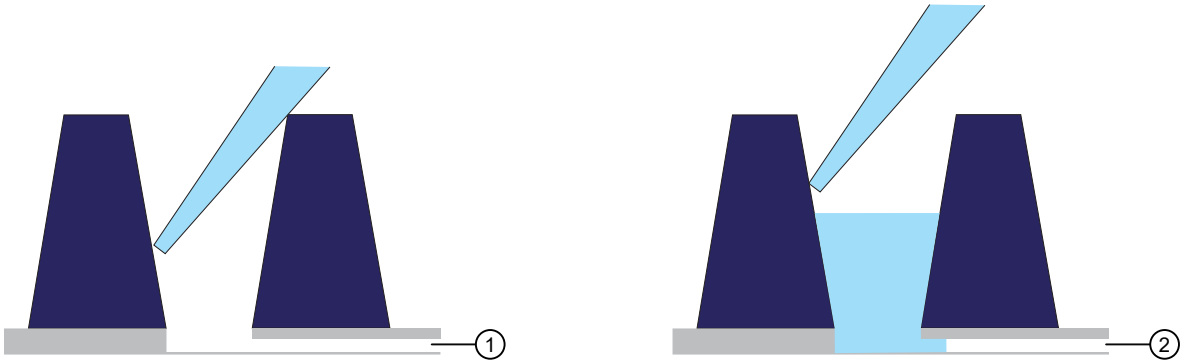
① Columns 1–4 and column X
② A–D represent wells A1–D1 associated with column 1
③ Array associated with well 4C

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.

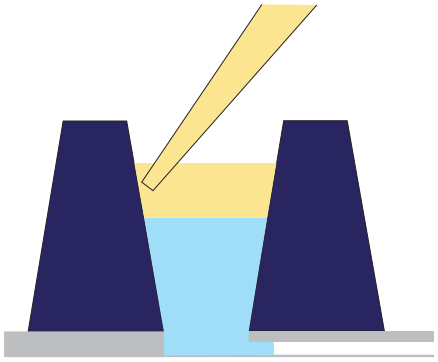
IMPORTANT! Do not contact bottom of well with the pipette tip or puncture the thin film at the bottom of the well.



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.

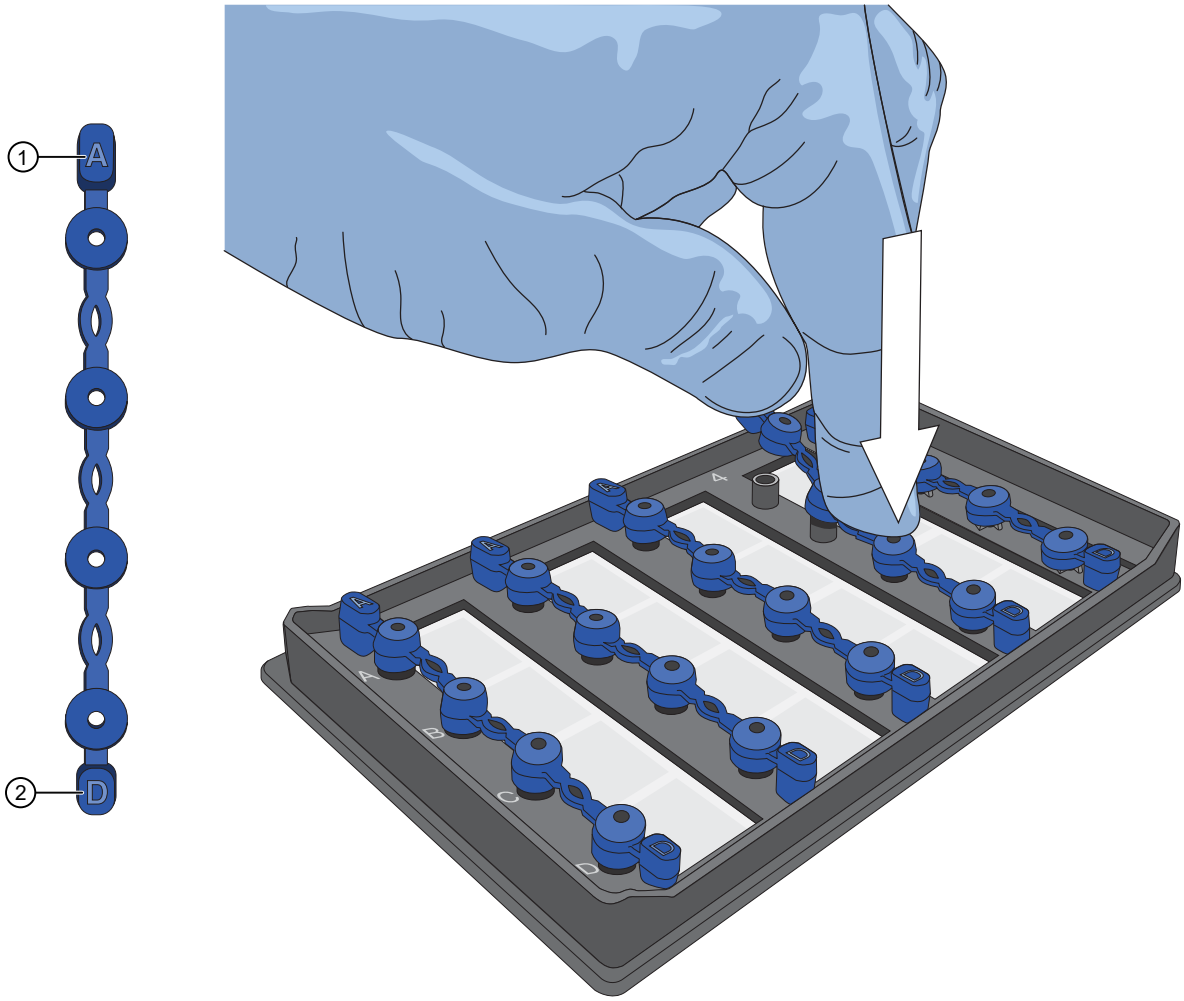


Figure 2 Place the MAP plate gasket strips firmly into place

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

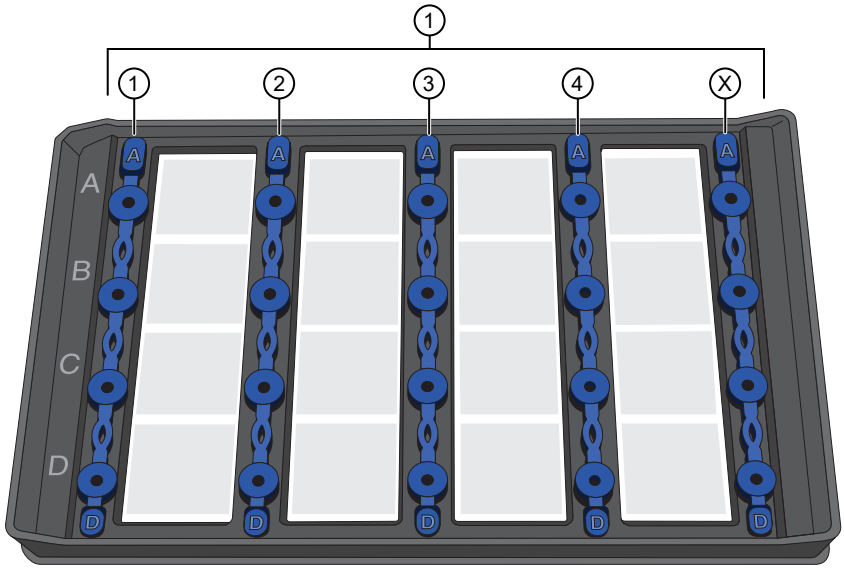


Figure 3 MAP plate with MAP plate gasket strips in place

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 14.

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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.

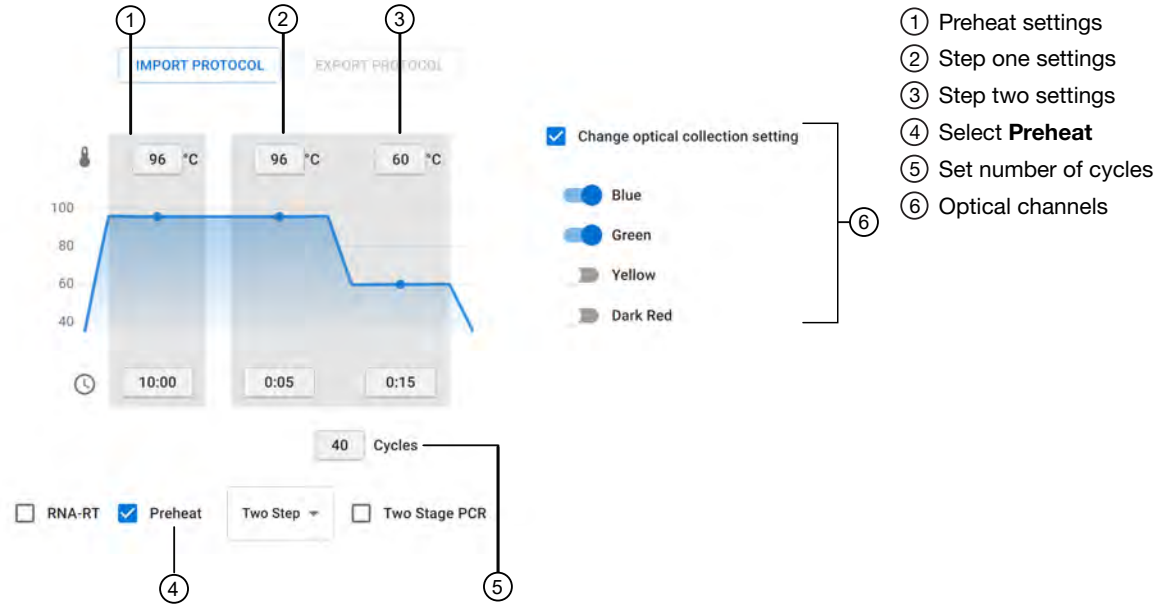

Figure 4 Thermal protocol

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 following optical channels to the on position. Toggle the remaining optical channels to the off position:
   - **FAM**
   - **VIC**
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.	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 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

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 following channels.
   - **FAM**
   - **VIC**
5. For all other channels in the **Analysis** column, set the toggle to the off position to exclude them from analysis.
6. Select the relevant sample grouping option for your experiment.
   - **Individual**—Each sample has a separate result entry.
   - **Replicates**—The results show the Mean, Standard Deviation, and the CV% of the concentration for all the samples in the group.
   - **Pooling**—The results treat all the samples in the group as one large sample.

   In this example, a group is created for the KRAS 554 assay.

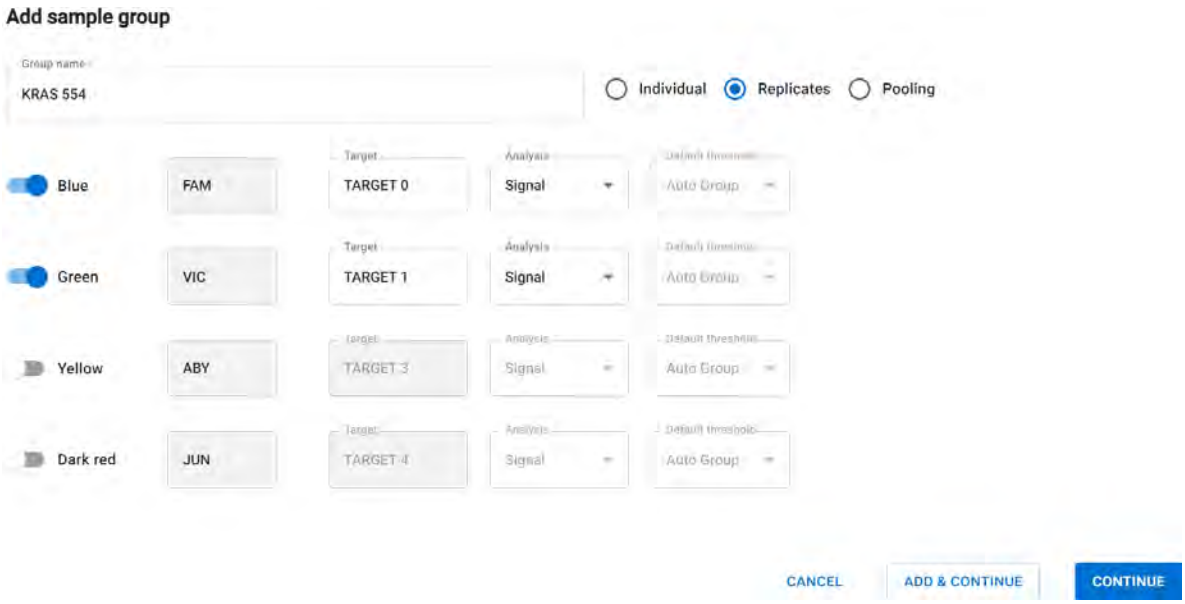

Figure 5 GROUP settings for the new group

7. Select **CONTINUE** to return to the **SETUP** tab, then click **SAVE**.

8. On the **SETUP** tab, in the sample plate select samples to be included for analysis.

9. In the **Sample group** dropdown, select the new group name to assign the selected samples to the group.

   In this example, samples in the first column of the plate are assigned to the KRAS 554 assay group.

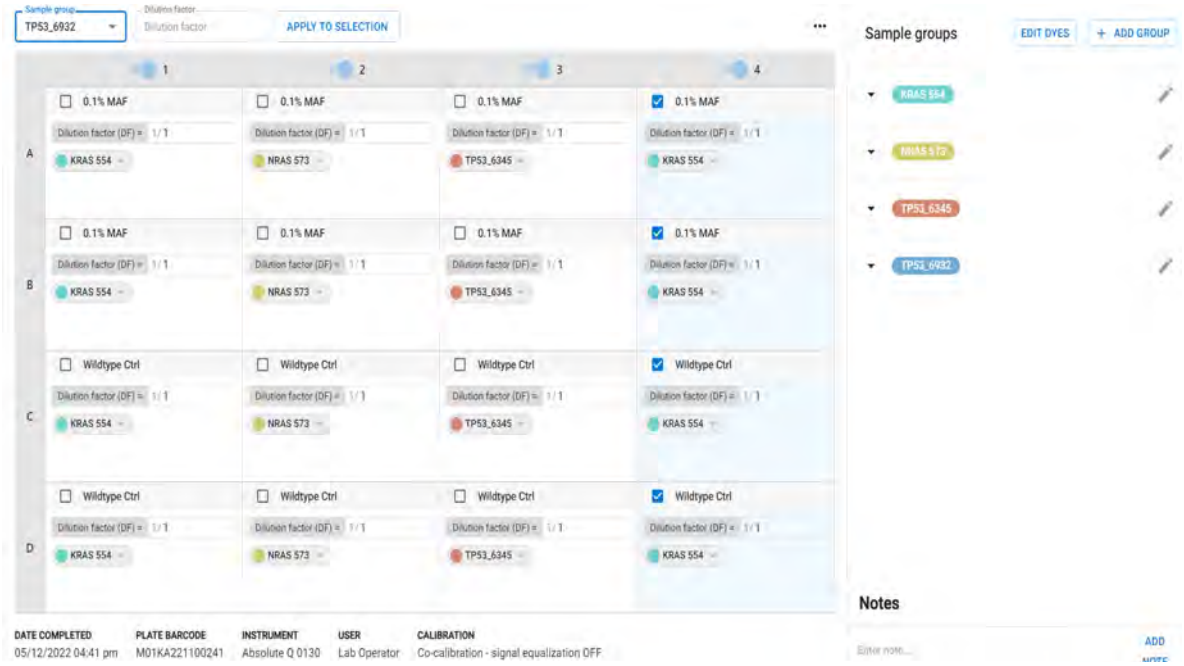

Figure 6 Assign samples to the group

10. To add information that identifies the sample, click the sample name field on the sample well, then enter a description in the label field.

    In this example, the sample in column 1, row A is selected for edit. Samples A and B are labelled as **0.1% MAF**, while samples C and D are labelled as **Wildtype Control** for the KRAS 554 assay.

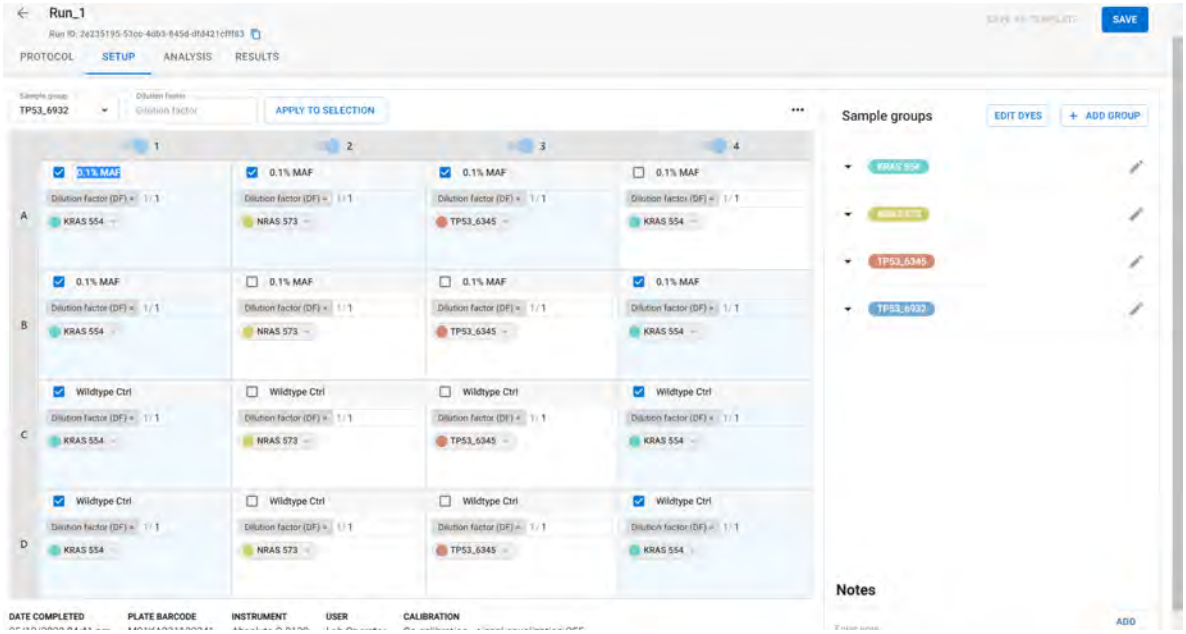

Figure 7 Label samples

11. Click away from the sample name field to save the sample name.

12. Click **SAVE** to save your changes.

Post-analysis procedure

Adjust the threshold

When the run has completed, it is critical to inspect the wild-type control that was run with the sample.

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. Select to view the 2D scatter plot of each sample in the group.

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. Manually adjust thresholds to gate the wild-type population in the VIC™ channel and the mutant population in the FAM™ channel.
   a. Click **MANUAL**.
   b. 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.
   c. Click **SAVE**.

   In the example below, the wild-type population (purple) is observed in the VIC™ channel, and the mutant population (orange and green) is observed in the FAM™ channel.

Figure 8 Adjust thresholds on wild-type control samples

Normalize the background and calculate the mutant allele frequency percentage (%MAF)

To detect a target mutant allele at low abundance against wild-type background, use the wild-type control (CEPH gDNA control) to establish the background signal in the FAM™ channel, as described in the following procedure.

1. From the concentration table on the **RESULTS** tab in the QuantStudio™ Absolute Q™ Digital PCR Software, record the concentrations of the unknown sample (units = cp/µL) in the FAM™ and VIC™ channels, and the concentration of the wild-type control in the FAM™ channel.

2. Correct the FAM™ concentration (units = cp/µL) of the unknown sample using the following equation:

$$FAM_{corrected} = FAM_{unknown} - FAM_{wildtype} \qquad (Eq. 1)$$

where $FAM_{corrected}$ is the background-corrected FAM™ concentration, $FAM_{unknown}$ is the FAM™ concentration of the unknown sample, and $FAM_{wildtype}$ is the FAM™ concentration of the wild-type control sample. $FAM_{corrected}$ corresponds to the mutant template concentration.

3. Calculate the percentage mutant concentration of the unknown sample using the following equation:

$$\%MAF = \frac{FAM_{corrected}}{VIC_{unknown} + FAM_{corrected}} \times 100\% \qquad (Eq. 2)$$

where *%MAF* is the mutant allele frequency and $VIC_{unknown}$ is the VIC™ concentration of the unknown sample.

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 21.

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**.