QuantStudio™ Absolute Q™ Digital PCR System

INSTALLATION, USE, AND MAINTENANCE

for use with:
QuantStudio™ Absolute Q™ Digital PCR Software v6.3 or later
Security, Auditing, and E-signature (SAE) v2.2 software or later

Catalog Number A52864
Publication Number MAN0028562
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: MAN0028562 C.0 (English)

Revision	Date	Description
C.0	6 November 2023	Catalog numbers for the QuantStudio™ Absolute Q™ AutoRun Digital PCR Suite were removed.
B.0	26 September 2023	• Content about the QuantStudio™ Absolute Q™ AutoRun Digital PCR Suite was removed. • The instructions to install the desktop software were updated.
A.0	30 June 2023	New document for use of the QuantStudio™ Absolute Q™ Digital PCR System and QuantStudio™ Absolute Q™ AutoRun Digital PCR Suite with v6.3 software.

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

Contents

1 Product information

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

Product description

The Applied Biosystems™ QuantStudio™ Absolute Q™ Digital PCR System enables precision quantification of target nucleic acid sequences. Using patented microfluidic array technology, QuantStudio™ Absolute Q™ MAP16 Digital PCR Plates (MAP plates) are loaded with digital PCR (dPCR) reagents and then processed by the QuantStudio™ Absolute Q™ Digital PCR Instrument. Depending on the dPCR assay and protocol, results can be provided in less than 90 minutes. The resulting data are visualized with the QuantStudio™ Absolute Q™ Digital PCR Software.

The Applied Biosystems™ QuantStudio™ Absolute Q™ Digital PCR Instrument, using software version 6.3 or later, supports lab automation with the use of a robot and provides a scalable, automation-ready workflow with tools for multi-plate analysis on co-calibrated instruments.

Instruments, software, accessories, and consumables

The following table describes the products that are required for installation and operation of the system.

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

Item	Cat. No.	Amount
Instrument system		
QuantStudio™ Absolute Q™ Digital PCR System • QuantStudio™ Absolute Q™ Digital PCR Instrument • Dell™ Tower computer with monitor, keyboard, and mouse • QuantStudio™ Absolute Q™ Digital PCR Software v6.3 or later	A52864	1 instrument, 1 desktop computer, 1 monitor, and accessories
Instrument accessories		
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 QuantStudio™ Absolute Q™ Isolation Buffer	A52865	1

(continued)

Item	Cat. No.	Amount
Instrument system		
QuantStudio Absolute Q MAP16 Plate Kit and Master Mix • 12 QuantStudio™ Absolute Q™ MAP16 Digital PCR Plates • 60 QuantStudio™ Absolute Q™ MAP plate gasket strips • 3 mL QuantStudio™ Absolute Q™ Isolation Buffer • 5 QuantStudio Absolute Q DNA Digital PCR Master Mix	A53301	1
QuantStudio™ Absolute Q™ Digital PCR Starter Kit[1]	A52732	1
Reagents		
Absolute Q™ DNA Digital PCR Master Mix (5X)	A52490	200 reactions
QuantStudio™ Absolute Q™ Isolation Buffer	A52730	(1) 3 mL bottle
Absolute Q™ 1-step RT Digital PCR Master Mix (4X)	A55146	200 reactions

[1] The kit is required for system installation. See *QuantStudio™ Absolute Q™ Digital PCR Starter Kit User Guide* (Pub No. MAN0025653).

Digital PCR Assays

Predesigned and custom dPCR assays are available for use in dPCR experiments. For more information contact your local sales representative or go to **http://www.thermofisher.com/dpcr-assays.html**.

For information on the use of predesigned dPCR assays, see the documentation provided with the assay available at **http://www.thermofisher.com/dpcr-assays.html**.

Use this guide to perform experiments with dPCR assays ordered from Thermo Fisher Scientific or with your unique custom assay protocols.

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.

Item	Source
Equipment	
Centrifuge, table top	**MLS**
Pipettes, P10, P20, and P200	**MLS**
Filter pipette tips, P10, P20, and P200	**MLS**

(continued)

Item	Source
Other consumables	
Low bind microcentrifuge tubes	**MLS**
Microcentrifuge tube rack	**MLS**
Nuclease-Free Water	**MLS**
Non-abrasive, lint-free wipe	**MLS**
70% ethanol in water	**MLS**

Recommended materials not supplied

Note: For information on the recommended kits for nucleic acid isolation, see **http://thermofisher.com/magmax**.

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.

Item	Source
Spectrophotometer	**MLS**
Qubit™ Flex Fluorometer	Q33327
KingFisher™ Apex with 24 Combi Head	5400940
KingFisher™ Apex with 96 Deep-Well Head	5400930

Software description

The QuantStudio™ Absolute Q™ Digital PCR System uses the following software.

- QuantStudio™ Absolute Q™ Digital PCR Software v6.3—Controls the instrument, performs user-defined experiments, analyzes data generated by the experiment. Parameters such as plate format, optical channels, and thermal conditions for an experiment can be modified as needed prior to the start of data generation. The software lets you to perform the following tasks.
  - Define the experiment, including sample types, sample groups, replicates, pool sample, dilutions, threshold parameters, experiment notes, and names
  - Create and edit protocols
  - Run and monitor protocols
  - View system status
  - View data in plot and tables
  - Analyze multiple runs simultaneously using studies

  - Generate run reports
  - Export data and reports
  - Insert and remove MAP plates
  - Install the shipping lock screw for transport of the instrument
  - Download instrument logs for system troubleshooting
- Security, Auditing, and E-signature (SAE) v2.2 (Optional)—Controls security and user access to the software and specific features. See Appendix E, “Use the software with Security, Auditing, and E-signature (SAE) v2.2”.

The software is installed during system installation. See “Download and install the desktop software” on page 129.

Hardware description

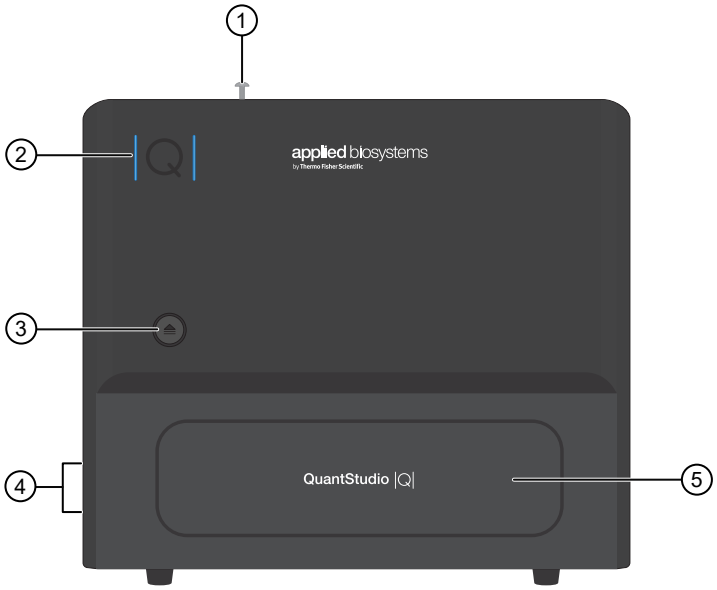
Overview of the Absolute Q™ instrument

The instrument is an integrated processing system compatible with QuantStudio™ Absolute Q™ MAP16 Digital PCR Plates.

A dedicated computer provided with the instrument uses the QuantStudio™ Absolute Q™ Digital PCR Software to operate the instrument and analyze data.

For information on installing the instrument, see Appendix D, “Install, update, and move the QuantStudio™ Absolute Q™ Digital PCR System”.

For information on maintaining the instrument, see Appendix F, “Maintain the instrument”.



1. Shipping lock screw
2. Status indicator light
3. Plate presentation tray open/close button
4. Power switch, power port, USB port
5. Plate presentation tray

The instrument has the following features and functions.

- The plate presentation tray is controlled using a button on the front panel or from within the software. Once a MAP plate is loaded into the tray, it is retracted into the instrument for automated processing.
- An internal barcode scanner verifies the barcodes on the MAP plates.
- An internal compressor and pneumatic subsystem drives the microfluidic array compartmentalization directly within the MAP plate using positive pressure.
- Liquid never contacts any parts in the instrument, so minimal maintenance and cleaning are required.
- The plate nest is thermally controlled to perform PCR thermal cycling.
- The fluorescent optical system is mounted above the MAP plate and scans the MAP plate in up to 5 optical channels before and after PCR.
- Each optical channel is associated with a color and a supported dye. See “QuantStudio™ Absolute Q™ Digital PCR Instrument Optical Dyes” on page 15.
- A computer integrated into the instrument manages critical runtime activities and stores recent data that have not yet been analyzed.
- During an experiment run, positive pressure is applied to drive and separate the reagent mix into pico-scale microchambers on the MAP plate before starting PCR. PCR occurs in parallel across the entire MAP plate. Each microchamber contains a discrete reaction.
- The microchamber arrays are scanned for fluorescence before and after PCR and are used for data analysis.

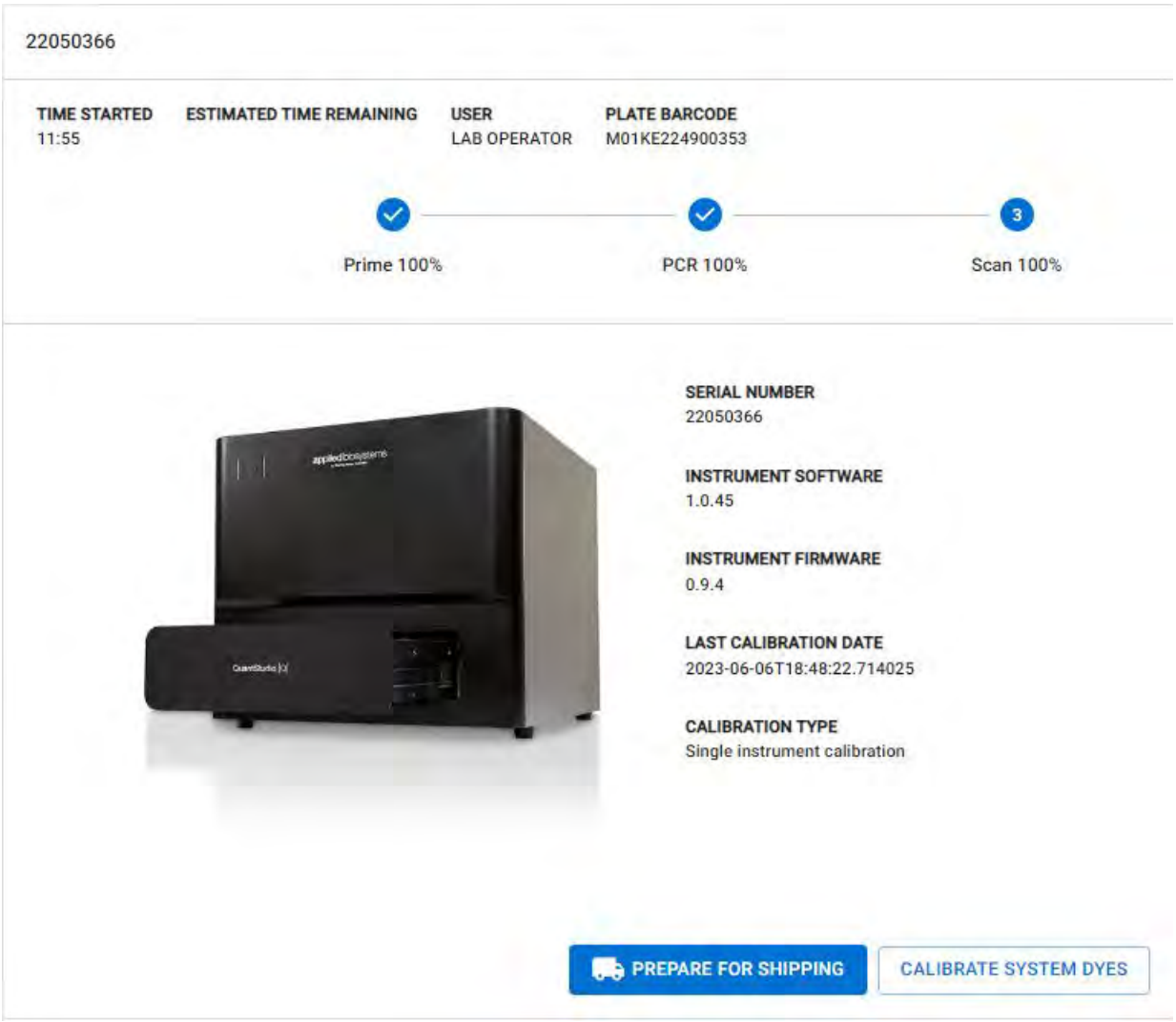
Instrument indicator status light key

The vertical bars of the Q symbol on the front of the instrument display the instrument status.

Appearance	Color	Status	Meaning
–|Q|–	White	Flashing	On, initializing – not ready.
|Q|	Blue	Steady	Idle, ready to connect to Absolute Q™ software.
~|Q|~	Blue	Pulsing	Running protocol.
–|Q|–	Yellow	Brief flashing	Plate door open button pushed while door is locked.
|Q|	Red	Steady	Error, see Appendix A, “Troubleshooting”.

Instrument information

Selecting the Instrument in the left pane of the Absolute Q™ software provides information on the instrument that is connected to the desktop computer.



The instrument software displays the following states for the device.

State	Description
READY	The instrument is ready to run an experiment.
RUNNING	The statistics of the run in progress are displayed. **Note:** A run can be interrupted from the **Instrument** page by selecting **STOP**.
ERROR	Displays an error if an error condition has occurred.
No instrument found	An instrument is not connected to the desktop computer.
UPDATE	Incompatible instrument configuration detected. See "Update the instrument software and firmware" on page 133.

QuantStudio™ Absolute Q™ Digital PCR Instrument Optical Dyes

The following optical dyes are supported for use when selecting optical channels when analyzing experiment runs.

For more information on optical configuration, see "QuantStudio™ Absolute Q™ Digital PCR Instrument Optical Configuration" on page 156.

Channel color	System dyes
Blue	FAM™ dye
Green	VIC™ dye *(recommended)* HEX™ dye[1,2]
Yellow	ABY™ dye
Red	ROX™ dye
Dark Red	CY™5 dye *(recommended)* JUN™ dye

[1] For information about HEX™ dye support, contact a Thermo Fisher service and support representative.

[2] HEX™ data from two instruments cannot be combined into a study, even if the systems are co-calibrated. For more information, see "Multi-plate analysis–Studies" on page 71.

QuantStudio™ Absolute Q™ MAP16 Digital PCR Plates description

The QuantStudio™ Absolute Q™ Digital PCR Instrument uses QuantStudio™ Absolute Q™ MAP16 Digital PCR Plates (MAP plates) for loading samples and running experiments.

IMPORTANT! When disposing of plates, follow all applicable waste regulations controlling the chemicals used in the experiment.

Each MAP plate has the following features.

- Contains 16 wells, 4 columns of 4 wells each, and each experiment must use at least one full column (4 samples).
- Contains 16 digital PCR microchamber arrays that each contain 20,480 fixed volume microchambers where dPCR is performed.
- Can be used in up to 4 experiments, depending on the number of columns used in each experiment. A MAP plate with unused columns can be used with subsequent experiments until all 4 columns have been used.
- Has a standard microtiter plate footprint and is compatible with most plate and liquid handlers.
- Has a label that includes a barcode, product number, and unique serial number. The instrument automatically reads the barcode when the MAP plate is inserted, and the unique serial number is tracked in the results.
- Requires 1 MAP plate gasket strip be placed on each column before insertion into the instrument, regardless of whether the column is being used for the experiment.

Take the following into consideration when using MAP plate gasket strips.

- Following use on a column that was used for sample testing, the MAP plate gasket strip cannot be reused.
- MAP plate gasket strips on unused columns can be reused until the column is used for sample testing.
- MAP plate gasket strips used on column X can be used for all runs on that MAP plate.

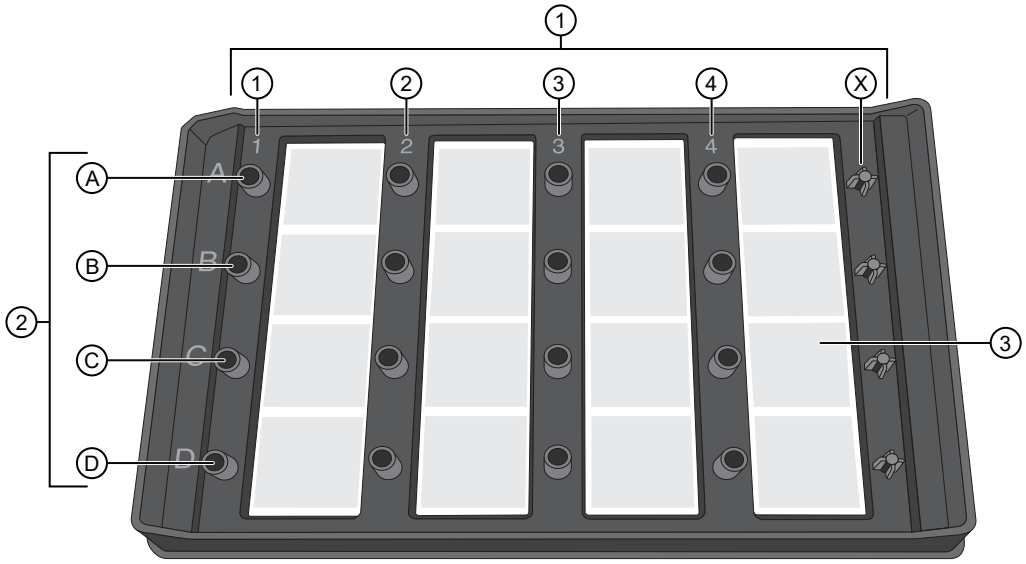


Figure 1 MAP plate without MAP plate gasket strips

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

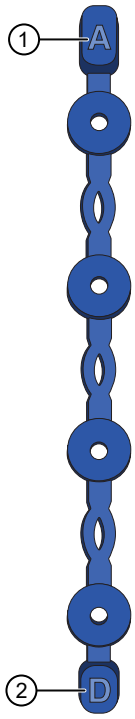


Figure 2 MAP plate gasket strip

① This end of the MAP plate gasket strip is placed on row A of the MAP plate
② This end of the MAP plate gasket strip is placed on row D of the MAP plate

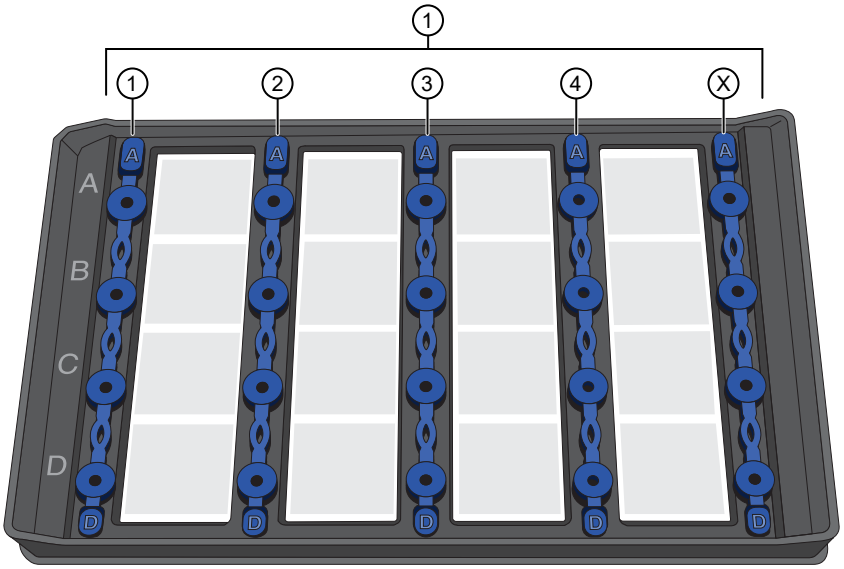


Figure 3 MAP plate with MAP plate gasket strips in place

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

The following figure shows the dimensions of a MAP plate.

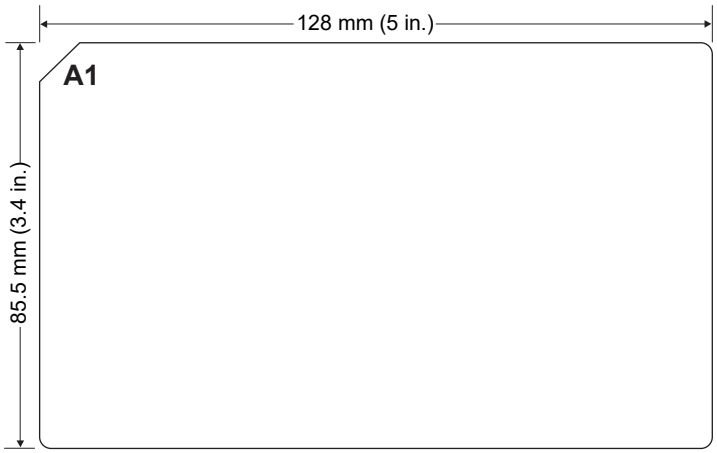


Figure 4 MAP plate dimensions

QuantStudio™ Absolute Q™ MAP16 Digital PCR Plate compatibility

IMPORTANT! The instrument is only compatible with QuantStudio™ Absolute Q™ MAP16 Digital PCR Plates. The instrument can malfunction with third-party plates, which could result in contamination and/or damage to the instrument.

- For best results, we strongly recommend that you use an Absolute Q™ Master Mix and QuantStudio™ Absolute Q™ Isolation Buffer.
- MAP plates are made of injection molded thermoplastic commonly used in other PCR vessels and are generally compatible with most existing reagent kits and components available from third parties. Compatibility of any untested third-party reagent is not guaranteed. Contact technical support for more information on tested reagents (see Appendix I, “Documentation and support”).

Network and password security requirements

Network configuration and security

The network configuration and security settings of your laboratory or facility (such as firewalls, anti-virus software, network passwords) are the sole responsibility of your facility administrator, IT, and security personnel. This product does not provide any network or security configuration files, utilities, or instructions.

If external or network drives are connected to the software, it is the responsibility of your IT personnel to ensure that such drives are configured and secured correctly to prevent data corruption or loss. It is the responsibility of your facility administrator, IT, and security personnel to prevent the use of any unsecured ports (such as USB, Ethernet) and ensure that the system security is maintained.

Password security

Thermo Fisher Scientific strongly recommends that you maintain unique passwords for all accounts in use on this product. All passwords should be reset upon first sign in to the product. Change passwords according to your organization's password policy.

It is the sole responsibility of your IT personnel to develop and enforce secure use of passwords.

QuantStudio™ Absolute Q™ Digital PCR Software security

By default, the QuantStudio™ Absolute Q™ Digital PCR Software does not require login credentials to access the software nor does it restrict access to functions within the software.

To require login credentials and modify access by user roles, see Appendix E, “Use the software with Security, Auditing, and E-signature (SAE) v2.2”.

Single plate workflow

This workflow represents running a single experiment on the QuantStudio™ Absolute Q™ Digital PCR Instrument.

Experiment workflow
Prepare an experiment (page 22)
Run digital PCR (page 34)
Analyze data (page 67)

Automation workflow

The QuantStudio™ Absolute Q™ Digital PCR Software lets you create dPCR runs that can be used on a system using automation. This workflow represents the steps in the Absolute Q™ software that are necessary for running dPCR runs using automation.

Automation workflow
Prepare an experiment (page 22)
Create and export a template (page 36) **or** **Create and export draft runs** (page 50)
Import template or draft run files into your automation system and perform a run
Export completed run data from your automation system
Import the run data into the Absolute Q™ software (page 60)
Proceed to analyze the (imported) run data (page 67)

2 Prepare an experiment

This chapter provides a general protocol for preparing experiments using custom dPCR assays ordered on **http://www.thermofisher.com/dpcr-assays.html** or with your unique custom dPCR assays. For predesigned dPCR assays, follow the instructions in the assay user guide provided with the assay.

Sample preparation workflow

This workflow represents preparing a single experiment on the QuantStudio™ Absolute Q™ Digital PCR Instrument.

Note: The procedure for sample preparation can vary depending on application and reagents.

Sample preparation workflow
DNA preparation guidlines (page 23) **or** **RNA preparation guidelines** (page 24)
Sample dilution guidelines (page 24)
Prepare the dPCR reaction mix for DNA (page 27) **or** **Prepare the dPCR reaction mix for RNA** (page 28)
Load the reaction mix into the MAP plate (page 30)
Run digital PCR (page 34)

DNA preparation guidlines

We recommend the following best practices for the preparation of DNA template genomic DNA (gDNA), complementary DNA (cDNA), for use in digital PCR (dPCR) experiments. Because dPCR experiment strategy and methodology can vary significantly, sample preparation and template quality must be assessed on an individual basis.

For information about DNA isolation kits, go to **https://www.thermofisher.com/us/en/home/life-science/dna-rna-purification-analysis.html**.

Quality of DNA

Use a gDNA or cDNA template that meets the following criteria.

- Is extracted from the raw material that you are testing with an optimized protocol.

  IMPORTANT! Salting-out procedures and crude lysates are not recommended.

  Failure to adhere to these recommendations can cause PCR amplification failures and clogging of the microfluidics both leading to poor or absent data collection.

  Note: Salting-out procedures and crude lysates are not recommended.

- Does not contain PCR inhibitors
- Has an $A_{260/230}$ and $A_{260/280}$ ratio between 1.7 and 1.9

The ratio of absorbency at 260 nm and 280 nm is used to assess the purity of DNA and RNA. A ratio of ~1.8 is generally accepted as “pure” for DNA. A ratio of ~2.0 is generally accepted as “pure” for RNA. If the ratio is appreciably lower in either case, it may indicate the presence of protein, phenol, or other contaminants that absorb strongly at or near 280 nm.

The ratio of absorbency at 260 nm and 230 nm is used as a secondary measure of nucleic acid purity. The 260/230 values for “pure” nucleic acid are often higher than the respective 260/280 values. Expected 260/230 values are commonly in the range of 2.0–2.2. If the ratio is appreciably lower than expected, it may indicate the presence of contaminants that absorb at 230 nm.

Quantity of DNA

The quantity of DNA template added to a dPCR reaction depends on the following factors.

- Concentration of gDNA or cDNA present in each sample
- Expected number of copies of the target sequence present in the genome or cDNA of your samples

Before performing digital PCR experiments, consider quantifying the amount of gDNA or cDNA in each sample.

We recommend one of the following methods for quantification, see “Recommended materials not supplied” on page 10.

- Quant-iT™ 1X dsDNA Assay Kit, High Sensitivity using the Qubit™ Flex Fluorometer
- Spectrophotometer

RNA preparation guidelines

We recommend the following best practices for the preparation of RNA template for use in digital PCR (dPCR) experiments. Because dPCR experiment strategy and methodology can vary significantly, sample preparation and template quality must be assessed on an individual basis.

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.

Sample dilution guidelines

If a target is present at a sufficiently high concentration in the sample of interest, it is possible that all reaction microchambers will be positive, which prevents the determination of the target concentration. In this case, the sample must be diluted prior to running the dPCR experiment. After you determine the dilution factor you can include it as part of sample setup; see “SETUP page (templates)” on page 41 or “SETUP page (DRAFT runs)” on page 54.

Determine the optimal dilution when the target is known

In a dPCR experiment, gDNA samples are diluted to a limiting quantity, to the extent that most individual PCR reactions contain either zero or one target molecule. If the target copy number per genome is known, dilute the extracted DNA to the optimal input range as described in the following sections.

- “Determine the target copy number per genome” on page 24
- “Dilute the extracted genomic DNA to the ideal input range” on page 25

Determine the target copy number per genome

This section provides example calculations for determining the target copy number per genome. Other calculation methods can be used. For information on the human genome, see *On the length, weight and GC content of the human genome*, Piovesan et al. BMC Res Notes (2019) 12:106 **https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6391780/pdf/13104_2019_Article_4137.pdf**.

1. If the source or species of the gDNA is known, using a genome size checker tool, determine the size of the genome. The lengths of several common genome models can be found at the following link.

   http://www.thermofisher.com/DNA-calculator

The size checker estimate of the single human genome is 3.2×10^9 bp (haploid).

2. Using the size of the genome determined in step 1, calculate the genome mass using the following formula.

   m = (n) (1.096×10^{-21} g/bp), where m is the genome mass, and n is the genome size in base pairs

   The following example calculates the mass of the human genome using the estimate of 3.2×10^9 bp (haploid) for (n).

   m = (3.2×10^9 bp) (1.096×10^{-21} g/bp)

   m = 3.5×10^{-12} g or 3.5 pg

3. Using the mass of the genome calculated in step 2, refer to a public database of genomic variants to identify the copies of the target sequence per single genome. For example, **http://dgv.tcag.ca/dgv/app/home**.

   The following example determines the genomic copy ratio to the mass of the human genome of the RNase P gene (single exon RPPH1 gene) located on chromosome 14 cytoband 14q11.2. (chr.14:20343370 on build GRCh38).

   RNase P gene copies per haploid human genome mass: 1 copy/3.5 pg

   In other words, 1 copy of the RNase P target sequence can be found in every 3.5 pg of human DNA. This example is relevant to any gene that is present at the normal rate of one copy per haploid genome (two copies per diploid genome) and provides a basis to perform a dPCR experiment to determine the optimal digital range.

Note: The digital range is the dynamic range of a dPCR instrument.

Dilute the extracted genomic DNA to the ideal input range

1. Based on the known target copy number per genome, dilute the samples to be within the dynamic range of the digital platform. The optimal digital range is such that each reaction well contains approximately 0.6–1.6 copies of the target sequence.

Note: A much wider range of concentrations can be run, however the concentration between 0.6 and 1.6 copies achieves the greatest confidence.

   To calculate the desired dilution point, divide the target copies per reaction by the volume of each microchamber's reaction, for example 0.6 to 1.6 copies divide by 0.000432 µl.

2. Take the resulting copies/µL of reaction mix and divide by the copies per picogram value determined in "Determine the target copy number per genome" on page 24 to arrive at a genomic pg/µL concentration target.

   For example:

$$\frac{12{,}926.96\ pg\ gDNA}{\mu L\ \text{Reaction Mix}} = \frac{1.6\ cp\ of\ RNase\ P}{0.000432\ \mu L} \cdot \frac{3.5\ pg\ gDNA}{1\ \text{copies}\ RNase\ P}$$

3. Once your target concentration is identified, construct a dilution series to dilute your genomic material to the correct final concentration. For an example of serial dilution, see Table 1.

IMPORTANT! Be sure to include the final addition of your sample into the reaction mix as the final dilution step.

Table 1 Serial dilution example

Tube	µL from previous dilution	µL diluent	Total tube µL	Conc. (pg/µL)	Total dilution factor
Stock	—	—	—	6.10E+07	4.7E+02
D1	3.28	196.72	200	1.00E+06	
D2	10.00	190.00	200	5.00E+04	
Reaction mix	2.59	7.41	10	12962.96	

4. The total dilution factor shown in Table 1 can be used during run setup to automatically back-calculate the stock concentration from the dPCR concentration results. The dilution factor can be calculated by dividing the stock concentration by the final reaction mix concentration.

Determine the optimal dilution when the target is unknown

If the target copy number per genome is unknown (for example, for a locus of unknown copies per genome or RNA of unknown expression level), we recommend that you determine the optimal dilution by preparing a dilution series of the sample that includes three to four data points above and below the expected digital range. This helps ensure that one of the data points is within the optimal digital range.

The quantification cycle (C_q) value is a function of concentration, therefore 1 copy target sequence in different reaction volumes produces different C_q values. Additionally, the actual C_q value in real time PCR always depends on the primary analysis parameters set by the user (for example, baseline, and threshold).

If tested using real-time PCR, the C_q values can be used to estimate the target molecule input for the points of the dilution series prior to dPCR.

- 1 copy in total volume of 20 µL produces C_q of ~38 (96-well plate)
- 1 copy in total volume of 10 µL produces C_q of ~37 (384-well plate)
- 1 copy in total volume of 1.5 µL produces C_q of ~34.5 (TaqMan™ Array Card)
- 1 copy in total volume of 33 nL produces C_q of ~29 (OpenArray™ Plate)

Prepare the dPCR reaction mix for DNA

This section provides general information for using the Absolute Q™ DNA Digital PCR Master Mix (5X) and your dPCR assay to prepare a dPCR reaction mix.

For information on preparing the dPCR reaction mix for a pre-designed Absolute Q™ dPCR assay, see the documentation provided with the assay.

Note: MAP plates are made of injection molded thermoplastic commonly used in other PCR vessels and are generally compatible with most existing reagent kits and components available from third parties. Compatibility of any untested third-party reagent is not guaranteed. Contact technical support for more information on tested reagents (see Appendix I, "Documentation and support").

Gather the following materials.

- Absolute Q™ DNA Digital PCR Master Mix (5X)
- Nuclease-free water
- Digital PCR assay (40X or 20X)

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

   Note: Store reagents on ice when not in use.

2. Vortex the Absolute Q™ Master Mix (5X) and dPCR assay (40X or 20X) at high speed for 10 seconds.

3. Using a benchtop centrifuge, centrifuge the DNA sample at 10,000 × *g* or the highest speed available for 1 minute, then transfer the supernatant to the reaction mix as indicated in step 4.

4. Combine the following reagents in the order listed.

Reagent	Final Concentration	Volume per reaction	Volume per reaction with 10% overage[1]
Nuclease-free water	–	Fill to 9 µL	Fill to 10 µL
Absolute Q™ DNA Digital PCR Master Mix (5X)	1X	1.8 µL	2 µL
Digital PCR assay (40X or 20X)[2]	1X	0.23 µL (40 X) or 0.45 µL (20X)	0.25 µL (40 X) or 0.50 µL (20X)
DNA Sample	1–11,000 copies/ µL[3]	Variable	Variable
Total	**–**	**9 µL**	**10 µL**

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

[2] If you are using a dPCR assay with a stock concentration other then 40X or 20X you must manually calculate the volumes based on the concentration you are using.

[3] A DNA copy and dilution calculator can be found at **http://www.thermofisher.com/DNA-calculator**.

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. Centrifuge at 1,000 × *g* for up to 1 minute to collect the contents at the bottom of the tube.

Proceed to “Load the reaction mix into the MAP plate” on page 30.

Prepare the dPCR reaction mix for RNA

This section provides general information for using the Absolute Q™ 1-Step RT-dPCR Master Mix and your dPCR assay to prepare a dPCR reaction mix.

For information on preparing the dPCR reaction mix for a pre-designed Absolute Q™ dPCR assay, see the documentation provided with the assay.

Note: MAP plates are made of injection molded thermoplastic commonly used in other PCR vessels and are generally compatible with most existing reagent kits and components available from third parties. Compatibility of any untested third-party reagent is not guaranteed. Contact technical support for more information about tested reagents (see Appendix I, “Documentation and support”).

Gather the following materials.

- Absolute Q™ 1-Step RT-dPCR Master Mix
- Nuclease-free water
- Digital PCR assay (20X)

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™ 1-Step RT-dPCR Master Mix.
- Store prepared reactions on ice or at 4°C for up to one hour.

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.

Note:

- Thaw RNA templates on ice or at 4°C.
- Bring the reaction mix to room temperature before loading the MAP plate.

1. Vortex the Absolute Q™ 1-Step RT-dPCR Master Mix 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)
Nuclease-free water	—	For 10 µL total reaction volume	For 40 µL total reaction volume
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
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.
   - Pipet up and down 10–20 times to mix. Avoid creating bubbles.
   - Pulse vortex 3–5 times for 1 second each.
4. Using a benchtop centrifuge, centrifuge at 10,000 x *g* for one minute to collect the contents at the bottom of the tube.

Proceed to “Load the reaction mix into the MAP plate” on page 30.

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 + 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.

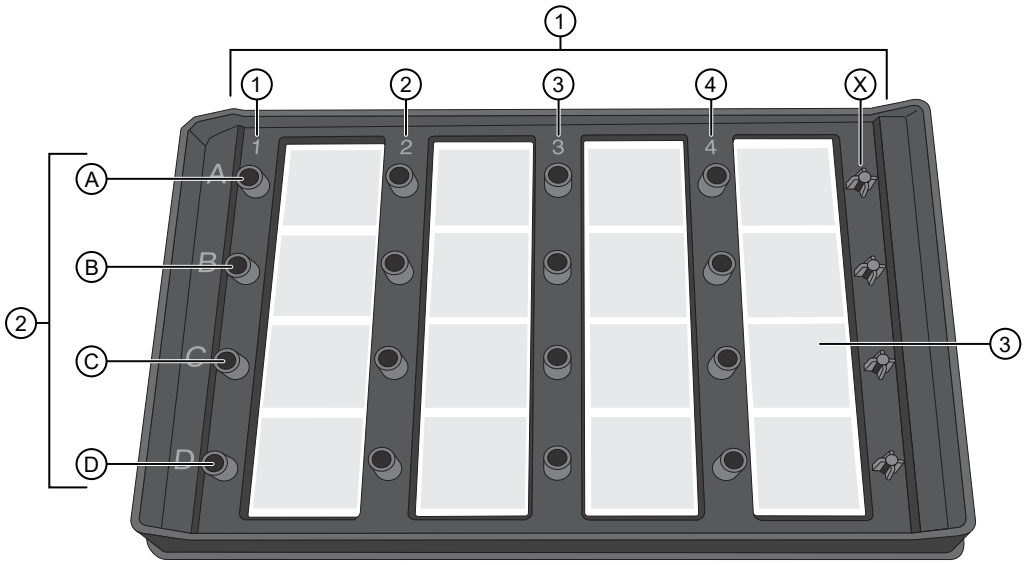


Figure 5 MAP plate without MAP plate gasket strips

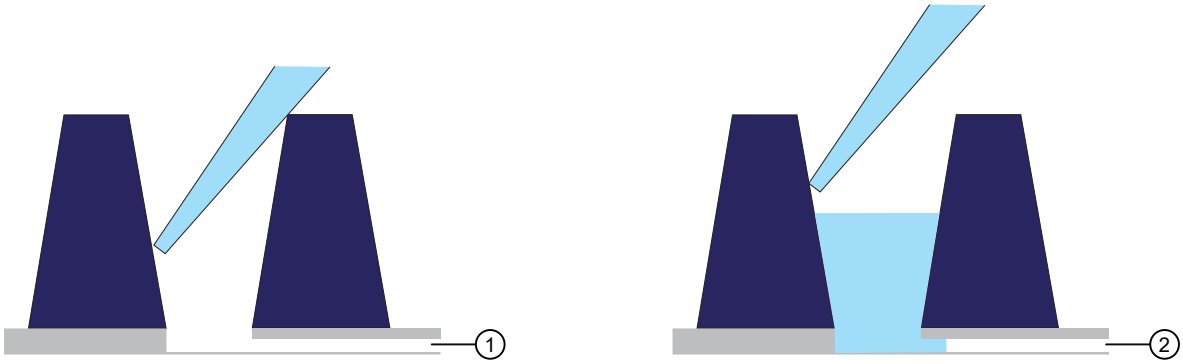
① Columns 1–4 and column X
② A–D represent wells A1–D1 associated with column 1
③ Array associated with well 4C

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.

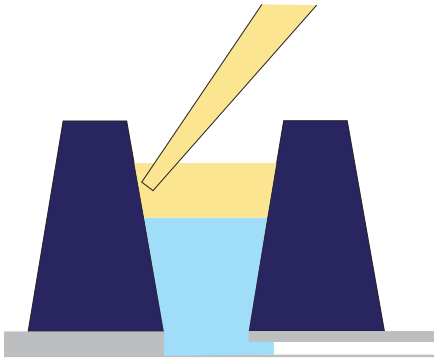
IMPORTANT! Do not contact bottom of well with the pipette tip or puncture the thin film at the bottom of the well.



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.

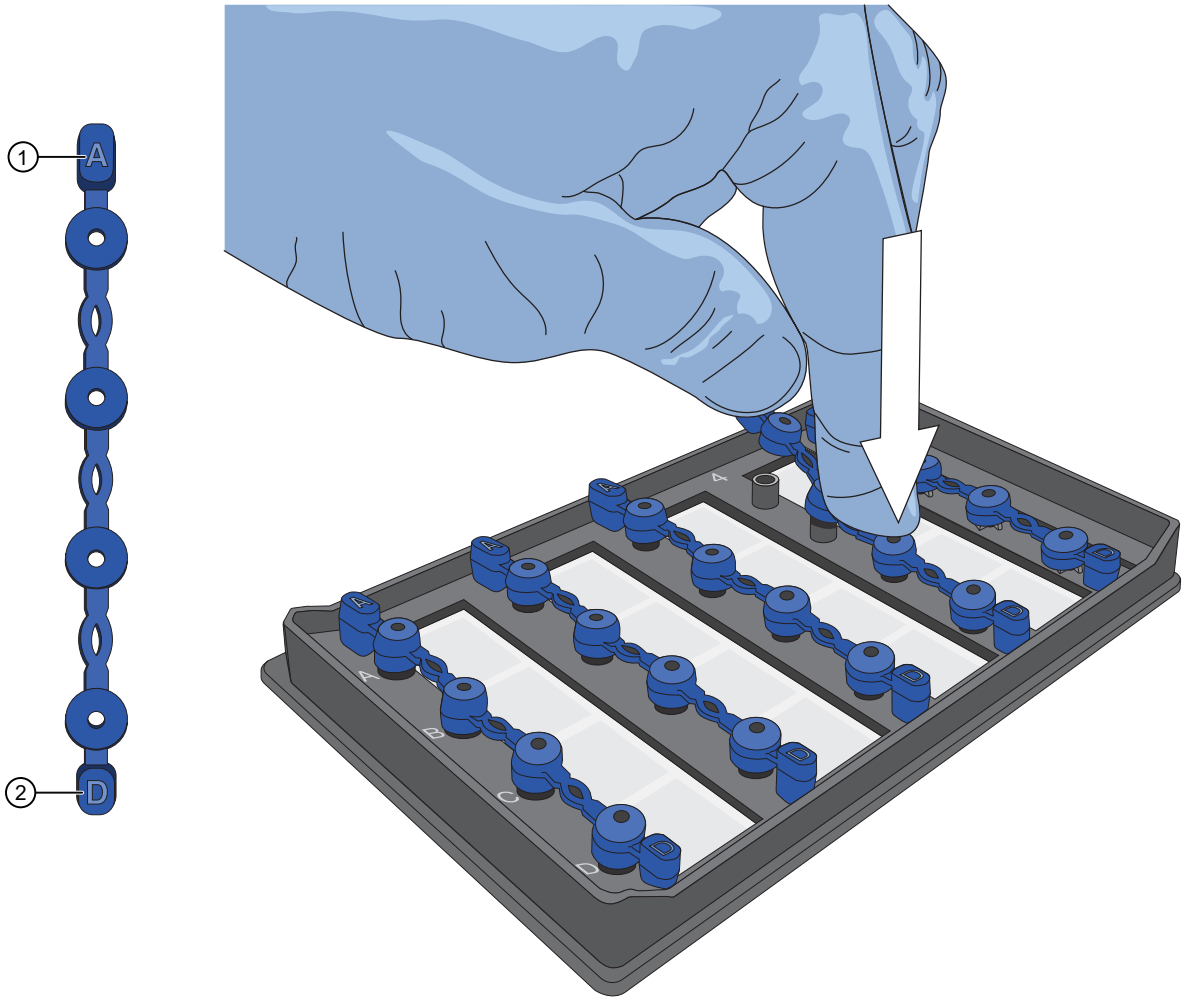


Figure 6 Place the MAP plate gasket strips firmly into place

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

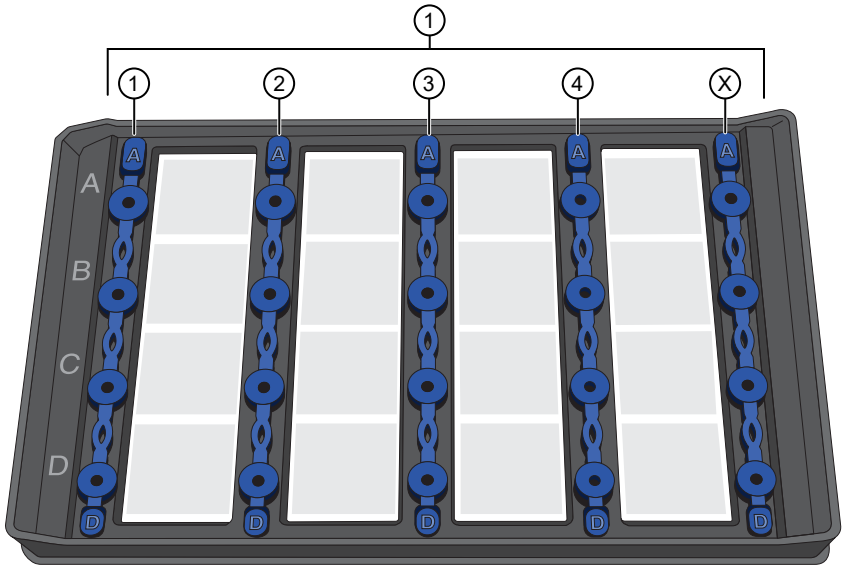


Figure 7 MAP plate with MAP plate gasket strips in place

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 digital PCR

This chapter provides information for running experiments on the QuantStudio™ Absolute Q™ Digital PCR Instrument.

Power on the instrument and computer

IMPORTANT! Prior to powering on the QuantStudio™ Absolute Q™ Digital PCR Instrument, confirm that the shipping lock screw has been removed. Failure to do so can damage the instrument. See "Uninstall the shipping lock screw" on page 135.

1. Confirm that the power cable is connected to an appropriate power source.
2. Power on the instrument by moving the power switch located on the left side near the back of the instrument to the I position.

   Note: The instrument makes a humming noise as it charges the internal compressor.

   The bars of the instrument symbol flash white to indicate that the system is initializing. This takes approximately 30 seconds.

   The instrument is ready when the status lights are a steady blue.
3. Confirm that the USB 3.0 cable is connected from the instrument to the USB 3.0 port on the dedicated computer.
4. Power on the dedicated computer and monitor, then start the software.

   Note: The instrument is ready when ready status appears under the instrument on the **Instrument** page in the QuantStudio™ Absolute Q™ Digital PCR Software.

Dashboard

When you start the QuantStudio™ Absolute Q™ Digital PCR Software, the **Dashboard** page opens and lets you perform the following tasks.

The left pane lets you access Absolute Q™ functions from any page in the software.

- —Click to return to the **Dashboard**
- —Click to open the **Runs** page. See “Manage runs” on page 50.
- —Click to open the **Templates** page. See “Manage Templates” on page 36.
- —Click to open the **Studies** page. See “Multi-plate analysis–Studies” on page 71.
- —Click to open the **Instrument** page. See“Instrument information” on page 14.
- —Click to access the user manual, end user license agreement (EULA), and information about the currently installed version of software.
- —Click to access the **System Settings** page. See “System settings” on page 131

The **Dashboard** provides the following shortcuts to software functions and system information.

- **Create new run**—Opens the **Runs** page where you can access the CREATE RUN feature. See “Create a run from the RUNS page” on page 51.
- **View completed runs**—Opens the **Runs**, **COMPLETED** tab that lists all completed runs. See “Open a completed run” on page 69.
- **Generate batch runs**—Opens the **Templates** page where you can create batch runs from templates. See “Generate batch runs from a template” on page 47.
- **View studies**—Opens the **Studies** page where you can view existing studies. See “Open a study for analysis” on page 75.
- **Recent runs**—Lists information about the most recent runs, select a run to see more information on the **Runs** page.
- **Connected Instrument**—Provides information about the connected instrument. This information only appears when an instrument is connected.

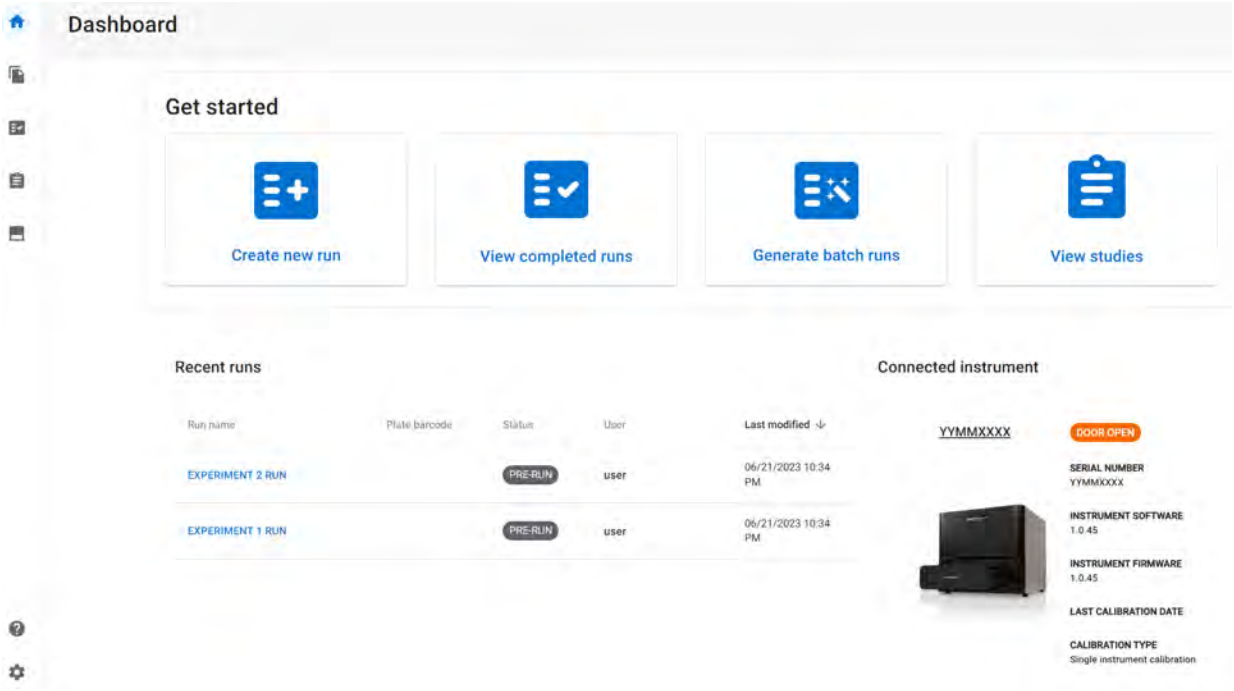


Figure 8 Dashboard with a connected instrument

Manage Templates

Templates include protocol parameters and plate setup configurations that can be used for creating new runs. The software is preconfigured with a default template.

The sections that follow provide information about creating and managing templates from the **Templates** page.

You can perform the following tasks from the **Templates** page.

- View information about the template including whether it can be edited (read-only), number of samples defined in the experiment, the user who created the template, and the date the template was last modified.
- Open a template to view and edit protocol and plate setup parameters. See “Edit a template” on page 44.
- **IMPORT TEMPLATES**—Use to import one or more templates. See “Import templates” on page 45.
- **CREATE TEMPLATE**—Use to create a template with specific protocol and plate setup parameters. See “Create a template” on page 37.
- **GENERATE BATCH RUNS**—Use to create batch runs from a single template. See “Generate batch runs from a template” on page 47.
- **EXPORT**—Use to export one or more templates. See “Export templates” on page 46.
- **RENAME**—Use to rename a template. See “Rename a template” on page 46.
- **DELETE**—Use to delete one or more templates. See “Delete templates” on page 47.
- Create a run from a template. See “Create a run from a template” on page 47.

Create a template

This section provides information about creating template procotol paramaters and plate setup configurations for use in creating runs.

1. From the left pane 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.

   Proceed to the **PROTOCOL** page to continue template creation by defining the PCR parameters of the protocol for the template. See “PROTOCOL page (templates)” on page 37.

PROTOCOL page (templates)

The **PROTOCOL** page within the template function lets you perform the following tasks when configuring the protocol.

- Define PCR parameters—Use the controls to set protocol specific parameters, see the steps in this section for detailed instructions.
- **Change optical collection setting**—Set optical channel (optional). Disabling optical channels prevents data from being collected from those channels. Any existing sample groups using these channels are affected.
- **IMPORT PROTOCOL**—Import an existing protocol.
- **EXPORT PROTOCOL**—Export the protocol.
- **GENERATE BATCH RUNS**—Create multiple runs (batch runs) containing plate-specific information, such as a barcode and/or sample information from a template. Batch runs can be used both in non-automated and automated configurations. See “Generate batch runs from a template” on page 47.
- **MAKE A COPY**—Make a copy of the template

During template creation, the **PROTOCOL** page appears after you have created the template. See “Create a template” on page 37.

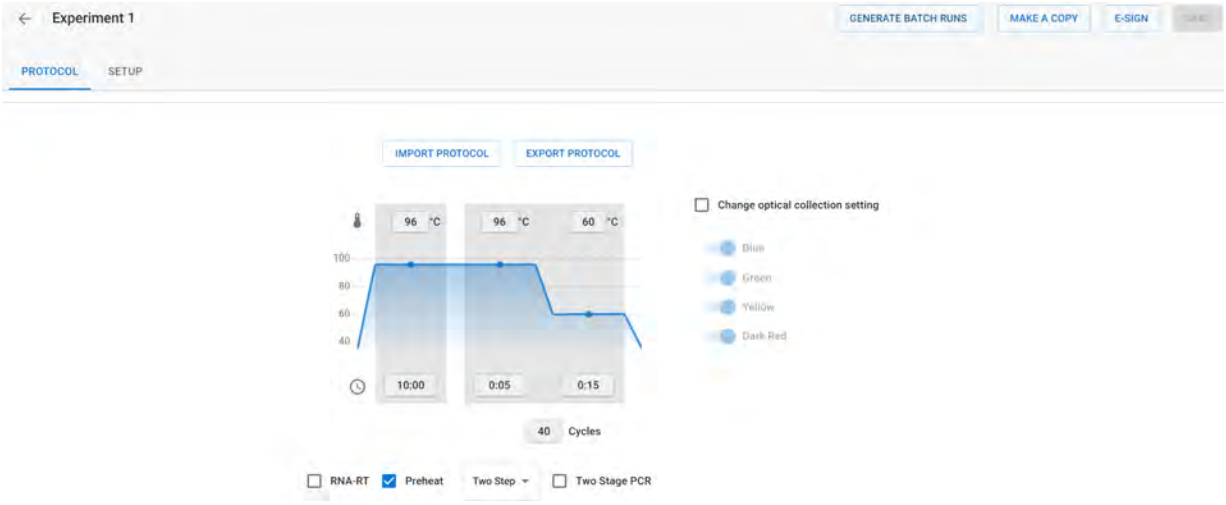


Figure 9 Protocol page

1. Modify PCR parameters if needed.

Parameter	Actions
Temperature	Perform one of the following actions. • Enter a value in the temperature fields. • Drag the slider bars to adjust the temperature.
Dwell times	Enter in seconds or minutes and seconds in mm:ss format.
Cycles	Set the number of cycles by entering a value into the **Cycles** field.
RNA-RT	Select **RNA-RT** to add an extra temperature step for RNA reverse transcription to cDNA for RNA samples. Not required for DNA samples.
Preheat	Select **Preheat** to add a preheat step. Sometimes called hot start, preheating the samples before PCR helps to reduce non-specific binding at lower temperatures.

(continued)

Parameter	Actions
Two or three-step cycling	From the cycling option dropdown list, select **Two Step** or **Three Step** cycling.
Two-stage PCR cycle	Select **Two Stage PCR** to add a second PCR cycle stage.

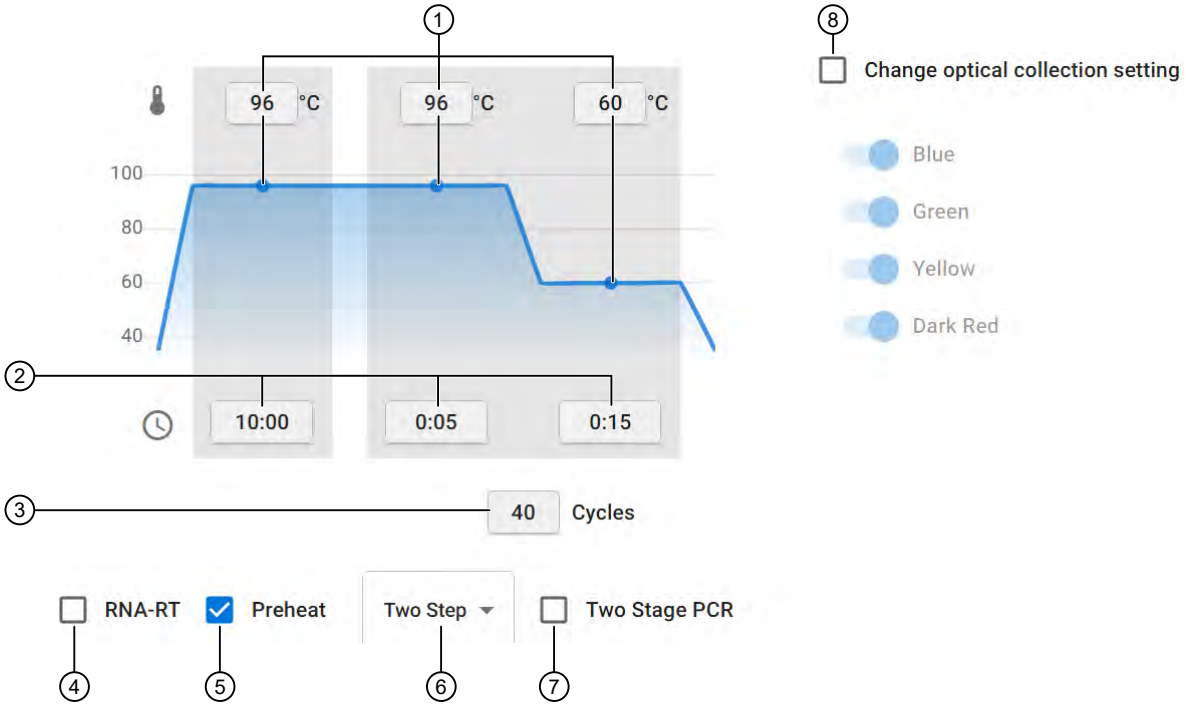


Figure 10 Protocol parameters

1. Temperature settings fields and slider bar
2. Time fields
3. Cycles field
4. RNA-RT setting
5. Preheat setting
6. Two Step or Three Step cycling option
7. Two Stage PCR setting
8. Change optical collection setting

2. *(Optional)* All optical channels are selected by default. Perform the following actions to change optical channel settings.

IMPORTANT! Disabling optical channels prevents data from being collected from those channels. Any existing sample groups using these channels are affected. To include a run in a study, all runs must have the same optical collection setting. For information about studies, see "Multi-plate analysis–Studies" on page 71.

a. Click **Change optical collection setting**.

b. When prompted to confirm changing the optical selection setting, click **CHANGE OPTICAL COLLECTION SETTING**.

c. Set the optical channels by toggling the channels on or off.

Note: If a channel is disabled, a message appears on the **PROTOCOL** page indicating that the protocol has disabled channels.

Note: If **Change optical collection setting** option is disabled, the software resets to the default setting of all channels in the on position.

3. *(Optional)* Import an existing protocol by performing the following actions.
   a. Click **IMPORT PROTOCOL**, then click **IMPORT FILE** to open the **File browser**, then navigate to the location of the AQUA file to import.
   b. Select the file, then select **Open**.
4. *(Optional)* Export the protocol by performing the following actions.
   a. Click **EXPORT PROTOCOL** to open the **File browser**, then navigate to the location where you want to save the exported protocol AQUA file.
   b. Click **Export**.
5. When the protocol settings are complete, perform one of the following actions.
   - Select the **SETUP** tab to proceed to MAP plate setup. If changes were made, you are prompted to save the changes.
   - Click **SAVE** to save changes, then select **SETUP** to proceed to MAP plate setup.

   The **SETUP** page opens. For information on MAP plate setup, proceed to “SETUP page (templates)” on page 41.
6. *(Optional)* Create a copy of the current template with a new name.
   a. Click **MAKE A COPY**.
   b. When prompted, type the new name in the **Template name** field, then click **MAKE COPY**.
7. (Optional) Click **GENERATE BATCH RUNS** to create multiple runs from this template. See “Generate batch runs from a template” on page 47.

Note: While batch runs can be created from the **PROTOCOL** page, this task is typically done after plate setup is complete.

SETUP page (templates)

The **SETUP** page within the templates function lets you perform the following tasks for setting up the MAP plate.

- Name the samples—Sample names are user-assigned identifiers for the contents of each loaded well of a plate.
- Set the dilution factor—The dilution factor is the total dilution from the sample to the reaction mix and must be set with a value ≥1. This value is the denominator used in the dilution factor field.

  For example, if 2 µL of the sample is put into a 10 µL reaction mix, the dilution factor is [2 µL sample/ 10 µL reaction = 1/5]. In this case, the dilution factor denominator is 5.

  If instead the stock solution is diluted by a factor of 100, then 2 µL of the diluted sample is used in a 10µl reaction mix, then the dilution factor is [(1 µL stock / 100 µL dilution) × (2 µL dilution/ 10 µL reaction) =1/500]. In this case, the dilution factor denominator is 500.

  Note: The dilution factor field accepts scientific notation, for example 10E+10.

- Assign groups—Groups determine what type of analysis is applied to all samples within a group.
- **ADD GROUP**—Create more groups. See “Manage groups” on page 63.
- **EDIT DYES**—Reassign dyes to the green and dark red channels for this run.
- **Export plate setup**—Use to export a blank or configured plate setup CSV file to modify outside software and import for use.
- **Import plate setup**—Use to import plate existing setup files.
- **GENERATE BATCH RUNS**—Create multiple runs containing plate-specific information, such as a barcode and/or sample information from a template. Batch runs can be used both in non-automated and automated configurations. See “Generate batch runs from a template” on page 47.
- **MAKE A COPY**—Make a copy of the template.
- **Notes**—Add notes regarding the plate setup.

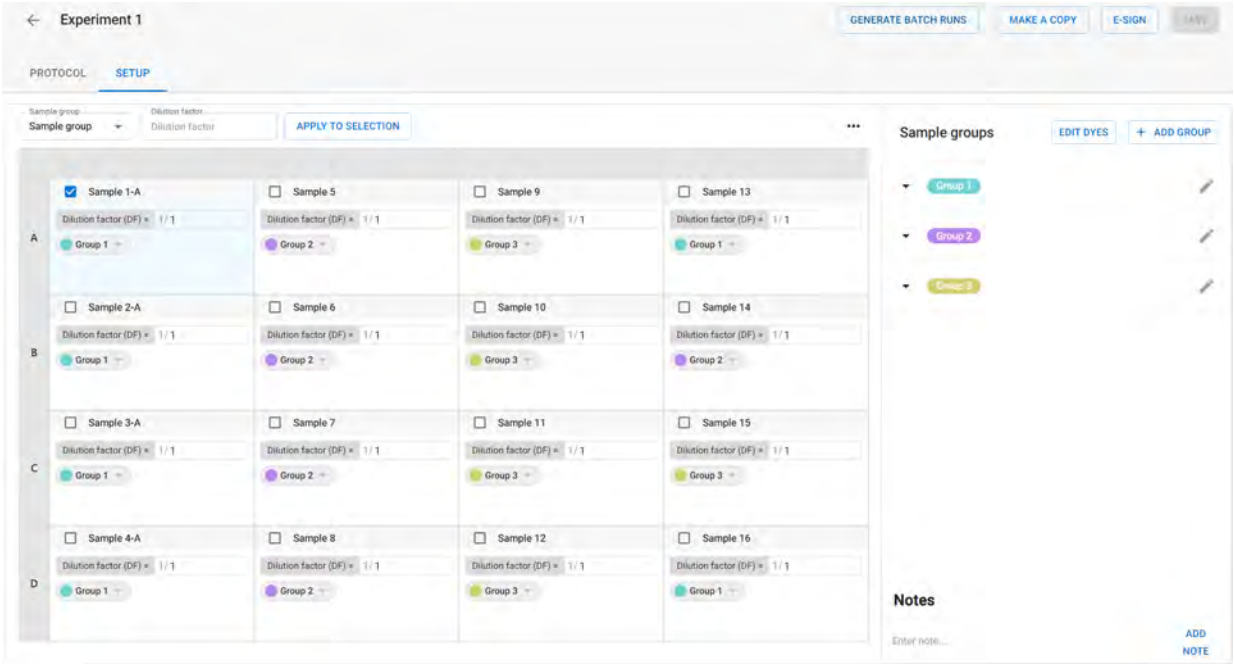


Figure 11 SETUP page for templates

1. To access the **SETUP** page, perform one of the following actions based on the type of analysis you are doing.

Analysis type	Action
Single-plate analysis	See “Open a completed run” on page 69.
Multi-plate analysis	See “Open a study for analysis” on page 75.

2. Modify the plate settings if needed.

Setting	Action
Sample name	Sample wells are preconfigured with a default name. To change the name, perform the following action. • Click the sample well name, then type the new sample name.
Dilution factor	Sample wells are preconfigured with a dilution factor of 1. To change the dilution factor, perform one of the following actions. • For a single sample well, enter a value in the **Dilution factor** field of the well. • For multiple sample wells, enter a value in the **Dilution factor** field above the plate table, then click **APPLY TO SELECTED**.
Group	By default, all sample wells are assigned to the preconfigured **Group 1**. To change the group, perform one of the following actions. • For a single sample well, click the dropdown arrow by the group name, then select a group from the list. • For multiple sample wells, click the dropdown arrow in the **Sample group** field above the plate table, select the group, then click **APPLY TO SELECTED**. To add or edit a group, see "Manage groups" on page 63.
EDIT DYES	Select **EDIT DYES**, to change the dye assignments for the green and dark red channels for this run.

3. *(Optional)* Click ··· in the upper-right corner of the sample table to access the **Import plate setup** and **Export plate setup** options.

Option	Actions
Import plate setup—Use to import an existing plate setup. You can use the imported plate setup as-is, or modify as needed.	1. Click ···, then select **Import plate setup**. 2. In the **File browser**, navigate to the location of the plate setup CSV file to be imported. 3. Select the plate setup file, then click **Open**.
Export plate setup—Use to export the plate setup file to modify or use as-is in other templates or runs.	1. Click ···, then select **Export plate setup**. 2. In the **File browser**, navigate to the location of where to export the plate setup file. 3. Click **Save**.

4. *(Optional)* In the **Notes** area, **Enter note...** field, type notes related to this plate setup, then click **ADD NOTE**.

5. Click **SAVE** to save the protocol and MAP plate configurations.

6. *(Optional)* Create a copy of the current template with a new name.
   a. Click **MAKE A COPY**.

b. When prompted, type the new name in the **Template name** field, then click **MAKE COPY**.

7. (Optional) Click **GENERATE BATCH RUNS** to create multiple runs for this template. See “Generate batch runs from a template” on page 47.

Edit a template

1. From the left pane click to open the **Templates** list page.
2. Use the search field to find a template or select a template from the list.
3. From the **Select action** dialog, select **Edit template**, then click **EDIT TEMPLATE**.
4. Select the **PROTOCOL** tab to modify the protocol using the following options if needed.

Option	Actions
Manually set the protocol parameters.	1. Set the temperature by performing one of the following actions. • Enter a value in the temperature fields. • Drag the slider bars to adjust the temperature. 2. Enter dwell times in mm:ss format. 3. Set the number of **Cycles** by entering a value into the **Cycles** field. 4. *(Optional)* Select **RNA-RT** to add an extra temperature step for RNA reverse transcription to cDNA for RNA samples. Not required for DNA samples. 5. *(Optional)* Select **Preheat** to add a preheat step. Sometimes called hot start, preheating the samples before PCR helps to reduce non-specific binding at lower temperatures. 6. *(Optional)* Select the **Two Step** dropdown to select 2 or 3 step cycling. 7. *(Optional)* In the cycling option dropdown list, select **Two Stage PCR** to add a second PCR cycle stage. 8. *(Optional)* Change optical channel settings by selecting **Change Optical Collection Setting** and toggle channels off or on if needed. **IMPORTANT!** Disabling optical channels prevents data from being collected from those channels. Any existing sample groups using these channels are affected. To include a run in a study, all runs must have the same optical collection setting. For information about studies, see “Multi-plate analysis–Studies” on page 71.
Import an existing protocol.	1. Click **IMPORT PROTOCOL**, then navigate to the location of the AQUA file to import. 2. Select the file, then click **Open**.
Emport an exiting protocol.	1. Click **EXPORT PROTOCOL**, then navigate to the location to export the file then click **Save**.

5. Select the **SETUP** tab to modify the MAP plate using the following options if needed.

Setting	Action
Sample name	Sample wells are preconfigured with a default name. To change the name, perform the following action. • Click the sample well name, then type the new sample name.
Dilution factor	To change the dilution factor, perform one of the following actions: • For a single sample well, enter a value in the **Dilution factor** field of the well. • For multiple sample wells, select the wells, then enter a value in the **Dilution factor** field above the plate table, then click **APPLY TO SELECTED**.
Group	To change sample group assisnments, do one of the following: • For a single sample well, click the dropdown arrow by the group name, then select a group from the list. • For multiple sample wells, click the dropdown arrow in the **Sample group** field above the plate table, select the group, then click **APPLY TO SELECTED**. To add or edit a group, see “Manage groups” on page 63.
Notes	Type notes related to this plate setup, then click **ADD NOTE**.

6. Click **SAVE** to save the protocol and MAP plate configurations.

7. *(Optional)* Create a copy of the current template with a new name.
   a. Click **MAKE A COPY**.
   b. When prompted, type the new name in the **Template name** field, then click **MAKE COPY**.

Import templates

Use the **IMPORT TEMPLATES** option to import existing templates.

1. From the left pane click to open the **Templates** list page.
2. Click **IMPORT TEMPLATES**.
3. From the **Import templates** dialog, perform one of the following actions.

Option	Action
Drag-and-drop template files from a location on your file system.	1. Using **File Explorer**, find the DPT file in your file system, then drag-and-drop the file into the **Import templates** dialog box. 2. When the import completes, in the **Import Status** dialog, click **CLOSE**.
Browse to find the DPT file.	1. In the **Import templates** dialog, click **IMPORT FILES**. 2. In the **File browser**, navigate to the location of the template DPT file or files to be imported. 3. Select the template file or files, then click **Open**. 4. When the import completes, in the **Import Status** dialog, click **CLOSE**.

Note: If there are templates that cannot be imported, an error appears in the **Import Status** dialog. Make note of the error so that you can take corrective action on the template, then click **CLOSE** to continue.

The template list refreshes to include the imported templates.

Export templates

Use the **EXPORT** option to export templates for use on other systems or software installed independently from the instrument.

1. From the left pane click to open the **Templates** list page.
2. Use the search field to find the templates for export, then select one or more templates from the list.
3. Click **EXPORT**, then in the **File browser**, navigate to the location of where to export the template file or files.
4. Click **Select Folder**.
5. When the export completes, in the **Export Status** dialog, click **CLOSE**.

Note: If there are templates that cannot be exported, an error appears in the **Export Status** dialog. Make note of the error so that you can take corrective action on the template, then click **CLOSE** to continue.

Rename a template

Only one template can be renamed at a time.

Note: To create a copy of an existing template with a new name, use the **MAKE A COPY** option within the edit template function, see “Edit a template” on page 44 .

1. From the left pane click to open the **Templates** list page.
2. Use the search field to find a template or select a template from the list.
3. Click **RENAME**.
4. When prompted, enter the new name, then click **RENAME**.

   The **Templates** list is refreshed with the renamed template.

Delete templates

Multiple templates can be deleted at one time.

1. From the left pane click to open the **Templates** list page.
2. Use the search field to find the templates to be deleted.
3. Select the template or templates to be deleted, then click **DELETE**.
4. When prompted, confirm that the selected templates or templates are to be deleted by performing one of the following actions.
   - To delete the template(s), click **DELETE TEMPLATE** or **DELETE (# of templates selected) TEMPLATES**.
   - To cancel the delete action, click **DO NOT DELETE**.

Create a run from a template

1. From the left pane click to open the **Templates** list page.
2. Use the search field to find a template or select a template from the list.
3. In the **Select action** dialog, select **Create run from template**.
4. When prompted, enter a name for the run, then click **CREATE RUN**.
   The **PROTOCOL** page opens. To continue with configuring protocol settings, see “PROTOCOL page (runs)” on page 51.

Generate batch runs from a template

Use the **GENERATE BATCH RUNS** function to create multiple runs from the same template. These runs can be run manually or on an automation system that uses a robot to load the plates into the instrument. Each batch run is generated from a single template.

When using a system with automation, when the batch run is complete, you must import the run results generated on the automation system into the Absolute Q™ software for analysis.

After you determine how many runs that you want to generate from the template, you must create sample assignment files and/or plate barcode files that define plate information for each plate in the batch run.

Note: Examples of these files can be downloaded from the **Generate batch runs** screen.

Sample Assignment file

- A 2-column CSV file that defines the well number and associated sample name for each well on the plate
- Each CSV file represents one plate which corresponds to one run

Plate Barcode file

- A 1-column TXT file that defines the barcode numbers for plates in the run.
- The sample names for each well are inherited from the template.

Note: You can use a combination of sample assignment files and plate barcode files in a batch run providing that the files do not contain duplicate barcode information.

1. From the left pane click to open the **Templates** list page.
2. Use the search field to find a template or select a template from the list.
3. Perform one of the following actions.
   - Select a template, then click **GENERATE BATCH RUNS**.
   - Open a template, then click **GENERATE BATCH RUNS**.

   Generate batch runs screen opens, the name of the selected template appears in the **Select template file** field.
4. Use the following options to define plate information for the batch run.

Option	Action
ADD BARCODES & SAMPLE ASSIGNMENT FILES—defines sample name by well.	1. Click **ADD BARCODES & SAMPLE ASSIGNMENT FILES**. 2. Use the **File browser** to locate and select the sample assignment CSV files for the plates for this batch run, then click **Open**.
ADD PLATE BARCODE FILE—defines the barcode numbers of plates used in the run.	1. Click **ADD PLATE BARCODE FILE**. 2. Use the **File browser** to locate and select the barcode TXT files for the plates for this batch run, then click **Open**.

The **Generate batch runs** page opens with information from the CSV or TXT files.

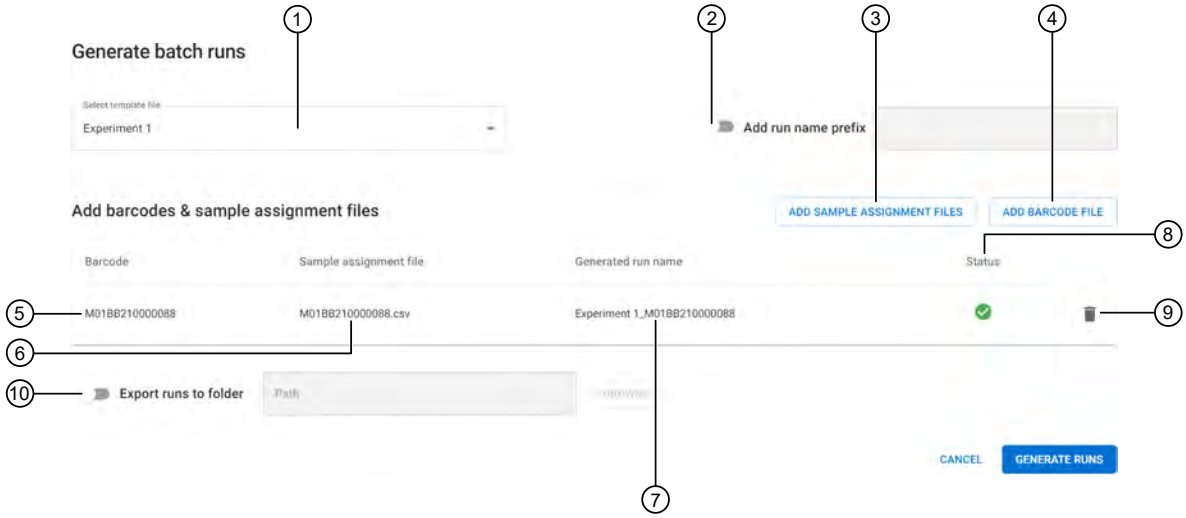


Figure 12

① **Select template file**—Displays the selected template. To change the template, select a template from the dropdown list.
② **Add run name prefix**—Toggle on to enter a prefix for the run names for this batch run.
③ **ADD SAMPLE ASSIGNMENT FILES** —Click to add more plates to the run using the sample CSV file format.
④ **ADD BARCODE FILE**—Click to add more plates to the run using the barcode TXT file format.
⑤ **Barcode**—Barcode number of the plate or plates.
⑥ **Sample Assignment File**—For sample assignment files, the name of the file including the path to the location of the file on the network. For barcodes, the name is inherited from the template file.
⑦ **Generated Run Name**—System-assigned run name that contains the format <template_name_barcode>
⑧ **Status** column—✓ indicates a valid file format, ⚠ indicates an issue with the file that must be corrected.
⑨ Select 🗑 to delete the row.
⑩ **Export runs to folder**—Toggle on to designate a location for the batch file information. (For implementations using automation).

5. *(Optional)* To change the template, select a template name from the **Select template file** dropdown.

6. *(Optional)* To add a prefix to the run name, toggle the **Add run name prefix** option to the on position, then type the desired text in the **Prefix** field.

7. *(Optional)* Click **ADD SAMPLE ASSIGNMENT FILES** or **ADD BARCODE FILE** to add more plates to the run.

8. *(Optional)* If a file has a warning status, in the **Status** column, click ⚠ for information regarding the issue. Take the appropriate corrective action.

9. *(Optional)* For implementations using automation, toggle the **Export runs to folder** option to the on position, then browse to select a file system location where the automation system can retrieve the information to perform the batch run.

10. Click **GENERATE RUNS** to create the DPR files for each plate.

    The **Generate run status** dialog opens that shows the status of the creation of the runs for the batch run, click **CLOSE** to continue.

When the batch run generation is complete, the runs appear on the **Runs** page on the **DRAFT** tab.

11. *(Optional)* For implementations using automation, completed run data must be imported from the automation system to be used in analysis. For information about importing completed batch run data, see “Import runs” on page 60.

Note: Runs created in the software, but performed on an automation system, have a draft status until the run data from the automation system is imported.

12. *(Optional)* For implementations not using automation, experiments are run one plate at a time. For information about initiating a single plate run, see “Start a single plate run” on page 58 .

Manage runs

The **Runs** page provides information about the three run types, **DRAFT**, **COMPLETED**, and **CALIBRATION**.

This section provides information about managing **DRAFT** runs.

For information about managing **COMPLETED** runs, see “Single-plate analysis” on page 69 and “Open a completed run” on page 69.

IMPORTANT! **CALIBRATION** runs are only performed by qualified Thermo Fisher representatives.

DRAFT runs can have the following statuses.

- **PRE-RUN**—The run has not been started
- **IN PROGRESS**—The run has been started and is not yet complete.

You can perform the following tasks from the **DRAFT** page.

- View information about the draft run including plate barcode number, status of the run, user who created the run, the date the run was last modified date, and the exported date.
- Open a draft run to modify protocol and plate setup parameters. See “Edit a draft run from the Runs page” on page 57.
- **CREATE RUN**—Use this option to create a new run. See “Create a run from the RUNS page” on page 51.
- **IMPORT RUN**—Use this option to import runs created on other Absolute Q™ systems or runs generated on an automated system. See “Import runs” on page 60.
- **START RUN**—Use this option to start a run. This option is only available if an instrument is attached to the dedicated computer. See “Start a single plate run” on page 58.
- **EXPORT**—Use this option to export run for use on other Absolute Q™ systems. See “Export runs” on page 61
- **RENAME**—Use this option to rename a run. See “Rename a run” on page 62
- **DELETE**—Use this option to delete one or more runs. See “Delete a run” on page 62.

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 a template.

   The **PROTOCOL** page opens. To continue with configuring protocol settings, see “PROTOCOL page (runs)” on page 51.

PROTOCOL page (runs)

The **PROTOCOL** page within the **DRAFT Runs** function lets you perform the following tasks when configuring the protocol.

- Define PCR parameters on **DRAFT** runs—Use the controls to set protocol specific pararmeters, see the steps in this section for detatiled instructions.
- **Change optical collection setting**—Set optical channel (optional). Disabling optical channels prevents data from being collected from those channels. Any existing sample groups using these channels are affected.
- **IMPORT PROTOCOL**—Import an existing protocol.
- **EXPORT PROTOCOL**—Export the protocol.
- **SAVE AS TEMPLATE**—Save the run as a new template
- **E-SIGN**—For implementations using Security, Auditing, and E-signature (SAE) v2.2 software. See Appendix E, “Use the software with Security, Auditing, and E-signature (SAE) v2.2”.

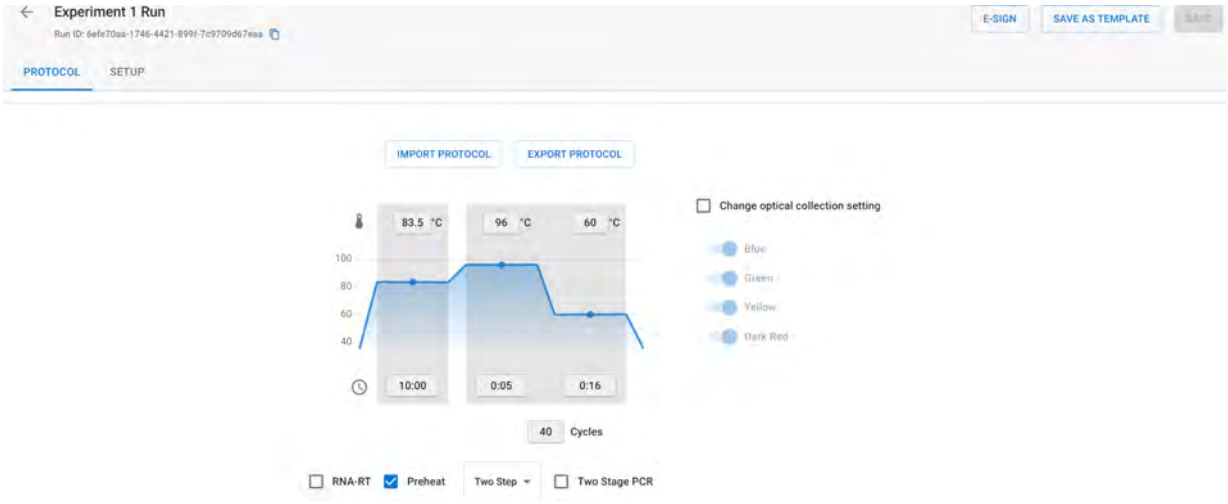


Figure 13 PROTCOL page for draft runs

1. Modify PCR parameters if needed.

Parameter	Actions
Temperature	Perform one of the following actions. • Enter a value in the temperature fields. • Drag the slider bars to adjust the temperature.
Dwell times	Enter in seconds or minutes and seconds in mm:ss format.
Cycles	Set the number of cycles by entering a value into the **Cycles** field.
RNA-RT	Select **RNA-RT** to add an extra temperature step for RNA reverse transcription to cDNA for RNA samples. Not required for DNA samples.
Preheat	Select **Preheat** to add a preheat step. Sometimes called hot start, preheating the samples before PCR helps to reduce non-specific binding at lower temperatures.
Two or three-step cycling	From the cycling option dropdown list, select **Two Step** or **Three Step** cycling.
Two-stage PCR cycle	Select **Two Stage PCR** to add a second PCR cycle stage.

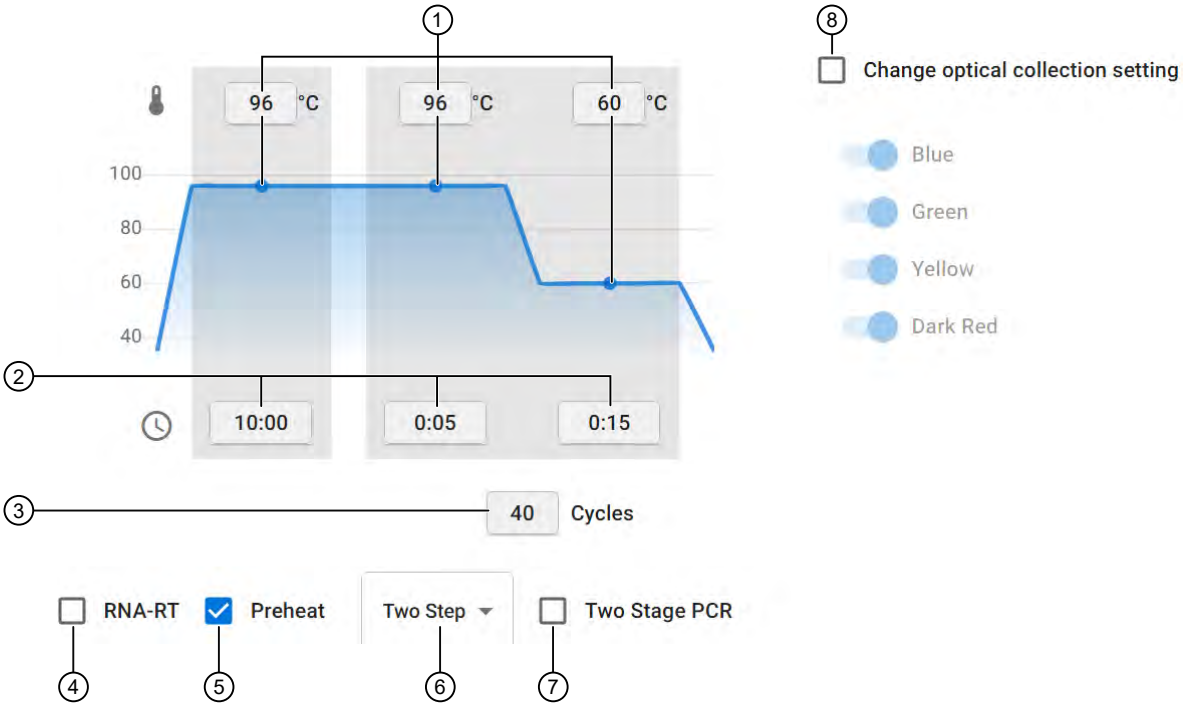


Figure 14 Protocol parameters

① Temperature settings fields and slider bar
② Time fields
③ Cycles field
④ RNA-RT setting
⑤ Preheat setting
⑥ Two Step or Three Step cycling option
⑦ Two Stage PCR setting
⑧ Change optical collection setting

2. *(Optional)* All optical channels are selected by default. Perform the following actions to change optical channel settings.

   IMPORTANT! Disabling optical channels prevents data from being collected from those channels. Any existing sample groups using these channels are affected. To include a run in a study, all runs must have the same optical collection setting. For information about studies, see “Multi-plate analysis–Studies” on page 71.

   a. Click **Change optical collection setting**.

   b. When prompted to confirm changing the optical selection setting, click **CHANGE OPTICAL COLLECTION SETTING**.

   c. Set the optical channels by toggling the channels on or off.

   Note: If a channel is disabled, a message appears on the **PROTOCOL** page indicating that the protocol has disabled channels.

   Note: If **Change optical collection setting** option is disabled, the software resets to the default setting of all channels in the on position.

3. *(Optional)* Import an existing protocol by performing the following actions.
   a. Click **IMPORT PROTOCOL**, then click **IMPORT FILE** to open the **File browser**, then navigate to the location of the AQUA file to import.

   b. Select the file, then select **Open**.

4. *(Optional)* Export the protocol by performing the following actions.
   a. Click **EXPORT PROTOCOL** to open the **File browser**, then navigate to the location where you want to save the exported protocol AQUA file.

   b. Click **Export**.

5. *(Optional)* Create a template of the run with a new name.
   a. Click **SAVE AS TEMPLATE**.

   b. When prompted, type the new name in the **Template name** field, then click **SAVE AS TEMPLATE**.

6. *(Optional)* For implementations using Security, Auditing, and E-signature (SAE) v2.2 software, click **E-SIGN** to provide an e-signature. See, “Sign data in the software” on page 149.

7. When protocol settings are complete, select the **SETUP** tab to continue run creation by configuring the MAP plate.
   The **SETUP** page opens. To continue with MAP plate configuration, see “SETUP page (DRAFT runs)” on page 54.

SETUP page (DRAFT runs)

The **SETUP** page in the **Runs DRAFT** page lets you perform the following tasks for setting up the MAP plate.

- Include or exclude columns for use in the run.
- Name the samples—Sample names are user-assigned identifiers for the contents of each loaded well of a plate.
- Set the dilution factor—The dilution factor is the total dilution from the sample to the reaction mix and must be set with a value ≥1. This value is the denominator used in the dilution factor field.

  For example, if 2 µL of the sample is put into a 10 µL reaction mix, the dilution factor is [2 µL sample/ 10 µL reaction = 1/5]. In this case, the dilution factor denominator is 5.

  If instead the stock solution is diluted by a factor of 100, then 2 µL of the diluted sample is used in a 10µl reaction mix, then the dilution factor is [(1 µL stock / 100 µL dilution) ×*(2 µL dilution/ 10 µL reaction) =1/500]. In this case, the dilution factor denominator is 500.

  Note: The dilution factor field accepts scientific notation, for example 10E+10.

- Assign groups—Groups determine what type of analysis is applied to all samples within a group.
- **ADD GROUP**—Create more groups. See “Manage groups” on page 63.
- **EDIT DYES**—Reassign dyes to the green and dark red channels for this run.
- **Export plate setup**—Use to export a blank or configured plate setup CSV file to modify outside software and import for use.
- **Import plate setup**—Use to import plate existing setup files.
- **Plate barcode**—Assign the barcode of the plate that is to be used for the run.
- **Notes**—Add notes regarding the plate setup.

- **SAVE AS TEMPLATE**—Save the run as a template.
- **E-SIGN**—For implementations using Security, Auditing, and E-signature (SAE) v2.2 software. See Appendix E, “Use the software with Security, Auditing, and E-signature (SAE) v2.2”.
- **PROTOCOL**—View or modify the protocol settings used for this draft run. See “PROTOCOL page (runs)” on page 51.

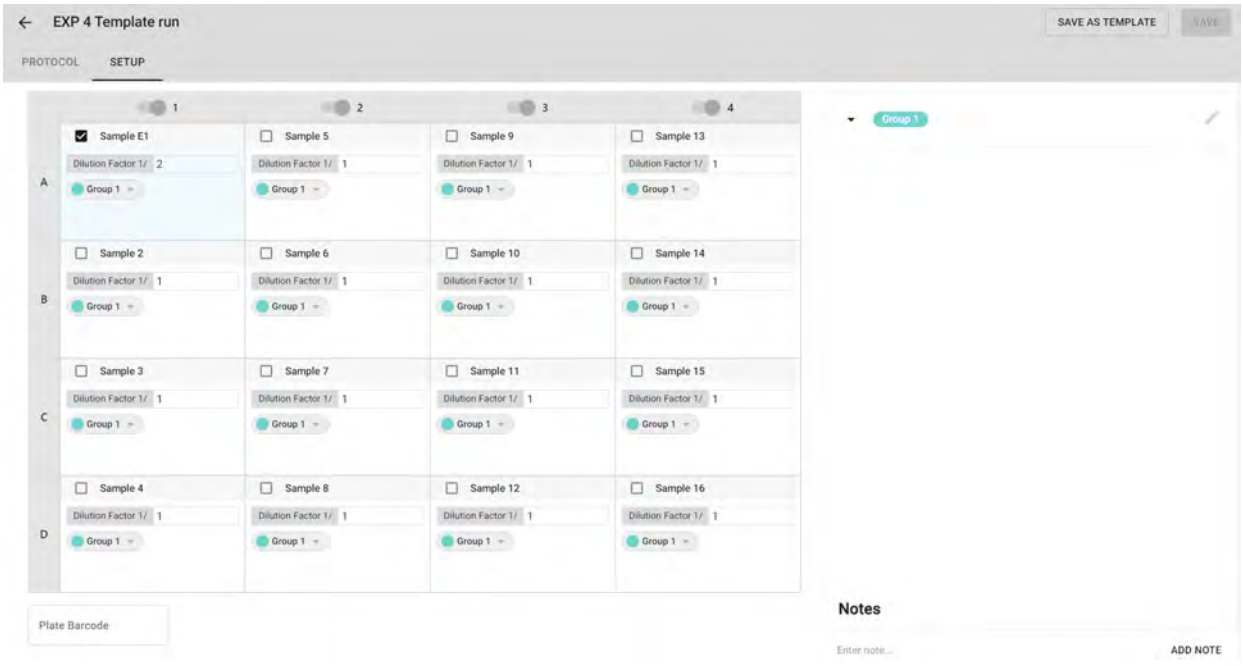


Figure 15 SETUP page for draft runs

1. To access the **SETUP** page, perform one of the following actions based on the type of analysis you are doing.

Analysis type	Action
Single-plate analysis	See “Open a completed run” on page 69.
Multi-plate analysis	See “Open a study for analysis” on page 75.

2. In the sample plate area, at the top of each column of the plate, include or exclude the column by setting the toggle switch on or off.

IMPORTANT! Failure to deselect the columns that are not in use prevents them from being used in a subsequent run.

3. Modify the plate settings if needed.

Setting	Action
Sample name	Sample wells are preconfigured with a default name. To change the name, perform the following action. • Click the sample well name, then type the new sample name.
Dilution factor	Sample wells are preconfigured with a dilution factor of 1. To change the dilution factor, perform one of the following actions. • For a single sample well, enter a value in the **Dilution factor** field of the well. • For multiple sample wells, enter a value in the **Dilution factor** field above the plate table, then click **APPLY TO SELECTED**.
Group	By default, all sample wells are assigned to the preconfigured **Group 1**. To change the group, perform one of the following actions. • For a single sample well, click the dropdown arrow by the group name, then select a group from the list. • For multiple sample wells, click the dropdown arrow in the **Sample group** field above the plate table, select the group, then click **APPLY TO SELECTED**. To add or edit a group, see “Manage groups” on page 63.
EDIT DYES	Select **EDIT DYES**, to change the dye assignments for the green and dark red channels for this run.

4. *(Optional)* Click ··· in the upper-right corner of the sample table to access the **Import plate setup** and **Export plate setup** options.

Option	Actions
Import plate setup—Use to import an existing plate setup. You can use the imported plate setup as-is, or modify as needed.	1. Click ···, then select **Import plate setup**. 2. In the **File browser**, navigate to the location of the plate setup CSV file to be imported. 3. Select the plate setup file, then click **Open**.
Export plate setup—Use to export the plate setup file to modify or use as-is in other templates or runs.	1. Click ···, then select **Export plate setup**. 2. In the **File browser**, navigate to the location of where to export the plate setup file. 3. Click **Save**.

5. *(Optional)* In the **Notes** area, **Enter note...** field, type notes related to this plate setup, then click **ADD NOTE**.

6. *(Optional)* In the **Plate Barcode** field, enter the barcode for the plate associated with this run.

Note: The instrument scans the plate for the barcode when the run is started. If your process is to ensure that a specific plate is used for this run, enter the barcode for the plate here. If the barcode entered here does not match the scanned number, the software returns an error. If this occurs, either load the correct plate, or return to the **SETUP** page to correct the barcode number.

7. *(Optional)* Create a template of the current run parameters.
   a. Click **SAVE AS TEMPLATE**.
   b. When prompted, type the new name in the **Template name** field, then click **SAVE AS TEMPLATE**.
8. When run configuration is complete, click **SAVE** .
9. *(Optional)* For implementations using Security, Auditing, and E-signature (SAE) v2.2 software, click **E-SIGN** to provide an e-signature. See, “Sign data in the software” on page 149.
10. *(Optional)* Click **START RUN** to start the run.

Note: This function is only available if the desktop computer is attached to an instrument.

Edit a draft run from the Runs page

This section provides information about editing the protocol and plate setup parameters on a draft run.

Note: Protocol parameters cannot be modified on completed runs. Protocol parameters can only be modified on draft runs with a **PRE-RUN** status.

1. From the left pane click to open the **Runs** list page.
2. On the **DRAFT** tab, use the search field to find a run or select a run from the list.
3. *(Optional)* Use the filter option to find and select a run.
   a. Click located above the run list.
   b. Select one or many filters from the filter options, then click **APPLY**.

   Note: If no filters are selected, all runs are displayed.

Filter	Option
STATUS	• **Active**—The run is in progress. • **Available**—The run has completed without errors. • **Obsolete**—The run has completed on the instrument but analysis has not started. • **Unknown**—The run was unsuccessful and no data are available for that run.
INSTRUMENT	Instruments associated with the run data either generated in or imported into the system.

   The run list refreshes to show runs with the selected filters.
   c. *(Optional)* Click the on the filter name to remove a filter selection from the list of runs.
4. *(Optional)* To modify protocol parameters on a draft run, see “PROTOCOL page (runs)” on page 51.
5. *(Optional)* To modify plate setup parameters, see “SETUP page (DRAFT runs)” on page 54.

Start a single plate run

This section provides information about starting a single plate run in a non-automated configuration.

The **START RUN** function is only available when the dedicated computer is connected to an instrument.

IMPORTANT! You must inspect and clean the plate nest before each run. See “Clean the Absolute Q™instrument and plate nest” on page 154.

IMPORTANT! Before running the protocol, ensure that your protocol parameters are defined correctly. Protocol parameters cannot be changed after the run. See “PROTOCOL page (runs)” on page 51.

1. From the left pane click to open the **Runs** list page.
2. Use the search field to find a run or select a run from the list.
3. To start a run, perform one of the following actions.
   - From the **RUNS** page, **DRAFT** tab, select a run with a **DRAFT** status, then click **START RUN**.
   - From the **RUNS** page, **SETUP** tab, 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.

4. In the **Plate barcode** field, add the barcode number of the MAP plate.
5. 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.

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

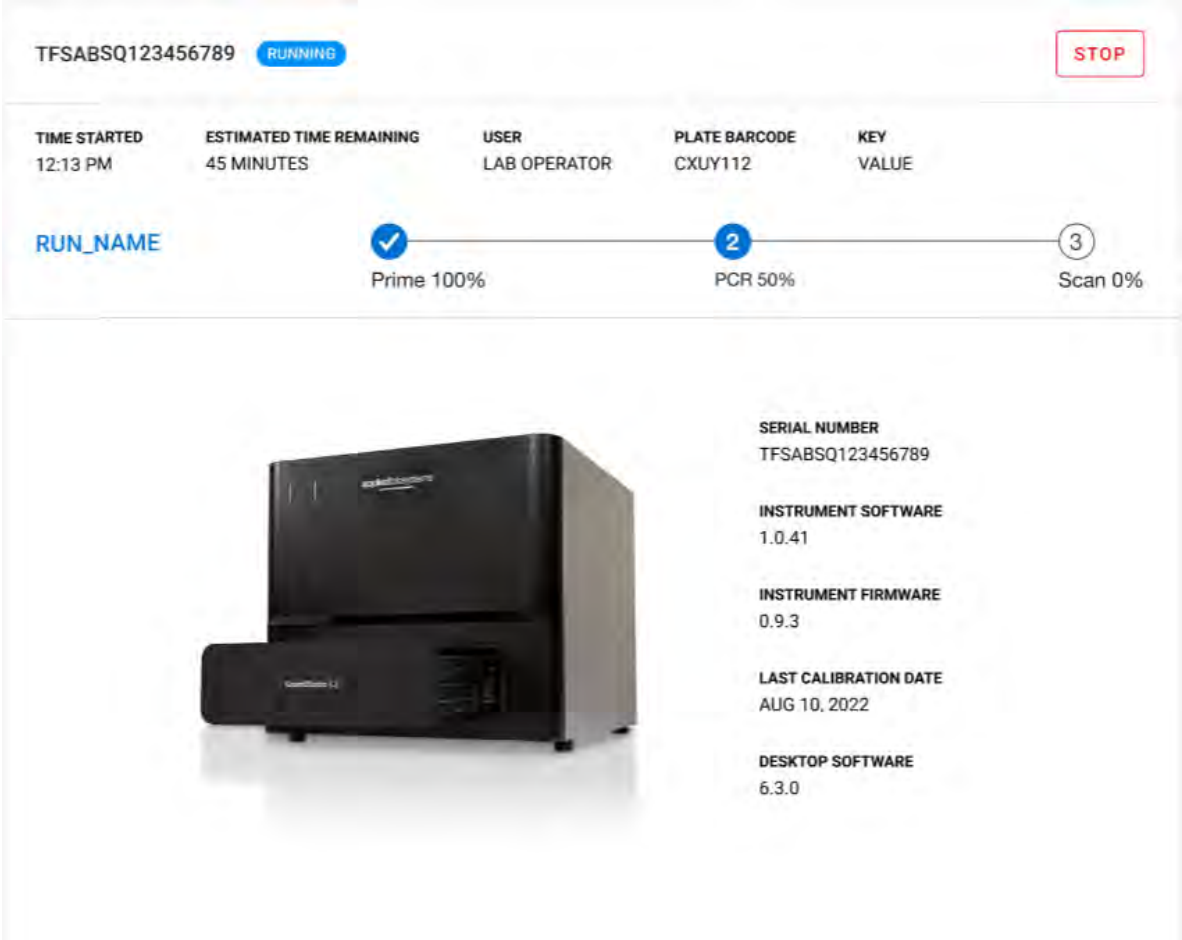
   The door closes and the MAP plate barcode is scanned.

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**.

7. *(Optional)* To check the status of the run, from the left pane, select ▬ to open the **Instrument** page.

   The status bar to the right of the run name shows the progress of the run.



Import runs

Use the **IMPORT RUN** option to import runs created on other Absolute Q™ systems or runs generated on an automated system. Runs can be imported from either the **DRAFT** or **COMPLETED** tabs. Finished runs have a ZST file extension.

Note: A run can be imported more than one time. Each time the run is imported it is treated as a separate run, it does not overwrite the previously imported data.

1. From the left pane click to open the **Runs** list page.
2. Click **IMPORT RUN**.
3. From the **Import runs** dialog, perform one of the following actions.

Option	Action
Drag-and-drop run files from a location on your file system.	1. Using **File Explorer**, find the DPR, ZIP, or ZST file or files in your file system, then drag-and-drop the file into the **Import runs** dialog box. 2. When the import completes, in the **Import Status** dialog, click **CLOSE**.
Browse to find the run files.	1. In the **Import runs** dialog, click **IMPORT FILES**. 2. In the **File browser**, navigate to the location of the find the DPR, ZIP, or ZST file or files to be imported. 3. Select the run file or files, then click **Open**. 4. When the import completes, in the **Import Status** dialog, click **CLOSE**.

Note: If there are runs that cannot be imported, an error appears in the **Import Status** dialog. Make note of the error so that you can take corrective action on the run, then click **CLOSE** to continue.

The run list refreshes to include the imported runs.

Export runs

Use the **EXPORT** option to export run files for use on other Absolute Q™ systems. Run files can be exported from either the **DRAFT** or **COMPLETED** tabs.

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

2. Use the following options to export runs that have not been started from the **DRAFT** tab or runs that have finished from **COMPLETED** tab.

Option	Action
Export draft run files—Runs that have not been run have a DPR file extension.	1. From the **DRAFT** tab, use the search field to find the runs for export, then select one or more runs. 2. Select **EXPORT**. 3. In the **File browser**, navigate to the location of where to export the run file or files. 4. Click **Select Folder**.
Export completed run files—Runs that have finished have a ZST file extension.	1. From the **DRAFT** tab, select the **COMPLETED** tab. 2. Use the search field to find the runs for export, or click to filter the runs by **STATUS**, **INSTRUMENT**, or **STUDY-COMPATIBLE RUNS**. 3. Select one or more runs. 4. Select **EXPORT**. 5. In the **File browser**, navigate to the location of where to export the run file or files. 6. Click **Select Folder**.

3. When the export completes, in the **Export Status** dialog, click **CLOSE**.

Note: If there are templates that cannot be exported, an error appears in the **Export Status** dialog. Make note of the error so that you can take corrective action on the template, then click **CLOSE** to continue.

Rename a run

Only one run can be renamed at a time. Runs can be renamed from either the **DRAFT** or **COMPLETED** tabs.

1. From the left pane click to open the **Runs** list page.
2. Use the following options to rename runs that have not been started from the **DRAFT** tab or runs that have finished from **COMPLETED** tab.

Option	Action
Rename draft run files—Runs that have not been run have a DPR file extension.	1. From the **DRAFT** tab, use the search field to find the runs to rename or select a run from the list. 2. Click **RENAME**. 3. When prompted, enter the new name, then click **RENAME**.
Rename completed run files—Runs that have finished have a ZST file extension.	1. From the **DRAFT** tab, select the **COMPLETED** tab. 2. Use the search field to find a run to rename, or click to filter the runs by **STATUS**, **INSTRUMENT**, or **STUDY-COMPATIBLE RUNS**. 3. Select a run, then click **RENAME**. 4. When prompted, enter the new name, then click **RENAME**.

The **Runs** list is refreshed and displays the renamed run.

Delete a run

Multiple runs can be deleted at a time. Runs can be deleted from either the **DRAFT** or **COMPLETED** tabs.

1. From the left pane click to open the **Runs** list page.
2. Use the following options to delete runs that have not been started from the **DRAFT** tab or runs that have finished from **COMPLETED** tab.

Option	Action
Delete draft run files—Runs that have not been run have a DPR file extension.	1. From the **DRAFT** tab, use the search field to find the runs to delete or select a run from the list. 2. Click **DELETE**. 3. Proceed to step 3.
Delete run files—Runs that have finished have a ZST file extension.	1. From the **DRAFT** tab, select the **COMPLETED** tab. 2. Use the search field to find a run to delete, or click to filter the runs by **STATUS**, **INSTRUMENT**, or **STUDY-COMPATIBLE RUNS**. 3. Select a run, then click **DELETE**. 4. Proceed to step 3.

3. When prompted, confirm that the selected run or runs are to be deleted by doing one of the following actions.
   - To delete the run(s), click **DELETE RUN** or **DELETE (# of runs selected) RUNS**.
   - To cancel the delete action, click **DO NOT DELETE**.

Manage groups

Groups are used to define the analysis and results type for reporting for individual samples or sets of samples. After a group has been defined, it can be edited or deleted.

Note: Only groups without samples can be deleted.

When samples are assigned to a group, they will all have the same definition for the following characteristics of the sample.

- Grouping options.
  - **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 samples in the group as one large sample.
- The target DNA associated with each fluorescent dye.
- The analysis type for each optical channel.
  - **CNV** (Copy Number Variation)—Reporting ratio of CNV/CNV Ref

    Note: A reference must be selected when using CNV. If multiple CNV are selected, they will share the same reference.
  - **CNV Ref** (Copy Number Variation Reference)—The reference target for CNV.

    Note: The reference target is a gene of known and stable copy number used to calculate the copy number for the gene of interest.
  - **Signal**—Absolute quantification.
- Default threshold setting.
  - **Auto Sample**—The software automatically assigns the threshold for each sample independently.
  - **Auto Group**—The software aggregates the samples in the group and automatically determines a common threshold.
  - **Manual**—Set a specific threshold for the channel.

See the following sections for more information.

- To create groups, see “Create groups” on page 64.
- To edit groups, see “Edit groups” on page 65.
- To delete groups, see “Delete groups” on page 65.
- To add samples to groups, see “Assign samples to groups” on page 66.

Create groups

Groups can be created from either the **Templates** or **Runs** pages.

1. In the left pane, perform one of the following actions.
   - Click to open the **Runs** list page.
   - Click to open the **Templates** list page.
2. Use the search field to find a run or template, or select a run or template from the list.
   When accessing this option from the **Templates** page, select **Edit Template**, then click **EDIT TEMPLATE**.
3. Select the **SETUP** tab.
4. In the **Sample groups** area, click **+ ADD GROUP**.
5. In the **Group name** field, enter a name for the group.
6. Select one of the following sample grouping options.
   - **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 of the samples in the group as one large sample.
7. *(Optional)* Toggle an optical channel on or off as needed for the sample group.
8. In the **Target** fields, enter the name of the DNA target for each active optical channel.
9. From the **Analysis** dropdown, select the analysis type for each optical channel.
   - **CNV** (Copy Number Variation)—Reporting ratio of CNV/CNV Ref

     Note: A reference must be selected when using CNV. If multiple CNV are selected, they will share the same reference.
   - **CNV Ref** (Copy Number Variation Reference)—The reference target for CNV.

     Note: The reference target is a gene of known and stable copy number used to calculate the copy number for the gene of interest.
   - **Signal**—Absolute quantification.
10. From the **Default Threshold** dropdown, select one of the following options.
    - **Auto Sample**—The software automatically assigns the threshold for each sample independently.
    - **Auto Group**—The software aggregates the samples in the group and automatically determines a common threshold.
    - **Manual**—Set a specific threshold for the channel.

Note: The default threshold setting is an initial value used in the analysis and can be changed. See Chapter 4, “Analyze data”.

11. Select one of the following options.
    - **CONTINUE** to return to the **SETUP** page, then click **SAVE**.
    - **ADD & CONTINUE** to add another group.

Edit groups

Groups can be edited from either the **Templates** or **Runs** pages.

1. In the left pane, perform one of the following actions.
   - Click to open the **Runs** list page.
   - Click to open the **Templates** list page.
2. Use the search field to find a run or template, or select a run or template from the list.

- When accessing this option from the **Templates** page, select **Edit Template**, then click **EDIT TEMPLATE**.

3. Select the **SETUP** tab.
4. In the **Sample Groups** area, click in the group to be edited.
5. Edit the group settings.
   For information about group settings, see “Create groups” on page 64.
6. Click **SAVE CHANGES**.

Delete groups

Groups can be deleted from either the **Templates** or **Runs** pages.

Only groups that do not contain samples can be deleted.

1. In the left pane, perform one of the following actions.
   - Click to open the **Runs** list page.
   - Click to open the **Templates** list page.
2. Use the search field to find a run or template, or select a run or template from the list.

- When accessing this option from the **Templates** page, select **Edit Template**, then click **EDIT TEMPLATE**.

3. Select the **SETUP** tab.
4. In the **Sample Groups** area, click in the group to be deleted.
5. Below the group information area, click **DELETE SAMPLE GROUP**.

Assign samples to groups

Assigning samples to groups defines the analysis and results type for reporting for individual samples or sets of samples.

Samples can be assigned to groups from the **Runs**, **Templates**, or **Studies** pages.

1. In the left pane, perform one of the following actions.
   - Click to open the **Runs** list page.
   - Click to open the **Templates** list page.
   - Click to open the **Studies** list page.
2. Use the search field to find a run, template, or study, or select one from the list.

- When accessing this option from the **Templates** page, select **Edit Template**, then click **EDIT TEMPLATE**.

3. From the **SETUP** tab, use one of the following actions to assign samples to a group.

Option	Action
Single sample well	In the sample well, click the dropdown arrow by the group name, then select a group from the list.
Multiple sample wells	1. Select the sample wells. 2. Click the dropdown arrow in the **Sample group** field above the plate table, then select the group. 3. Click **APPLY TO SELECTION**.

Note: By default, all sample wells are assigned to the preconfigured **Group 1**. For information on adding groups, see “Create groups” on page 64

4. Repeat step 3 as needed to assign all samples to groups.
5. When sample assignments are complete, click **SAVE**.

4 Analyze data

Software features

QuantStudio™ Absolute Q™ Digital PCR Software provides the following analysis options.

- Single-plate analysis—The **COMPLETED** tab on the **Runs** page shows single-plate experiment results organized by plate run. See “Single-plate analysis” on page 69.
- Multi-plate analysis—The **Studies** page shows multi-plate experiment results for a group of completed runs and allows the results of those runs to be analyzed together. See “Multi-plate analysis–Studies” on page 71.

Both options are organized by plate run and provide access to the **PROTOCOL**, **SETUP**, **ANALYSIS**, and **RESULTS** pages for analyzing and viewing experiment results.

- The **PROTOCOL** page provides the protocol settings used for a completed run and statistics regarding the run. See “PROTOCOL page (analysis)” on page 79.
- The **SETUP** page provides controls for analysis options including sample and target names, sample groups, replicate statistics, pooling, and copy number calculations. See “SETUP page (analysis)” on page 76.
- The **ANALYSIS** page shows relevant information by samples or groups and provides different options for viewing the data. See “ANALYSIS page” on page 80.
- The **RESULTS** page shows a summary of the run data and provides reporting and data exporting options. See “RESULTS page” on page 113.

Analysis workflow

The following is a typical workflow for analyzing run data.

Analysis workflow
Select a completed run (page 69) or Study (page 75) to begin analysis.
Make plate setup modifications before starting analysis if needed. See SETUP page (analysis) (page 76).
View the general analysis in the sample groups gallery and sample groups detail pages. See Sample Groups (page 80).
Investigate QC flags and adjust QC bounds if needed. See QC data and arrays (page 109).
View and adjust individual sample thresholds if needed. See Samples (page 98).
View the results and output data (page 113) and generate reports (page 115).
Repeat the previous steps if needed to finalize the results.

Single-plate analysis

The sections that follow provide information about viewing and analyzing single-plate runs from the **COMPLETED** tab.

You can perform the following tasks from the **COMPLETED** tab on the **Runs** page.

- View information about the run including plate barcode number, status, user who created the run, the date the run was last modified, and the exported date.
- Open a run to view run parameters, set analysis parameters, and view results of a single run. See “Open a completed run” on page 69
- **ADD TO STUDY**—Studies are a group of completed runs that allow the results of those runs to be analyzed together. See “Add completed runs to a study” on page 72.
- **EXPORT**—Use this option to export runs for use on other Absolute Q™ systems. See “Export runs” on page 61.
- **RENAME**—Use this option to rename a run. See “Rename a run” on page 62 .
- **DELETE**—Use this option to delete one or more runs. See “Delete a run” on page 62.
- **IMPORT RUN**—Use this option to import runs created on other Absolute Q™ systems or runs generated on an automated system. See “Import runs” on page 60.
- **CREATE RUN**—Use this option to create a new run. See “Create a run from the RUNS page” on page 51.

Open a completed run

This section provides information about managing **COMPLETED** runs.

COMPLETED runs can have the following statuses.

- **ABORTED**—The run execution was aborted on the instrument.
- **FINISHED**—The run completed without error.
- **ANALYZING**—The run is in the analysis phase.
- **ERROR**—An error has occurred during the run.

Use the **COMPLETED** tab to open a run and perform the following actions on completed runs.

- View or modify plate settings. See “SETUP page (analysis)” on page 76.
- View protocol information. See “PROTOCOL page (analysis)” on page 79.
- Perform analysis. See “ANALYSIS page” on page 80.
- View and export results. See “RESULTS page” on page 113.
- Start multi-plate analysis by adding runs to studies. See “Add completed runs to a study” on page 72.

1. From the left pane click to open the **Runs** list page.
   By default, the **Runs** page opens on the **COMPLETED** tab that shows the list of completed runs.
2. Use the search field to find a run or select a run from the list.
3. *(Optional)* Use the filter option to find and select a run.
   a. Click located above the run list.

b. Select one or many filters from the filter options, then click **APPLY**.

Note: If no filters are selected, all runs are displayed.

Filter	Option
STATUS	• **Analyzing**—The run is in progress. • **Completed**—The run has completed without errors. • **Waiting**—The run has completed on the instrument but analysis has not started. • **Error**—The run was unsuccessful and no data are available for that run.
INSTRUMENT	Instruments associated with the run data.
STUDY-COMPATIBLE RUNS	**Filter selections by study compatibility**—Select to filter the run list by the study inclusion criteria. For information about study criteria, see "Multi-plate analysis–Studies" on page 71.

The run list refreshes to show runs with the selected filters.

c. *(Optional)* Click the ⊗ on the filter name to remove a filter selection from the list of runs.

4. Select a run from the list to view information about that run, then perform any of the following actions.
   - View or modify plate settings. See "SETUP page (analysis)" on page 76.
   - View protocol information. See "PROTOCOL page (analysis)" on page 79.
   - Perform analysis. See "ANALYSIS page" on page 80.
   - View and export results. See "RESULTS page" on page 113.
   - Start multi-plate analysis by adding runs to studies. See "Add completed runs to a study" on page 72.

Multi-plate analysis–Studies

The sections that follow provide information about viewing and analyzing multi-plate runs from the **Studies** page.

Studies are a group of completed runs from multiple plates that enable the results of those runs to be analyzed together.

The following information should be taken into consideration when working with studies.

- Studies can contain up to 24 runs.
- Changes made to runs added to a study do not affect the original run.
- Samples from runs in a study that have identical group setups are merged into a single group for analysis.

IMPORTANT! To be included in a study, runs must meet the following criteria.

- All runs must be created with the same software version.
- Each run must have completed successfully and have a FINISHED status.
- Each run must have used the same optical collection settings in the run protocol.
- The same instrument must be used to collect the data.

Studies are created using the following options.

- From the **Runs** page, see “Add completed runs to a study” on page 72.
- From the **Studies** page, see “Create a study” on page 72.

The sections that follow provide information about viewing and analysing multi-plate runs from the **Studies** page.

To open a study to view run parameters, set analysis parameters, and view results of a study, see “Open a study for analysis” on page 75

You can perform the following tasks from the **Studies** page.

- **CREATE A STUDY**—Use this option to create a study. See “Create a study” on page 72.
- **IMPORT**—Use this option to import studies created on other Absolute Q™ systems. See “Import studies” on page 74.
- **EXPORT**—Use this option to export studies for analysis on other Absolute Q™ systems. See “Export studies” on page 74.
- **RENAME**—Use this option to rename a study. See “Rename a study” on page 75.
- **DELETE**—Use this option to delete one or more studies. See “Delete a study” on page 75.

Manage studies

The following sections provide information about managing studies.

You can perform the following tasks when managing studies.

- Create a study in the **Studies** page, see “Create a study” on page 72.
- Add completed runs to a study, see “Add completed runs to a study” on page 72.
- **IMPORT STUDIES**—Use this option to import studies created on other Absolute Q™ systems. See “Import studies” on page 74.
- **EXPORT STUDIES**—Use this option to export studies for analysis other Absolute Q™ systems. See “Export studies” on page 74.
- **RENAME**—Use this option to rename a study. See “Rename a study” on page 75.
- **DELETE**—Use this option to delete a study. See “Delete a study” on page 75.

Create a study

Studies can be created from the **Studies** page or from completed runs on the **COMPLETED** page.

For information about creating a study from the **COMPLETED** tab on the **Runs** page, see “Add completed runs to a study” on page 72.

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

   Note: If there are no existing studies, you are prompted to **GO TO COMPLETED RUNS** to create your first study by adding runs from the runs list.

2. Click **CREATE STUDY** from upper-right corner of **Studies** list page.

3. When prompted, click **GO TO COMPLETED RUNS**, to add runs to the study.
   The **COMPLETED** tab on the **Runs** page opens. To continue with adding runs to a study, see “Add completed runs to a study” on page 72.

Add completed runs to a study

Add multiple runs to a study to analyze the samples in those runs as a group.

Completed runs are added to studies from the **Runs**, **COMPLETED** tab.

IMPORTANT! To be included in a study, runs must meet the following criteria.

- All runs must be created with the same software version.
- Each run must have completed successfully and have a FINISHED status.
- Each run must have used the same optical collection settings in the run protocol.
- The same instrument must be used to collect the data.

1. From the **Runs**, **COMPLETED** tab, perform one of the options to identify the first run to include in the study.

Option	Action
Use the search field to find a run.	Enter run information in the search field. Search for runs using the following options. • Run name • Plate barcode • Instrument name
Use the filter option to find completed runs.	1. Click ▼ located above the run list 2. From the **Filter runs** dialog, select **Finished**, then click **APPLY**

2. Select a run from the filtered run list.

3. Use the filter option to find and select runs that meet study-compatible criteria.
   a. Click ▼ located above the run list.
   b. From the **Filter runs** dialog, select **Filter selections by study compatibility**, then click **APPLY**.
      The run list refreshes to show runs that meet study-compatible criteria.
   c. *(Optional)* Click the ⊗ on the filter name to remove a filter selection from the list of runs.

4. Select the run or runs to be added, then click **ADD TO STUDY**.

5. In the **Add to Study** dialog, choose one of the following options.

Option	Actions
Create a new study.	1. Select **Create new study**. 2. In the **Study Name** field, enter a name for the study. 3. Click **ADD RUNS TO STUDY**.
Add to an existing study.	1. Select **Add to existing study**. 2. In the **Study Name** field, select a study from the dropdown list. 3. Click **ADD RUN TO STUDY**.

Import studies

Use the **IMPORT STUDIES** option to import studies created on other Absolute Q™ systems. Studies files have a ZIP extension.

1. From the left pane, click to open the **Studies** list page.
2. Click **IMPORT STUDIES**.
3. From the **Import studies** dialog, perform one of the following actions.

Option	Action
Drag-and-drop run files from a location on your file system.	1. Using **File Explorer**, find the ZIP file or files in your file system, then drag-and-drop the file into the **Import study** dialog box. 2. When the import completes, in the **Import Status** dialog, click **CLOSE**.
Browse to find the run files.	1. In the **Import studies** dialog, click **IMPORT FILES**. 2. In the **File browser**, navigate to the location of the find the ZIP file or files to be imported. 3. Select the study file or files, then click **Open**. 4. When the import completes, in the **Import Status** dialog, click **CLOSE**.

Note: If there are studies that cannot be imported, an error appears in the **Import Status** dialog. Make note of the error so that you can take corrective action on the run, then click **CLOSE** to continue.

The run list refreshes to include the imported studies.

Export studies

Use the **EXPORT** option to export studies for use on other Absolute Q™ systems.

1. From the left pane, click to open the **Studies** list page.
2. Use the search field to find the studies for export, then select one or more studies from the list.
3. Click **EXPORT**.
4. In the **File browser**, navigate to the location of where to export the study file or files.
5. Click **Select Folder** .

   If there are studies that cannot be exported, an **Export status** dialog opens that shows the status of the export, click **CLOSE** to continue.

Rename a study

Only one study can be renamed at a time.

1. From the left pane, click to open the **Studies** list page.
2. Use the search field to find a study or select a study from the list.
3. Click **RENAME**.
4. When prompted, enter the new name in the **Study name** field, then click **RENAME**.
   The **Studies** list is refreshed and displays the renamed template.

Delete a study

Multiple studies can be deleted at a time.

1. From the left pane, click to open the **Studies** list page.
2. Use the search field to find the studies to be deleted.
3. From the **Studies** list, select the study or studies to be deleted, then click **DELETE**.
4. When prompted, confirm that the selected study or studies are to be deleted by doing one of the following actions.
   - To delete the study or studies, click **DELETE STUDY** or **DELETE (# of studies selected) STUDIES**.
   - To cancel the delete action, click **DO NOT DELETE**.

Open a study for analysis

Use the **Studies** page to open a study to view runs that are grouped into a study and prepare them for analysis.

1. From the left pane, click to open the **Studies** list page.
2. Use the search field to find a study or select a study from the list.
3. *(Optional)* To show the list of runs contained in a study, click ▶.
4. Select a study from the list to view information about that study.
   The study opens on the **SETUP** page of the first run in the study.
5. *(Optional)* To view the setup of a different run, select the run from the **Runs** list.
6. *(Optional)* To remove a run from the study, in the **Runs** list, click ⋮, then click **Remove from study**.

7. To continue with analysis, perform any of the following actions.
   - View or modify plate settings. See “SETUP page (analysis)” on page 76.
   - View protocol information. See “PROTOCOL page (analysis)” on page 79.
   - Perform analysis. See “ANALYSIS page” on page 80.
   - View and export results. See “RESULTS page” on page 113.

SETUP page (analysis)

The **SETUP** page for completed runs provides the following information regarding each plate.

- The date and time the run completed.
- The plate barcode number.
- The instrument name of the instrument that was used.
- The name of the user shown is based on the implementation.
- For implementations using Security, Auditing, and E-signature (SAE) v2.2 software, the Run ID appears below the run name in the upper-left corner of the page. See Appendix E, “Use the software with Security, Auditing, and E-signature (SAE) v2.2”.

Implementation	User name
Standalone not using SAE	Unknown (local)
Standalone using SAE	The user name of the person who performed the run.
Runs imported from other systems	Unknown (remote)

You can make the following changes to the setup of the MAP plate on a completed run.

- Edit the sample names—Sample names are user-assigned identifiers for the contents of each loaded well of a plate.
- Set the dilution factor—The dilution factor is the total dilution from the sample to the reaction mix and must be set with a value ≥1. This value is the denominator used in the dilution factor field.

  For example, if 2 µL of the sample is put into a 10 µL reaction mix, the dilution factor is [2 µL sample/ 10 µL reaction = 1/5]. In this case, the dilution factor denominator is 5.

  If instead the stock solution is diluted by a factor of 100, then 2 µL of the diluted sample is used in a 10µl reaction mix, then the dilution factor is [(1 µL stock / 100 µL dilution) × (2 µL dilution/ 10 µL reaction) =1/500]. In this case, the dilution factor denominator is 500.

  Note: The dilution factor field accepts scientific notation, for example 10E+10.

- Assign groups—Groups determine what type of analysis is applied to all samples within a group.
- **ADD GROUP**—Create more groups. See “Manage groups” on page 63.
- **EDIT DYES**—Reassign dyes to the green and dark red channels for this run.
- **Export plate setup**—Use to export a blank or configured plate setup CSV file to modify outside software and import for use.
- **Import plate setup**—Use to import plate existing setup files.
- **Notes**—Add notes regarding the plate setup.

- **SAVE AS TEMPLATE**—Save the run as a template.
- **E-SIGN**—For implementations using Security, Auditing, and E-signature (SAE) v2.2 software. See Appendix E, “Use the software with Security, Auditing, and E-signature (SAE) v2.2”.

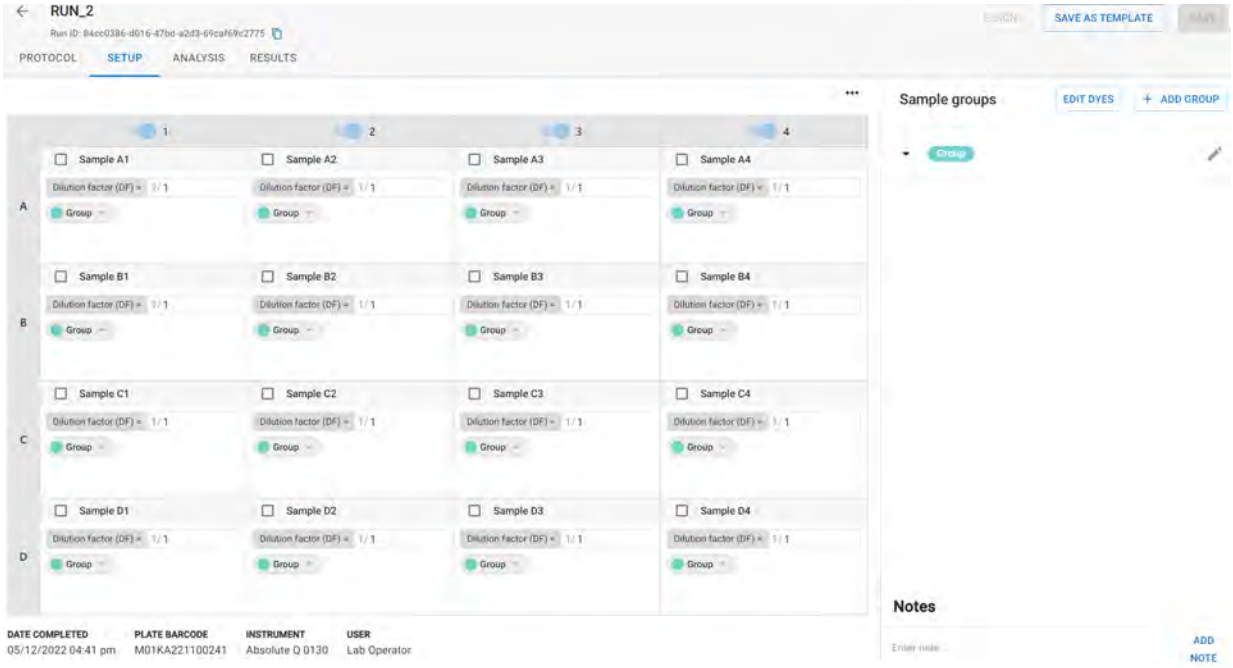


Figure 16 SETUP page for completed runs

1. To access the **SETUP** page, perform one of the following actions based on the type of analysis you are doing.

Analysis type	Action
Single-plate analysis	See “Open a completed run” on page 69.
Multi-plate analysis	See “Open a study for analysis” on page 75.

2. For multi-plate analysis (studies), from the **Run name** list, select a run.

3. Modify the plate settings if needed.

Setting	Action
Sample name	Sample wells are preconfigured with a default name. To change the name, perform the following action. • Click the sample well name, then type the new sample name.
Dilution factor	Sample wells are preconfigured with a dilution factor of 1. To change the dilution factor, perform one of the following actions. • For a single sample well, enter a value in the **Dilution factor** field of the well. • For multiple sample wells, enter a value in the **Dilution factor** field above the plate table, then click **APPLY TO SELECTED**.
Group	By default, all sample wells are assigned to the preconfigured **Group 1**. To change the group, perform one of the following actions. • For a single sample well, click the dropdown arrow by the group name, then select a group from the list. • For multiple sample wells, click the dropdown arrow in the **Sample group** field above the plate table, select the group, then click **APPLY TO SELECTED**. To add or edit a group, see “Manage groups” on page 63.
EDIT DYES	Select **EDIT DYES**, to change the dye assignments for the green and dark red channels for this run.

4. *(Optional)* Click ··· in the upper-right corner of the sample table to access the **Import plate setup** and **Export plate setup** options.

Option	Actions
Import plate setup—Use to import an existing plate setup. You can use the imported plate setup as-is, or modify as needed.	1. Click ···, then select **Import plate setup**. 2. In the **File browser**, navigate to the location of the plate setup CSV file to be imported. 3. Select the plate setup file, then click **Open**.
Export plate setup—Use to export the plate setup file to modify or use as-is in other templates or runs.	1. Click ···, then select **Export plate setup**. 2. In the **File browser**, navigate to the location of where to export the plate setup file. 3. Click **Save**.

5. *(Optional)* In the **Notes** area, **Enter note...** field, type notes related to this plate setup, then click **ADD NOTE**.

6. *(Optional)* Create a template of the current run parameters.
   a. Click **SAVE AS TEMPLATE**.
   b. When prompted, type the new name in the **Template name** field, then click **SAVE AS TEMPLATE**.

7. *(Optional)* For implementations using Security, Auditing, and E-signature (SAE) v2.2 software, click **E-SIGN** to provide an e-signature. See, “Sign data in the software” on page 149.

8. When changes are complete, click **SAVE** .

PROTOCOL page (analysis)

The **PROTOCOL** page for a completed run provides the following information regarding the protocol.

- Protocol settings used for the run.
- The date and time the run completed.
- The plate barcode number.
- The instrument name of the instrument that was used.
- The name of the user shown is based on the implementation.

Implementation	User name
Standalone not using SAE	Unknown (local)
Standalone using SAE	The user name of the person who performed the run.
Runs imported from other systems	Unknown (remote)

You can perform the following tasks in the **PROTOCOL** page on a completed run.

- Export the protocol.
- Save the protocol as a template.

- **EXPORT PROTOCOL**—Export the protocol.
- **SAVE AS TEMPLATE**—Make a new template
- **E-SIGN**—For implementations using Security, Auditing, and E-signature (SAE) v2.2 software. See Appendix E, “Use the software with Security, Auditing, and E-signature (SAE) v2.2”.

1. *(Optional)* Export the protocol by performing the following actions.
   a. Click **EXPORT PROTOCOL** to open the **File browser**, then navigate to the location where you want to save the exported protocol AQUA file.
   b. Click **Export**.

2. *(Optional)* Create a template of the run with a new name.
   a. Click **SAVE AS TEMPLATE**.
   b. When prompted, type the new name in the **Template name** field, then click **SAVE AS TEMPLATE**.

3. *(Optional)* For implementations using Security, Auditing, and E-signature (SAE) v2.2 software, click **E-SIGN** to provide an e-signature. See, “Sign data in the software” on page 149.

ANALYSIS page

The **ANALYSIS** page displays plots for sample groups and samples in gallery view or detail view. The sample list provides information about individual samples contained in a group and the ability to modify the sample data included in the plots.

Analysis by **Sample Group** lets you compare channels across the samples in a group and modify sample data on the group level. For information about analyzing runs by sample group, see “Sample Groups” on page 80.

Analysis by **Sample** lets you identify samples that have unusual QC values and lets you curate the data for analysis by individual sample. For information on analyzing sample data, see “Samples” on page 98.

Sample Groups

Viewing by **Sample Group** lets you compare channels across the samples in a group.

The sample group–gallery view plot provides and overview of the data in all samples, by channel, for each group, and lets you perform the following actions on sample data.

- Hide or unhide a sample for inclusion in analysis
- Omit or include a sample for inclusion in analysis
- Investigate QC alerts
- View sample data detail
- Download data plots

See the following sections for more information about the sample group–gallery view.

- For 1D Scatter plots, see “View and modify sample groups–1D Scatter plot–gallery view” on page 81.
- For 2D Scatter plots, see “View and modify sample groups–2D Scatter plot–gallery view” on page 89.
- For investigating QC data, see “QC data and arrays” on page 109.

The sample group–detail view plot provides detailed information by sample in a group for the selected channel and lets you perform the following actions on sample data.

- Hide or unhide a sample for inclusion in analysis
- Omit or include a sample for inclusion in analysis
- Investigate QC alerts
- View sample data detail
- Adjust thresholds for samples in a group by channel
- Adjust the data range to zoom in or out on an area of the plot
- Download data plots

Note: The sample group–detail view is accessed from the gallery view.

See the following sections for more information about the sample group–detail view.

- For 1D Scatter plots, see “View and modify sample groups–1D Scatter plot–detail view” on page 84.
- For 2D Scatter plots, see “View and modify sample groups–2D Scatter plot–detail view” on page 93.
- For investigating QC data, see “QC data and arrays” on page 109.

View and modify sample groups–1D Scatter plot–gallery view

This section provides information about viewing and modifying sample groups in the 1D Scatter plot gallery view.

For information about 2D Scatter plots, see “View and modify sample groups–2D Scatter plot–gallery view” on page 89

The sample group–detail view is accessed from the gallery. For information about the 1D Scatter plot sample group–detail view, see “View and modify sample groups–1D Scatter plot–detail view” on page 84.

Figure 17 Sample Group—1D Scatter–gallery view

Before performing analysis, complete any modifications that are needed to the sample setup of the plate or plates you are analyzing. See “SETUP page (analysis)” on page 76.

1. To access the **ANALYSIS** page, perform one of the following actions based on the type of analysis you are doing.

Analysis type	Action
Single-plate analysis	See “Open a completed run” on page 69.
Multi-plate analysis	See “Open a study for analysis” on page 75.

The run opens on the **ANALYSIS** page. By default the first sample group is selected in the **Analyze by** area and the plots are in 1D Scatter format.

The **Analyze by** area lets you modify the data for analysis by sample group or sample.

2. In the **Analyze by** area, click **Sample Group**, then select the group to be analyzed.

   The run results information for that group are displayed by channel in the gallery area. This information is an overview of the data of all samples contained in the group, presented in a single plot by channel. Individual sample data are shown in the sample list.

3. *(Optional)* To change the information in the gallery, select one of the following options.

Option	Description
	Show Rejects—Display or hide microchambers that have been rejected from the analysis results. Showing rejects does not impact the analysis or results calculations.
	2D Scatter plot type

4. In the **View sample** dropdown, set the view of the sample list by selecting one of the following options.

Option	Description
Ungrouped	Lists the samples contained in the group.
By omitted	Sorts the list of samples by omitted and included samples.
By run[1]	Groups the samples in the list by run.
By instrument[1]	Groups the samples in the list by instrument.
By plate barcode[1]	Groups the samples in the list by plate barcode.

[1] This option is only available in the **Studies** page.

5. *(Optional)* To modify the sort of the information in the sample list, use the settings in the column headings.

   The sample list lets you identify samples that have unusual QC values, review microchamber count information, and lets you perform the following actions on sample data to curate the data for analysis. Selecting a sample from the sample list lets you view and modify sample data at the sample level. For information about accessing sample information, see “Samples” on page 98.

   - Hide or unhide a sample for analysis
   - Omit or include a sample for analysis
   - Change the sort options of sample data
   - View sample data to investigate QC alerts

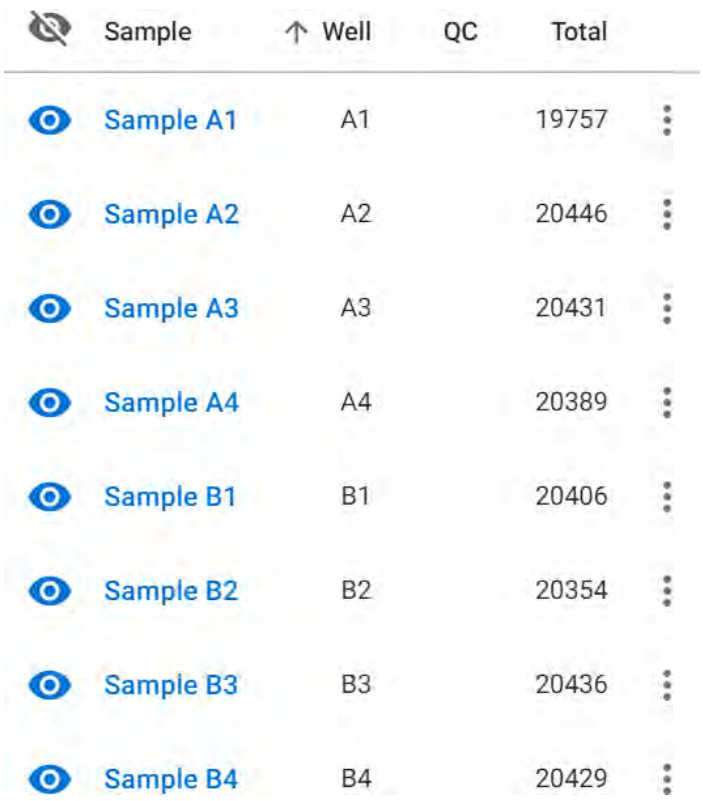


Figure 18 Sample list

Column	Action
—Toggle show or hide for all samples in the group.	Click to show or hide all the samples in the list.
Sample—Sample name.	Click the directional arrow to set the sort to ascending or descending order.
Well—Location of the sample on the plate.	
QC—Flags samples that have either failed or have unusual data. 1. —The sample failed and is excluded from analysis. 2. —The sample has unusual data that needs investigation.	
Total—Total number of accepted microchamber reactions less the number that have been rejected.	

6. *(Optional)* Use sample list options to modify sample inclusion or access sample detail information. When the changes are complete, click **SAVE** to recalculate the data and update the channel plots.

   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 plots and tables.

Option	Action
	Click to show or hide this sample.
Sample name	Click to access the sample detail PLOTS and QC pages and for this sample. Use to investigate samples that are flagged for QC and to modify thresholds for specific samples. See “QC data and arrays” on page 109 and “Samples” on page 98.
	Click to omit or include a sample in the analysis.

7. *(Optional)* To download a plot, click  , navigate to the location to save the file, then click **Save**.

For information about adjusting thresholds on sample groups, see “View and modify sample groups–1D Scatter plot–detail view” on page 84.

View and modify sample groups–1D Scatter plot–detail view

This section provides information about viewing and modifying sample groups in the 1D Scatter plot–detail view.

For information about 2D Scatter plots, see “View and modify sample groups–2D Scatter plot–detail view” on page 93.

For information about the sample group 1D Scatter plot–gallery view, see “View and modify sample groups–1D Scatter plot–gallery view” on page 81.

Before performing analysis, complete any modifications that are needed to the sample setup of the plate or plates you are analyzing. See “SETUP page (analysis)” on page 76.

1. To access the **ANALYSIS** page, perform one of the following actions based on the type of analysis you are doing.

Analysis type	Action
Single-plate analysis	See “Open a completed run” on page 69.
Multi-plate analysis	See “Open a study for analysis” on page 75.

   The run opens on the **ANALYSIS** page. By default the first sample group is selected in the **Analyze by** area and the plots are in 1D Scatter format.

   The **Analyze by** area lets you modify the data for analysis by sample group or sample.

2. In the **Analyze by** area, click **Sample Group**, then select the group to be analyzed.

   The run results information for that group are displayed by channel in the gallery area. This information is an overview of the data of all samples contained in the group, presented in a single plot by channel. Individual sample data are shown in the sample list.

3. *(Optional)* To change the information in the gallery, select one of the following options.

Option	Description
Show Rejects	Show Rejects—Display or hide microchambers that have been rejected from the analysis results. Showing rejects does not impact the analysis or results calculations.
	2D Scatter plot type

4. In the **View sample** dropdown, set the view of the sample list by selecting one of the following options.

Option	Description
Ungrouped	Lists the samples contained in the group.
By omitted	Sorts the list of samples by omitted and included samples.
By run[1]	Groups the samples in the list by run.
By instrument[1]	Groups the samples in the list by instrument.
By plate barcode[1]	Groups the samples in the list by plate barcode.

[1] This option is only available in the **Studies** page.

5. *(Optional)* To modify the sort of the information in the sample list, use the settings in the column headings.

   The sample list lets you identify samples that have unusual QC values, review microchamber count information, and lets you perform the following actions on sample data to curate the data for analysis. Selecting a sample from the sample list lets you view and modify sample data at the sample level. For information about accessing sample information, see “Samples” on page 98.

   - Hide or unhide a sample for analysis
   - Omit or include a sample for analysis
   - Change the sort options of sample data
   - View sample data to investigate QC alerts

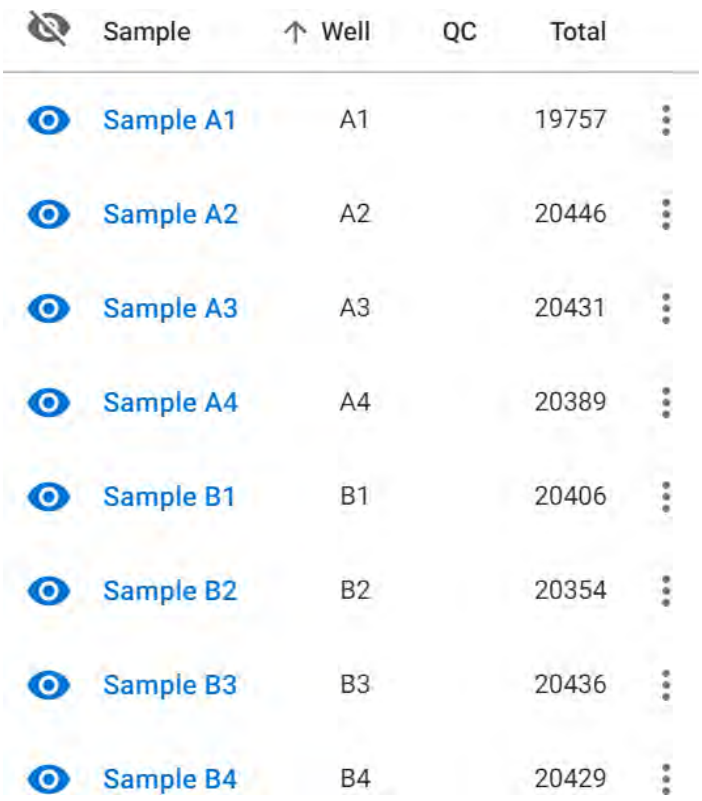


Figure 19 Sample list

Column	Action
—Toggle show or hide for all samples in the group.	Click to show or hide all the samples in the list.
Sample—Sample name.	Click the directional arrow to set the sort to ascending or descending order.
Well—Location of the sample on the plate.	
QC—Flags samples that have either failed or have unusual data. 1. —The sample failed and is excluded from analysis. 2. —The sample has unusual data that needs investigation.	
Total—Total number of accepted microchamber reactions less the number that have been rejected.	

6. *(Optional)* Use sample list options to modify sample inclusion or access sample detail information. When the changes are complete, click **SAVE** to recalculate the data and update the channel plots.

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 plots and tables.

Option	Action
	Click to show or hide this sample.
Sample name	Click to access the sample detail PLOTS and QC pages and for this sample. Use to investigate samples that are flagged for QC and to modify thresholds for specific samples. See “QC data and arrays” on page 109 and “Samples” on page 98.
	Click to omit or include a sample in the analysis.

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

Figure 20 Sample Group—1D Scatter–detail view

8. Use the following options to view data on the detail view plot.

Option	Action
View complete plot data.	Use the scroll bars on the right side and the bottom of the plot to adjust the view of the plot.
View information about a sample.	Hover over the sample column in the plot to show information about that sample. • **Sample**: Sample name • **Well**: Well location on the plate • **Threshold**: Threshold of the sample • **Total**: Total number of accepted microchamber reactions less the number that have been rejected • **Plate barcode**: Barcode number of the plate • **Run**: Name of the run • **Instrument**: Name of the instrument used for the run
View sample detail for a specific sample in the plot.[1]	1. Left-click a sample column in the plot. 2. Click **View Sample**. The sample detail page opens. For information about sample detail, see "Samples" on page 98.
Hide a sample from the plot area. [1]	1. Left-click a sample column in the plot. 2. Click **Hide Sample**. The plot refreshes and displays the data without the sample. The sample displays as hidden in the sample list. 3. *(Optional)* To include the hidden sample, click in the sample list.
Omit a sample from the plot area. [1]	1. Left-click a sample column in the plot. 2. Click **Omit Sample**. The plot refreshes and displays the data without the sample. The sample displays as omitted in the sample list. 3. *(Optional)* To include the omitted sample, click in the sample list, then click **Include sample**.

[1] This action can also be performed from the sample list.

9. *(Optional)* Change the plot type using the plot type option buttons.

10. *(Optional)* Change the channel for the detail view by selecting the desired channel at the top-left portion of the plot area.

11. *(Optional)* 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**.

12. *(Optional)* To magnify an area in the plot, adjust the upper and lower ends of the sample range.
    a. In the field on the upper-end of the y-axis, enter the desired value.
    b. In the field on the lower-end of the y-axis, enter the desired value.
    c. Click **SAVE**.
       The plot refreshes and the selected range is displayed.
    d. *(Optional)* To return the plot to the default range, click **Default range**.
13. *(Optional)* To download a plot, click ⬇, navigate to the location to save the file, then click **Save**.
14. *(Optional)* To return to the gallery view, click **BACK TO GALLERY**.

For information about viewing QC data and arrays, see “View and modify QC data” on page 109.

View and modify sample groups–2D Scatter plot–gallery view

This section provides information about viewing and modifying sample groups in the 2D Scatter plot gallery view.

The 2D scatter provides a visual correlation between two channels for a sample or sample group.

For information about 1D Scatter plots, “View and modify sample groups–1D Scatter plot–gallery view” on page 81.

The sample group–detail view is accessed from the gallery view. For information about the 2D Scatter plot sample group–detail view, see “View and modify sample groups–2D Scatter plot–detail view” on page 93.

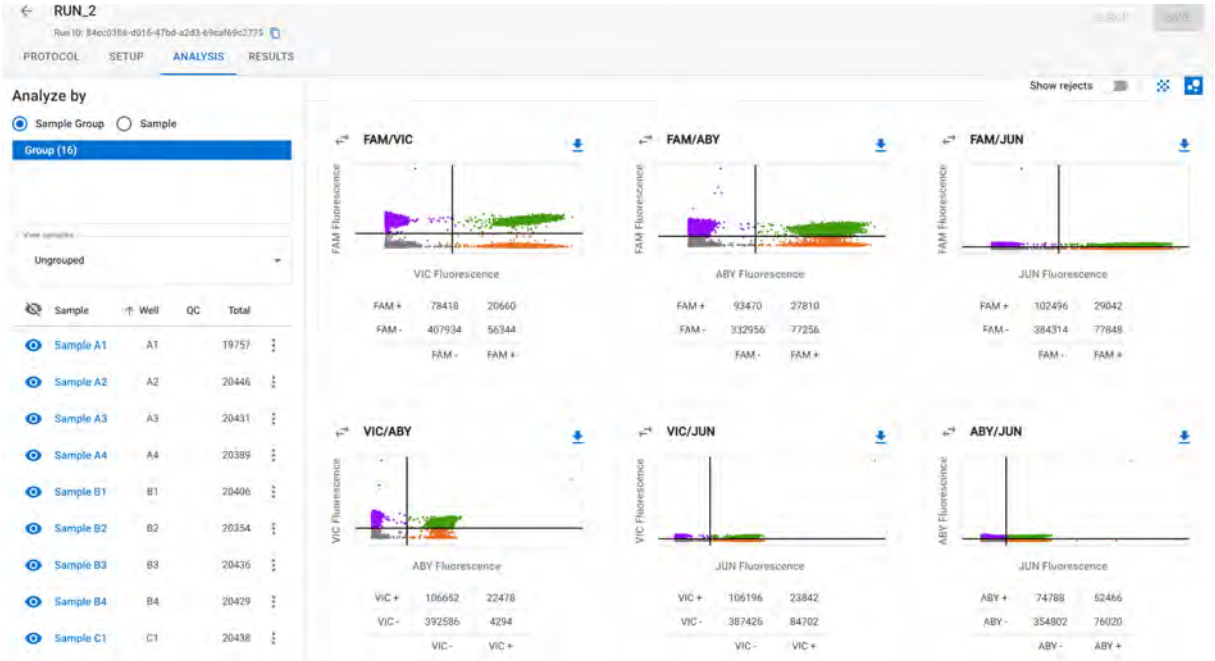


Figure 21 Sample Group—2D Scatter–gallery view

Before performing analysis, complete any modifications that are needed to the sample setup of the plate or plates you are analyzing. See “SETUP page (analysis)” on page 76.

1. To access the **ANALYSIS** page, perform one of the following actions based on the type of analysis you are doing.

Analysis type	Action
Single-plate analysis	See “Open a completed run” on page 69.
Multi-plate analysis	See “Open a study for analysis” on page 75.

   The run opens on the **ANALYSIS** page. By default the first sample group is selected in the **Analyze by** area and the plots are in 1D Scatter format.

   The **Analyze by** area lets you modify the data for analysis by sample group or sample.

2. To change the plot type to 2D Scatter, click .

   The plots in the gallery view update to the 2D Scatter format.

3. In the **Analyze by** area, click **Sample Group**, then select the group to be analyzed.

   The run results information for that group are displayed by channel in the gallery area. This information is an overview of the data of all samples contained in the group, presented in a single plot by channel. Individual sample data are shown in the sample list.

4. *(Optional)* To change the information in the gallery, select one of the following options.

Option	Description
Show Rejects	Show Rejects—Display or hide microchambers that have been rejected from the analysis results. Showing rejects does not impact the analysis or results calculations.
	1D Scatter plot type

5. In the **View sample** dropdown, set the view of the sample list by selecting one of the following options.

Option	Description
Ungrouped	Lists the samples contained in the group.
By omitted	Sorts the list of samples by omitted and included samples.
By run[1]	Groups the samples in the list by run.
By instrument[1]	Groups the samples in the list by instrument.
By plate barcode[1]	Groups the samples in the list by plate barcode.

[1] This option is only available in the **Studies** page.

6. *(Optional)* To modify the sort of the information in the sample list, use the settings in the column headings.

   The sample list lets you identify samples that have unusual QC values, review microchamber count information, and lets you perform the following actions on sample data to curate the data for analysis. Selecting a sample from the sample list lets you view and modify sample data at the sample level. For information about accessing sample information, see “Samples” on page 98.

   - Hide or unhide a sample for analysis
   - Omit or include a sample for analysis
   - Change the sort options of sample data
   - View sample data to investigate QC alerts

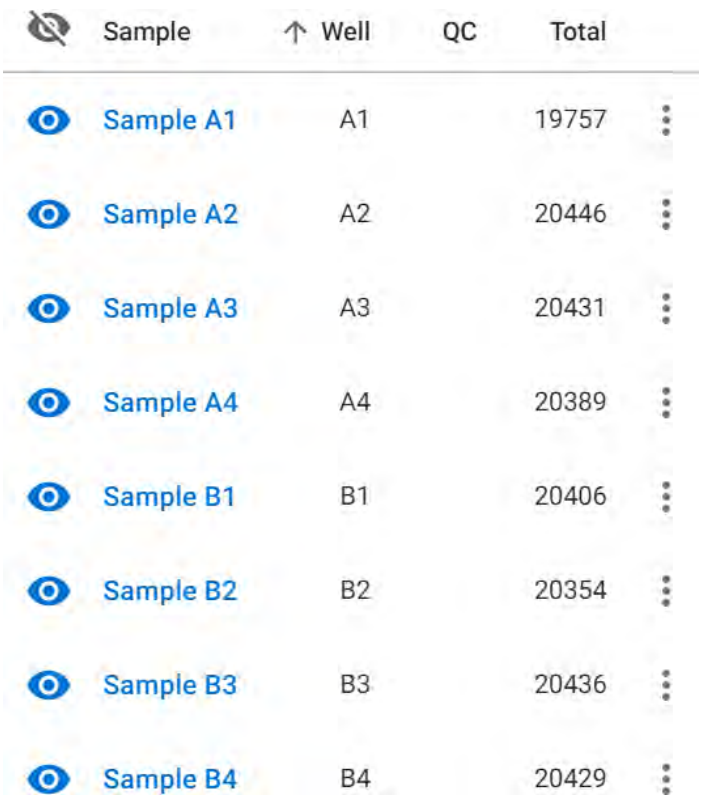


Figure 22 Sample list

Column	Action
—Toggle show or hide for all samples in the group.	Click to show or hide all the samples in the list.
Sample—Sample name.	Click the directional arrow to set the sort to ascending or descending order.
Well—Location of the sample on the plate.	
QC—Flags samples that have either failed or have unusual data. 1. —The sample failed and is excluded from analysis. 2. —The sample has unusual data that needs investigation.	
Total—Total number of accepted microchamber reactions less the number that have been rejected.	

7. *(Optional)* Use sample list options to modify sample inclusion or access sample detail information. When the changes are complete, click **SAVE** to recalculate the data and update the channel plots.

   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 plots and tables.

Option	Action
	Click to show or hide this sample.
Sample name	Click to access the sample detail PLOTS and QC pages and for this sample. Use to investigate samples that are flagged for QC and to modify thresholds for specific samples. See “QC data and arrays” on page 109 and “Samples” on page 98.
	Click to omit or include a sample in the analysis.

8. *(Optional)* To download a plot, click  , navigate to the location to save the file, then click **Save**.

9. *(Optional)* For implementations using Security, Auditing, and E-signature (SAE) v2.2 software, click **E-SIGN** to provide an e-signature. See, “Sign data in the software” on page 149.

For information about adjusting thresholds on sample groups, see “View and modify sample groups–2D Scatter plot–detail view” on page 93.

View and modify sample groups–2D Scatter plot–detail view

This section provides information about viewing and modifying sample groups in the 2D Scatter plot–detail view.

For information about 1D Scatter plots, “View and modify sample groups–1D Scatter plot–detail view” on page 84.

For information about the sample group 2D Scatter plot–gallery view, see “View and modify sample groups–2D Scatter plot–gallery view” on page 89.

Before performing analysis, complete any modifications that are needed to the sample setup of the plate or plates you are analyzing. See “SETUP page (analysis)” on page 76.

1. To access the **ANALYSIS** page, perform one of the following actions based on the type of analysis you are doing.

Analysis type	Action
Single-plate analysis	See “Open a completed run” on page 69.
Multi-plate analysis	See “Open a study for analysis” on page 75.

   The run opens on the **ANALYSIS** page. By default the first sample group is selected in the **Analyze by** area and the plots are in 1D Scatter format.

   The **Analyze by** area lets you modify the data for analysis by sample group or sample.

2. To change the plot type to 2D Scatter, click  .

   The plots in the gallery view update to the 2D Scatter format.

3. In the **Analyze by**, click **Sample Group**, then select the group to be analyzed.

   The run results information for that group are displayed in 6 channel combinations in the gallery area. This information is an overview of the data of all samples contained in the group, presented in individual plots showing two channels. These combinations can be changed as needed to view all possible combinations. To interchange the positions of the channels on the x and y-axis' of a plot, in the upper-left corner of the plot, click ⇄.

4. *(Optional)* To change the information in the gallery, select one of the following options.

Option	Description
Show Rejects	Show Rejects
	1D Scatter plot type

5. In the **View sample** dropdown, set the view of the sample list by selecting one of the following options.

Option	Description
Ungrouped	Lists the samples contained in the group.
By omitted	Sorts the list of samples by omitted and included samples.
By run[1]	Groups the samples in the list by run.
By instrument[1]	Groups the samples in the list by instrument.
By plate barcode[1]	Groups the samples in the list by plate barcode.

[1] This option is only available in the **Studies** page.

6. *(Optional)* To modify the sort of the information in the sample list, use the settings in the column headings.

   The sample list lets you identify samples that have unusual QC values, review microchamber count information, and lets you perform the following actions on sample data to curate the data for analysis. Selecting a sample from the sample list lets you view and modify sample data at the sample level. For information about accessing sample information, see “Samples” on page 98.

   - Hide or unhide a sample for analysis
   - Omit or include a sample for analysis
   - Change the sort options of sample data
   - View sample data to investigate QC alerts

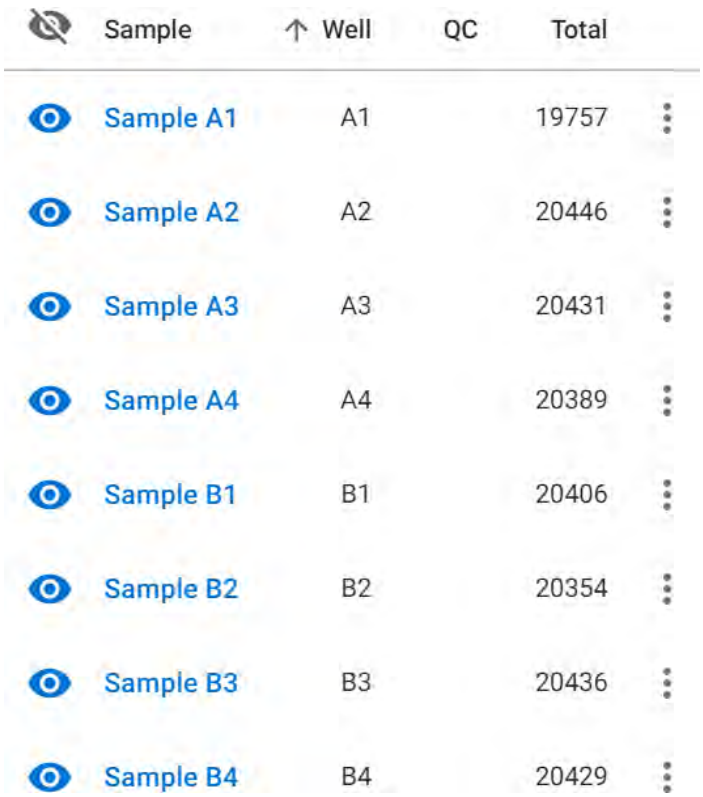


Figure 23 Sample list

Column	Action
—Toggle show or hide for all samples in the group.	Click to show or hide all the samples in the list.
Sample—Sample name.	Click the directional arrow to set the sort to ascending or descending order.
Well—Location of the sample on the plate.	
QC—Flags samples that have either failed or have unusual data. 1. —The sample failed and is excluded from analysis. 2. —The sample has unusual data that needs investigation.	
Total—Total number of accepted microchamber reactions less the number that have been rejected.	

7. *(Optional)* Use sample list options to modify sample inclusion or access sample detail information. When the changes are complete, click **SAVE** to recalculate the data and update the channel plots.

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 plots and tables.

Option	Action
	Click to show or hide this sample.
Sample name	Click to access the sample detail PLOTS and QC pages and for this sample. Use to investigate samples that are flagged for QC and to modify thresholds for specific samples. See “QC data and arrays” on page 109 and “Samples” on page 98.
	Click to omit or include a sample in the analysis.

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

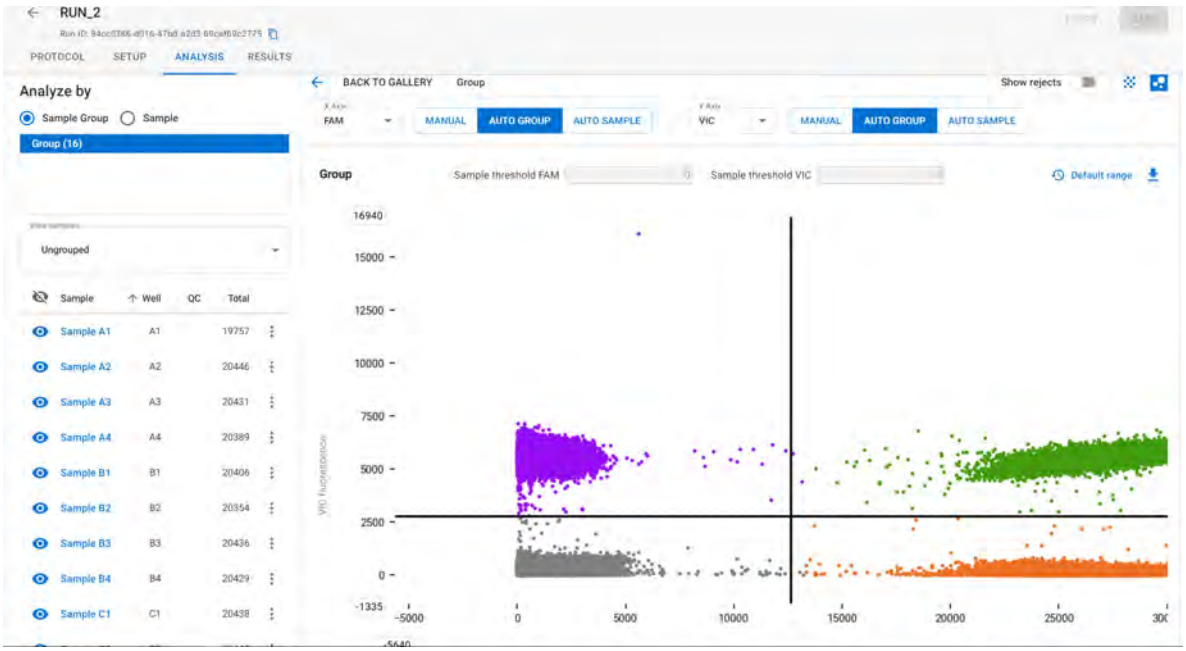


Figure 24 Sample Group—2D Scatter–detail view

9. Use the scroll bars on the right side and the bottom of the plot to adjust the view of the plot.

10. *(Optional)* To change the channel for the y-axis, select a channel from the **Y-Axis** dropdown.

11. *(Optional)* To change the channel for the x-axis, select a channel from the **X-Axis** dropdown.

12. *(Optional)* To change the threshold of the sample data for each axis, 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. **Note:** If the **AUTO SAMPLE** threshold is selected for an axis, the threshold for that channel will not be displayed in this view.	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 for the axis in the plot to the desired value. • Enter a value for the channel in the **Group threshold** field at the top of the table. 3. Click **SAVE**.

13. *(Optional)* To magnify an area in the plot, adjust the upper and lower ends of the sample range on either axis if needed.
    a. For the y-axis, in the field on the upper-end of the y-axis, enter the desired value.
    b. For the y-axis, in the field on the lower-end of the y-axis, enter the desired value.
    c. For the x-axis, in the field on the left-end of the x-axis, enter the desired value.
    d. For the x-axis, in the field on the right-end of the x-axis, enter the desired value.
    e. Click **SAVE**.
       The plot refreshes and the selected range is displayed.
    f. *(Optional)* To return the plot to the default ranges, Click **Default range**.

Note: You can adjust the thresholds in the magnified plot if needed. See step 12.

14. *(Optional)* To download a plot, click ⬇, navigate to the location to save the file, then click **Save**.

15. *(Optional)* For implementations using Security, Auditing, and E-signature (SAE) v2.2 software, click **E-SIGN** to provide an e-signature. See, “Sign data in the software” on page 149.

16. *(Optional)* To return to the gallery view, click **BACK TO GALLERY**.

Samples

Viewing by **Sample** lets you visualize and understand the behavior of a single sample. The sample view lets you perform the following actions at the sample level.

- Investigate why a QC flag indicates a sample might be questionable (Warning icon).

  Used when a sample has been identified as suspect and you want to determine if the sample should be omitted or if the QC threshold can be adjusted for this sample to make it usable.
- Investigate why a sample looks unusual (even if no warning) and lets you set the threshold of an individual sample within a group if that sample needs to be treated differently.

The sample–gallery view plot provides and overview of the data in the sample by channel and lets you perform the following actions on sample data.

- View sample data by channel
- Download data plots
- Investigate QC alerts

See the following sections for information about sample-gallery view.

- For 1D Scatter plots, see “View by sample–1D Scatter plot–gallery view” on page 99.
- For 2D Scatter plots, see “View by sample–2D Scatter plot–gallery view” on page 103.
- For investigating QC data, see “QC data and arrays” on page 109.

The sample–detail view plot provides detailed information by sample for the selected channel and lets you perform the following actions on sample data.

- Adjust thresholds for samples by channel
- Adjust the data range to zoom in or out on an area of the plot
- Download data plots
- Investigate QC alerts

Note: The sample–detail view is accessed from the gallery view.

See the following sections for information about the sample–detail view.

- For 1D Scatter plots, see “View and modify samples–1D Scatter plot–detail view” on page 100.
- For 2D Scatter plots, see “View and modify samples–2D Scatter plot–detail view” on page 106.
- For investigating QC data, see “QC data and arrays” on page 109.

View by sample–1D Scatter plot–gallery view

This section provides information about viewing samples in the 1D Scatter plot gallery view.

For information about adjusting thresholds on samples, see “View and modify samples–1D Scatter plot–detail view” on page 100.

For information about 2D Scatter plots, see “View by sample–2D Scatter plot–gallery view” on page 103.

For information about investigating QC data, see “QC data and arrays” on page 109.

The sample–detail view is accessed from the gallery. For information about the 1D Scatter plot sample–detail view, see “View and modify samples–1D Scatter plot–detail view” on page 100.

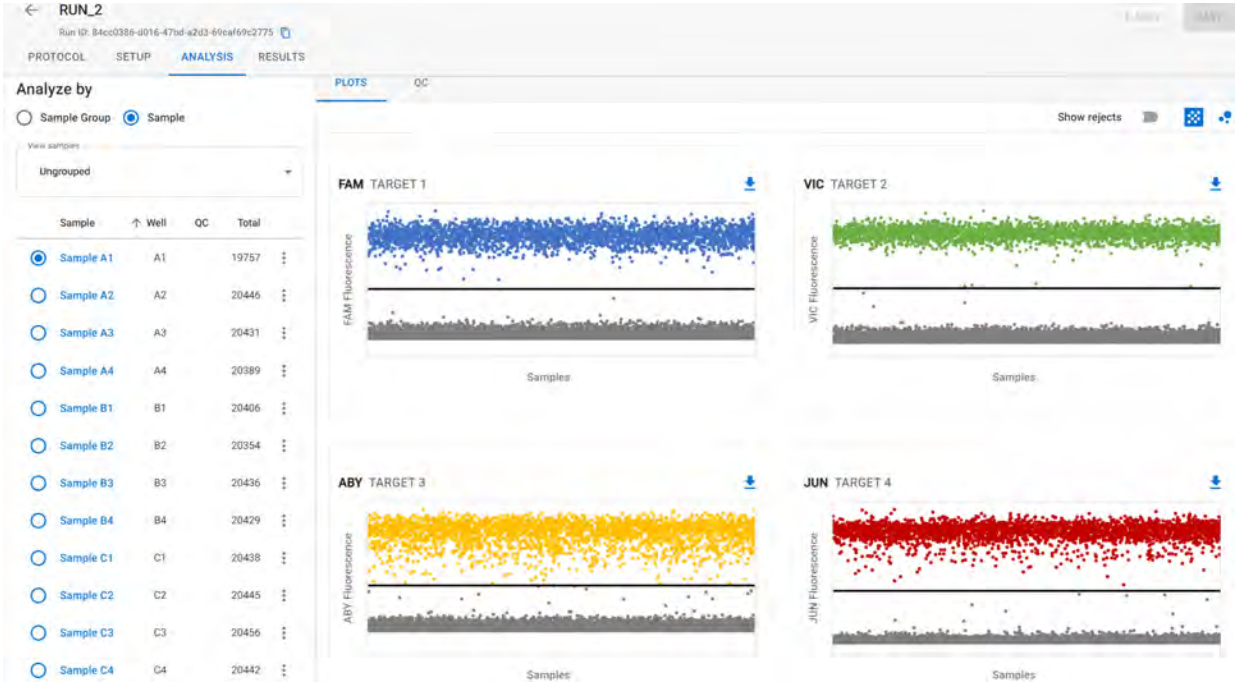


Figure 25 Sample —1D Scatter–gallery view

1. To access the **ANALYSIS** page, perform one of the following actions based on the type of analysis you are doing.

Analysis type	Action
Single-plate analysis	See “Open a completed run” on page 69.
Multi-plate analysis	See “Open a study for analysis” on page 75.

   The run opens on the **ANALYSIS** page. By default the first sample group is selected in the **Analyze by** area and the plots are in 1D Scatter format.

   The **Analyze by** area lets you modify the data for analysis by sample group or sample.

2. 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.

3. *(Optional)* To change the information in the gallery, select one of the following options.

Option	Description
Show Rejects	Show Rejects—Display or hide microreaction chambers that have been rejected from the analysis results. Showing rejects does not impact the analysis or results calculations.
	2D Scatter plot type

4. *(Optional)* To download a plot, click , navigate to the location to save the file, then click **Save**.

5. *(Optional)* For implementations using Security, Auditing, and E-signature (SAE) v2.2 software, click **E-SIGN** to provide an e-signature. See, “Sign data in the software” on page 149.

View and modify samples–1D Scatter plot–detail view

This section provides information about viewing and modifying samples in the 1D Scatter plot–detail view.

For information about 2D Scatter plots, see “View and modify samples–2D Scatter plot–detail view” on page 106.

For information about investigating QC data, see “QC data and arrays” on page 109.

For information about the sample 1D Scatter plot–gallery view, see “View by sample–1D Scatter plot–gallery view” on page 99.

Before performing analysis, complete any modifications that are needed to the sample setup of the plate or plates you are analyzing. See “SETUP page (analysis)” on page 76.

1. To access the **ANALYSIS** page, perform one of the following actions based on the type of analysis you are doing.

Analysis type	Action
Single-plate analysis	See “Open a completed run” on page 69.
Multi-plate analysis	See “Open a study for analysis” on page 75.

   The run opens on the **ANALYSIS** page. By default the first sample group is selected in the **Analyze by** area and the plots are in 1D Scatter format.

   The **Analyze by** area lets you modify the data for analysis by sample group or sample.

2. 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.

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

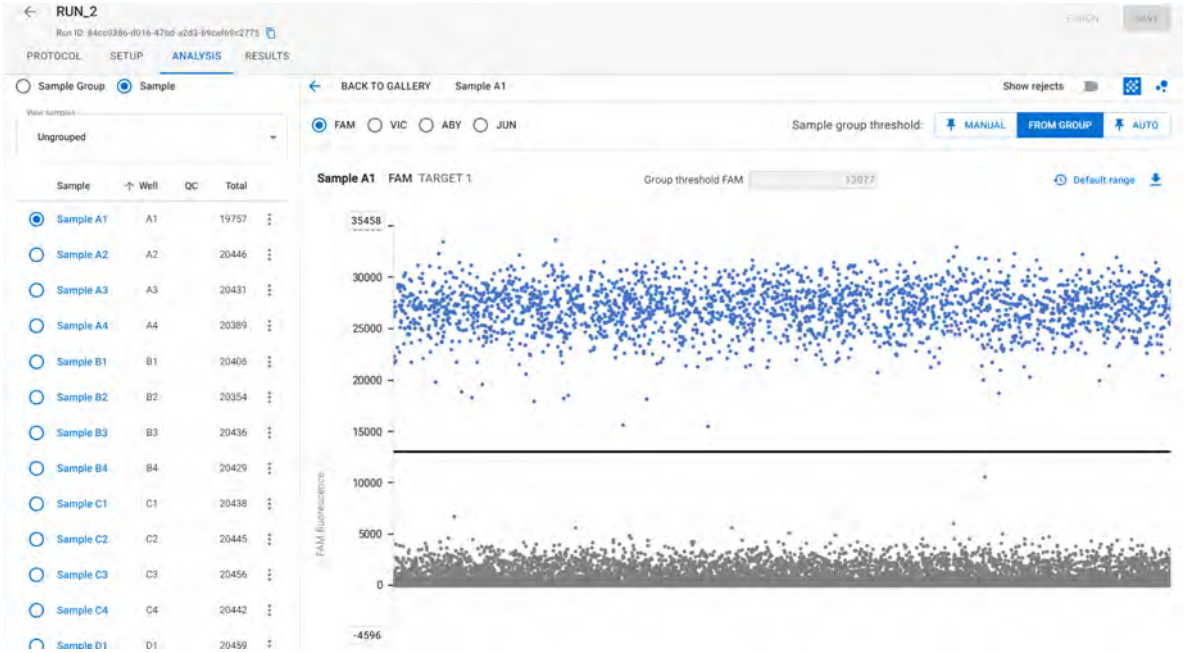


Figure 26 Sample—1D Scatter–detail view

4. Use the following options to view data on the detail view plot.

Option	Action
View complete sample data.	Use the scroll bars on the right side and the bottom of the plot to adjust the view of the plot.
View information about a sample.	Hover over the sample column in the plot to show information about that sample. • **Sample**: Sample name • **Well**: Well location on the plate • **Total**: Total number of accepted microchamber reactions less the number that have been rejected • **Plate barcode**: Barcode number of the plate • **Run**: Name of the run • **Instrument**: Name of the instrument used for the run

5. *(Optional)* Change the channel for the detail view by selecting the desired channel at the top-left portion of the plot area.

6. *(Optional)* 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**.

7. *(Optional)* To magnify an area in the plot, adjust the upper and lower ends of the sample range.
   a. In the field on the upper-end of the y-axis, enter the desired value.
   b. In the field on the lower-end of the y-axis, enter the desired value.
   c. Click **SAVE**.
      The plot refreshes and the selected range is displayed.
   d. *(Optional)* To return the plot to the default range, click **Default range**.
8. *(Optional)* To download a plot, click , navigate to the location to save the file, then click **Save**.
9. *(Optional)* To return to the gallery view, click **BACK TO GALLERY**.

View by sample–2D Scatter plot–gallery view

This section provides information about viewing samples in the 2D Scatter plot gallery view.

For information about adjusting thresholds on samples, see “View and modify samples–2D Scatter plot–detail view” on page 106.

For information about 1D Scatter plots, “View by sample–1D Scatter plot–gallery view” on page 99.

For information about investigating QC data, see “QC data and arrays” on page 109.

The sample–detail view is accessed from the gallery. For information about the 2D Scatter plot sample–detail view, see “View and modify samples–2D Scatter plot–detail view” on page 106.

1. To access the **ANALYSIS** page, perform one of the following actions based on the type of analysis you are doing.

Analysis type	Action
Single-plate analysis	See “Open a completed run” on page 69.
Multi-plate analysis	See “Open a study for analysis” on page 75.

   The run opens on the **ANALYSIS** page. By default the first sample group is selected in the **Analyze by** area and the plots are in 1D Scatter format.

   The **Analyze by** area lets you modify the data for analysis by sample group or sample.

2. 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.

3. To change the plot type to 2D Scatter plot, click .

   The plots in the gallery view update to the 2D Scatter format.

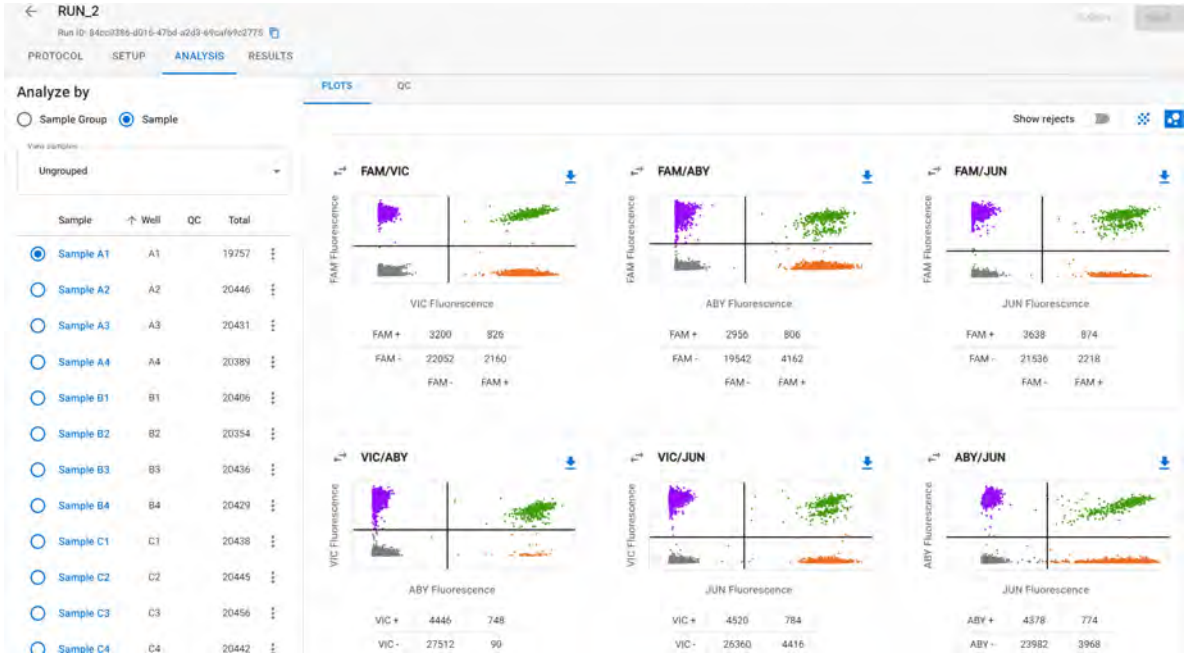


Figure 27 Sample—2D Scatter–gallery view

The sample results information for that sample are displayed in 6 channel combinations in the gallery area. This information is an overview of the data of the sample, presented in individual plots showing two channels. These combinations can be changed as needed to view all possible combinations, see step 4. Individual sample data are shown in the sample list.

4. *(Optional)* To interchange the positions of the channels on the x- and y-axis' of a plot, in the upper-left corner of the plot, click .

   The gallery refreshes and the plots with the new x- and y-axis values.

5. *(Optional)* To change the information in the gallery, select one of the following options.

Option	Description
Show Rejects	Show Rejects—Display or hide microreaction chambers that have been rejected from the analysis results. Showing rejects does not impact the analysis or results calculations.
	1D Scatter plot type

6. *(Optional)* To download a plot, click , navigate to the location to save the file, then click **Save**.

View and modify samples–2D Scatter plot–detail view

This section provides information about viewing and modifying samples in the 2D Scatter plot–detail view.

For information about 1D Scatter plots, see “View and modify samples–1D Scatter plot–detail view” on page 100.

For information about investigating QC data, see “QC data and arrays” on page 109.

For information about the sample 2D Scatter plot–gallery view, see “View by sample–2D Scatter plot–gallery view” on page 103.

Before performing analysis, complete any modifications that are needed to the sample setup of the plate or plates you are analyzing. See “SETUP page (analysis)” on page 76.

1. To access the **ANALYSIS** page, perform one of the following actions based on the type of analysis you are doing.

Analysis type	Action
Single-plate analysis	See “Open a completed run” on page 69.
Multi-plate analysis	See “Open a study for analysis” on page 75.

   The run opens on the **ANALYSIS** page. By default the first sample group is selected in the **Analyze by** area and the plots are in 1D Scatter format.

   The **Analyze by** area lets you modify the data for analysis by sample group or sample.

2. To change the plot type to 2D Scatter plot, click  .

   The plot updates to the 2D Scatter format.

3. 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.

4. *(Optional)* To interchange the positions of the channels on the x- and y-axis' of a plot, in the upper-left corner of the plot, click ⇄.
   The gallery refreshes and the plots with the new x- and y-axis values.

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

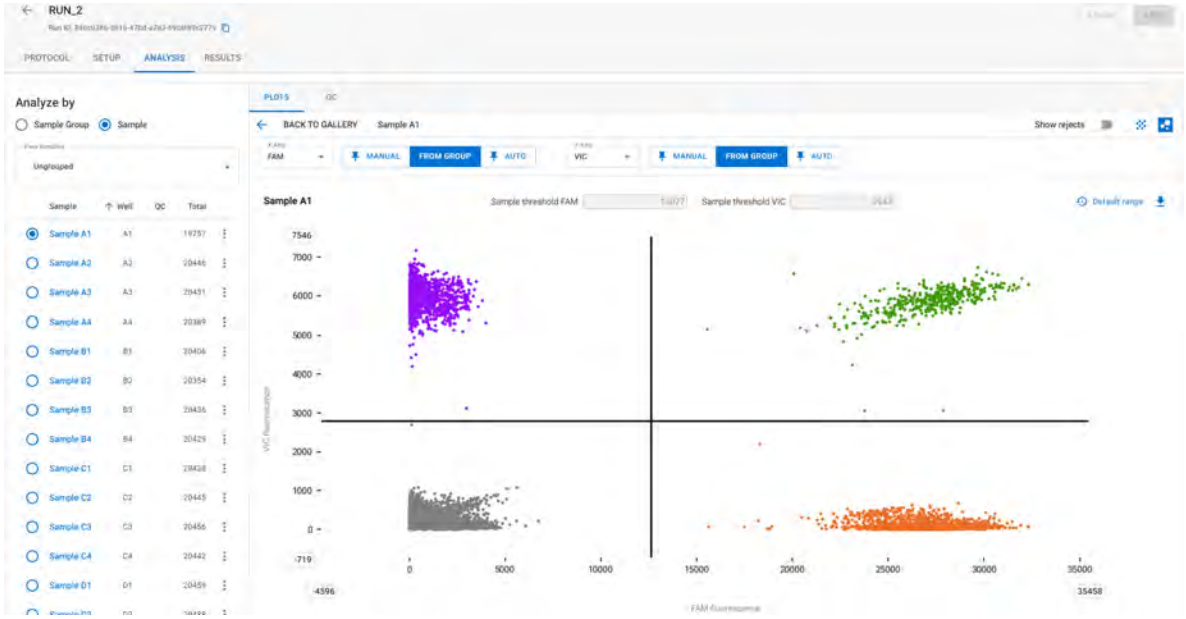


Figure 28 Sample 2D Scatter–detail view

6. Use the scroll bars on the right side and the bottom of the plot to adjust the view of the plot.

7. *(Optional)* To change the channel for the y-axis, select a channel from the **Y-Axis** dropdown.
8. *(Optional)* To change the channel for the x-axis, select a channel from the **X-Axis** dropdown.
9. *(Optional)* 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**.

10. *(Optional)* To magnify an area in the plot, adjust the upper and lower ends of the sample range on either axis if needed.
    a. For the y-axis, in the field on the upper-end of the y-axis, enter the desired value.
    b. For the y-axis, in the field on the lower-end of the y-axis, enter the desired value.
    c. For the x-axis, in the field on the left-end of the x-axis, enter the desired value.
    d. For the x-axis, in the field on the right-end of the x-axis, enter the desired value.
    e. Click **SAVE**.
    The plot refreshes and the selected range is displayed.
    f. (Optional) To return the plot to the default ranges, click **Default range**.
11. *(Optional)* To download a plot, click ⬇, navigate to the location to save the file, then click **Save**.
12. *(Optional)* To return to the gallery view, click **BACK TO GALLERY**.

QC data and arrays

The QC page displays the plot for the QC channel (ROX™) and array images for the sample. This quality control data helps ensure that only properly filled microchambers are used for analysis by evaluating the ROX™ signal for each microchamber. The top and bottom bounds are set by the software but can be manually adjusted. The QC plot should have a single level band indicating uniform filling. QC messages consist of failures and warnings. A sample that fails QC is rejected by the software and is not included in the analysis. A sample with a warning message alerts lets you there is something unusual about the sample that requires investigation to determine if the sample should be ommited or have the QC bound adjusted.

For information about viewing QC data and adjusting QC bounds, see “View and modify QC data” on page 109.

The QC page also contains array images for all channels of a sample. Microchambers with positive data points are colored. Microchambers with negative and rejected data points are gray scale. Often they have a low signal and appear black.

For information about viewing arrays, see “View arrays” on page 111.

View and modify QC data

This section provides information about viewing and modifying QC data on a sample.

If a sample fails it is unusable and is excluded from analysis by the software. A failed sample is designated with an ❗.

A sample with a warning indicates there is something unusual about the sample that warrants investigation to determine if the sample is usable by possibly adjusting the QC bounds, or if it should be omitted. A sample with a warning is designated with an ⚠.

For information about the QC failure and warning messages that can occur and recommended actions, see “QC failure and warning messages” on page 122.

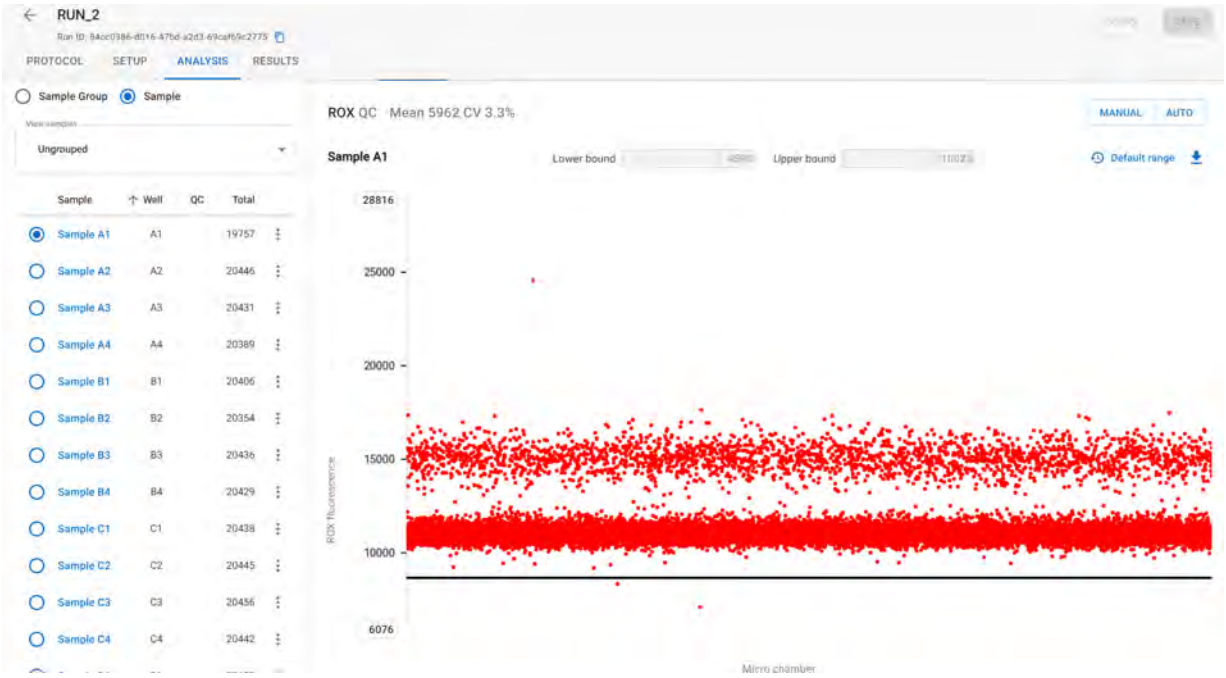


Figure 29 QC plot for the QC channel (ROX™)

1. From the sample list on the **Analysis** page, select the sample you want to investigate.
   By default the sample opens on the **PLOTS** page.

2. Click **QC** to open the QC page.
   The QC page displays the plot for the QC channel (ROX™) and array images for all channels in the sample.

3. Use the following options to view data on the QC plot of the sample.

Option	Action
View complete plot data.	Use the scroll bars on the right side and the bottom of the plot to adjust the view of the plot.
View information about the sample.	Hover over the sample in the plot to show information about that sample: • **Sample**: Sample name • **Well**: Well location on the plate • **Total**: Total number of accepted microchamber reactions less the number that have been rejected • **Plate barcode**: Barcode number of the plate • **Run**: Name of the run • **Instrument**: Name of the instrument used for the run

4. *(Optional)* To change the QC bounds 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—The software sets the QC bounds for the sample.	1. Click **AUTO**. 2. Click **SAVE**.
MANUAL—Manually set the QC bounds for the sample.	1. Click **MANUAL**. 2. Use one of the following options to set the QC bounds. • Drag the upper and lower bars in the plot to the desired value. • Enter a value in the **Upper bound** and the **Lower bound** fields at the top of the table. 3. Click **SAVE**. The plot refreshes and the new QC bounds are displayed.

5. *(Optional)* To magnify the plot, adjust the upper and lower ends of the sample range.
   a. In the field on the upper-end of the y-axis, enter the desired value.
   b. In the field on the lower-end of the y-axis, enter the desired value.
   c. Click **SAVE**.
      The plot refreshes and the selected range is displayed.
   d. *(Optional)* To return the plot to the default range, click **Default range**.
6. *(Optional)* To download a plot, click ⬇, navigate to the location to save the file, then click **Save**.

View arrays

This section provides information about viewing arrays.

The **QC** page contains array images for all channels of a sample. Microchambers with positive data points are colored. Microchambers with negative and rejected data points are gray scale. Often they have a low signal and appear black.

Figure 30 Arrays on the QC page

1. From the sample list on the **Analysis** page, select the sample you want to investigate.
   By default the sample opens on the **PLOTS** page.

2. Click **QC** to open the QC page.
   The QC page displays the plot for the QC channel (ROX™) and array images for all channels in the sample.

3. Using the scroll bar on the right side of the screen, scroll down to view array images.

4. *(Optional)* To view the full resolution of an array it must be downloaded to your computer. Use the steps that follow to download an image of the array.
   a. Click ⬇, navigate to the location to save the file, then click **Save**.
   b. View the array using an image viewer of your choosing.

5. *(Optional)* To download a plot, click ⬇, navigate to the location to save the file, then click **Save**.

Download plot data from the ANALYSIS page

The software lets you download plots from the gallery view and detail view in PNG format.

Note: These instructions assume that the data has been analyzed to produce the desired result. See, Chapter 4, "Analyze data".

1. To access the **ANALYSIS** page, perform one of the following actions based on the type of analysis you are doing.

Analysis type	Action
Single-plate analysis	See "Open a completed run" on page 69.
Multi-plate analysis	See "Open a study for analysis" on page 75.

2. To download Sample Group plot, use the following options.

Option	Actions
Download a plot from the **Sample Group** gallery view.	1. From the **Analyze by** area, click **Sample Group**. 2. In the gallery area, click ⬇ in the channel plot you want to download. 3. When prompted, navigate to the location to save the file,then click **Save**.
Download a plot from the **Sample Group** detail view.	1. From the **Analyze by** area, click **Sample Group**. 2. In the gallery area of the channel plot data, select the plot you want to see in the detail view. 3. When the detail view of the plot displays, then click ⬇. 4. *(Optional)* to change channels, select the channel at the top of the plot, then click ⬇. 5. When prompted, navigate to the location to save the file, then click **Save**.

3. To download a Sample plot, use the following options.

Option	Actions
Download a **Sample** QC plot.	1. From the **Analyze by** area, click **Sample** to access the QC page. 2. *(Optional)* To download the ROX plot, click ⬇ in the ROX area. 3. *(Optional)* To download an array plot for a channel, click ⬇ in the desired channel array. 4. When prompted, navigate to the location to save the file, then click **Save**.
Download a **Sample** plot from the gallery view.	1. From the **Analyze by** area, click **Sample**. The QC page opens. 2. Click **PLOTS** to access the plots gallery view. 3. In the sample table, select the sample you want to view. 4. In the gallery area, click ⬇ in the channel plot you want to download. 5. When prompted, navigate to the location to save the file,then click **Save**.
Download a **Sample** plot from the detail view.	1. From the **Analyze by** area, click **Sample**. The QC page opens. 2. In the sample table, select the sample you want to view. 3. Click **PLOTS** to access the plots gallery view. 4. In the gallery area of the channel plot data, select the plot you want to see in the detail view. 5. When the detail view of the plot displays, then click ⬇. 6. *(Optional)* To change channels, select the channel at the top of the plot, then click ⬇. 7. *(Optional)* To change samples, select the sample from the sample table, then click ⬇. 8. When prompted, navigate to the location to save the file,then click **Save**.

RESULTS page

The **RESULTS** page displays the results for samples and sample groups in a single table. The values are plotted together below the concentration table.

From the **RESULTS** page, you can perform the following actions.

- View statistical results by group and sample. See “View results” on page 114.
- View results plots by group or sample. See “View results” on page 114.
- Generate data reports. See“Generate reports” on page 115 .
- Download the data table in CSV format. See “Download data from the RESULTS page” on page 116.

The presentation of the **RESULTS** page is based on the data included for analysis on the **ANALYSIS** page.

For information about the formulas used to calculate results, see “Computation of results” on page 121.

Figure 31 View Results

View results

1. To access the **RESULTS** page, perform one of the following actions based on the type of analysis you are doing.

Analysis type	Action
Single-plate analysis	See “Open a completed run” on page 69.
Multi-plate analysis	See “Open a study for analysis” on page 75.

2. To view results, select from the following options.

Option	Actions
View results for all optical channels.	1. From the list of active optical channels above the concentration table, click **ALL** to view all channels. 2. Click ▸ next to a group name to show or hide the sample data for that group. 3. Use the scroll bar at the bottom of the concentration table to scroll through concentration table data for all channels. 4. Use the scroll bar on the side of the page to scroll down to see the plot data for all channels. 5. In the plot area, toggle between **Sample** and **Group** to change the display of the plot data. 6. Use the scroll bar at the bottom of the plot area to scroll through the plot data for all channels.
View results for a specific optical channel.	1. From the list of active optical channels above the concentration table, select the desired channel. 2. Click ▸ next to a group name to show or hide the sample data for that group. 3. Use the scroll bar on the side of the page to scroll down to see the concentration table data for the channel. 4. Use the scroll bar on the side of the page to scroll down to see the plot data for the channel. 5. In the plot area, toggle between **Sample** and **Group** to change the display of the plot data.

Generate reports

Use the **GENERATE REPORT** option to create and download reports as PDF files.

1. To access the **RESULTS** page, perform one of the following actions based on the type of analysis you are doing.

Analysis type	Action
Single-plate analysis	See “Open a completed run” on page 69.
Multi-plate analysis	See “Open a study for analysis” on page 75.

2. From the **RESULTS** page, click **GENERATE REPORT**.

   The **Generate report** dialog opens and **All Groups** are selected by default to be included in the report.

3. *(Optional)* Click **Select Groups**, then from the list of groups, select the checkbox next to each group to be included in the report. Any combination of groups can be selected.

4. *(Optional)* Click **QC Channel** to include the QC channel data for all samples.

5. Click **GENERATE REPORT**.

6. In the **File browser**, navigate to the location to download the report, then click **Save**.
7. When the report has successfully downloaded, click **CLOSE**.

Download data from the RESULTS page

The software lets you download results data from a run in CSV format. The compressed (.zip) file contains 3 tables in CSV format.

- Run summary statistics
- Raw fluorescence values
- Multi-channel overview

1. To access the **RESULTS** page, perform one of the following actions based on the type of analysis you are doing.

Analysis type	Action
Single-plate analysis	See “Open a completed run” on page 69.
Multi-plate analysis	See “Open a study for analysis” on page 75.

2. From the **RESULTS** page, click DOWNLOAD DATA.
3. In the **File browser**, navigate to the location to download the file, then click **Save**.
4. When the file has successfully downloaded, click **CLOSE**.

A

Troubleshooting

Troubleshooting Absolute Q™

Observation	Possible cause	Recommended action
QuantStudio™ Absolute Q™ Digital PCR Software is not connecting, front panel LEDs are white	Internal instrument software connection error.	Power off the instrument, then unplug both USB and power cables from instrument. Wait 10 seconds, then plug in the power cord and power on the instrument. When solid blue LED is seen, then plug in the USB cable.
QuantStudio™ Absolute Q™ Digital PCR Software is not connecting, front panel LEDs are blue	Poor USB cable connection.	Confirm that a USB 3.0 cable is used and that it is plugged into the USB 3.0 port on the desktop computer.
	Corrupt software.	Uninstall, then reinstall the software.
Front panel LEDs are red	Instrument error.	Power cycle the instrument using the power switch.
The Run status displays as DISCONNECTED	Port 8000 is blocked.	If a firewall or other application is using port 8000, remove it or use a different port for the firewall or other application.
Pressure leak error	Missing or damaged gaskets.	Make sure that all 5 columns of gaskets are present.
		Replace any damaged gaskets.
Instrument makes noise and LEDs are white one minute after power up	Instrument firmware startup error.	Power off the instrument. Unplug the power cable from the instrument. Wait 10 seconds. Plug the power cable back in and power on the instrument.
Barcode not found	Plate in backwards.	Well A1 should be at the top left of the plate tray.
	Missing or unreadable barcode label.	Enter the barcode manually if it is human readable.
Connection to the standalone SAE Administrator Console is lost	Power outage.	Restore power to the SAE Administrator Console.
	Cables have become disconnected.	Confirm all cables are properly connected.

Observation	Possible cause	Recommended action
Connection to the standalone SAE Administrator Console is lost *(continued)*	Hardware failure of the SAE Administrator Console.	Uninstall and reinstall the QuantStudio™ Absolute Q™ Digital PCR Software to continue use of the QuantStudio™ Absolute Q™ Digital PCR System without SAE. 1. Consult with your organization's policies and procedures regarding operation without SAE enabled before continuing. 2. At the desktop computer, shut down the QuantStudio™ Absolute Q™ Digital PCR Software. 3. Uninstall the QuantStudio™ Absolute Q™ Digital PCR Software. **IMPORTANT!** To prevent data loss, select **Keep** during uninstall to preserve the existing database. 4. Install the QuantStudio™ Absolute Q™ Digital PCR Software, see “Install the desktop software” on page 130. **IMPORTANT!** To prevent data loss, select **Update** during installation of the software to preserve the existing database.
Communication between the instrument and the Absolute Q™ computer is interrupted or inconsistent	Another application may be causing a communication conflict.	Ensure that only the QuantStudio™ Absolute Q™ Digital PCR Software and if applicable SAE Administrator Console are installed on the dedicated computer. Uninstall any other applications.
	Hardware failure.	Confirm all cables are properly connected
		Confirm that both devices have power.
		Contact **thermofisher.com/support**.

Field Service Archive files

Field Service Archive (FSA) files contain information regarding runs and instrument usage that can be used for troubleshooting unexpected run results and instrument performance. The following table provides information on the FSA files that can be captured from the QuantStudio™ Absolute Q™ Digital PCR System.

File type and file name format	Description
Data {run_name}_{short_run_id}_{YYYY_MM_DD}_data.fsa	• Used for troubleshooting issues with a run • Contains raw images and the run ZST file • Automatically created with each run • File size can be large, >1GB • 20 most recent files retained
Log {run_name}_{short_run_id}_{YYYY_MM_DD}_logs.fsa	• Used for troubleshooting issues with a run • Contains logs from the QuantStudio™ Absolute Q™ Digital PCR Software • Contains logs from the instrument computer software and hardware not related to the camera • Automatically created with each run • File size is ~120 MB • 70 most recent files retained
System {YYYY_MM_DD}_system.fsa	• Used for troubleshooting system issues not related to a run • Contains logs from the desktop computer and instrument computer at the time of capture • Created on demand • File size is ~120 MB • The file is not automatically deleted

Capture and transfer data and log FSA files

Data and log FSA files are used for troubleshooting unexpected results or instrument failure during a run.

Note: Only use these instructions when instructed by a Thermo Fisher support representative.

1. Capture and transfer data and log FSA files.
    1. On the desktop computer, open the **Start Menu**.
    2. Find the shortcut to the **QuantStudio Absolute Q 6 Field Service Archives** folder by performing one of the following actions.
        - In the search field, type ***QuantStudio Absolute Q 6 Field Service Archives***.
        - Scroll through the application list.
    3. Click on the shortcut to open the archive files folder, then select the data and log files for the run in question.
       For example:
        - *Absolute Q Starter Chemistry Run 31_8ea5ccfc_2022_04_01_data.fsa*
        - *Absolute Q Starter Chemistry Run 31_8ea5ccfc_2022_04_01_logs.fsa*
2. Send the files to your Thermo Fisher support representative for analysis using a file transfer program of your choosing.

Capture and transfer system FSA files

System FSA files are used for troubleshooting system issues not related to a run, for example if the plate tray is malfunctioning. A system FSA file is created on demand using the QuantStudio™ Absolute Q™ Digital PCR Software on the dedicated computer for the Absolute Q™ instrument.

Note: Only use these instructions when instructed by a Thermo Fisher support representative.

1. In the QuantStudio™ Absolute Q™ Digital PCR Software, select **System**, then in the **Download system logs** area, select **DOWNLOAD SYSTEM FSA**.
   The system log file is created and a **File Explorer** window opens with the system log file name pre-populated in the file name field.
2. Navigate to a folder of your choice, then click **Save**.
3. Send the files to your Thermo Fisher support representative for analysis using a file transfer program of your choosing.

B

Supplemental information

Computation of results

The following table provides information about the formulas used to calculate results used in analysis.

Table 3 Computation of results

Quantity	Formula(e)	Description
Lambda (copies/Reaction)	$\lambda = -ln(z/n)$	λ—the average number of molecules/microchamber or reaction z—the number of empty microchambers in the sample n—the number of accepted (non-rejected) microchambers
Lower and upper 95% confidence intervals for Lambda (copies/Reaction)	$\Delta \text{lower} = \lambda \left[1 - e^{\frac{-1.96\sqrt{e^{\lambda}-1}}{\lambda\sqrt{n}}}\right]$ $\Delta \text{upper} = \lambda \left[e^{\frac{1.96\sqrt{e^{\lambda}-1}}{\lambda\sqrt{n}}} - 1\right]$	
Precision (%)	$P = 100 * max\,(\Delta \text{lower}, \Delta \text{upper}) / \lambda$ Where, $\Delta \text{lower} = \lambda \left[1 - e^{\frac{-1.96\sqrt{e^{\lambda}-1}}{\lambda\sqrt{n}}}\right]$ $\Delta \text{upper} = \lambda \left[e^{\frac{1.96\sqrt{e^{\lambda}-1}}{\lambda\sqrt{n}}} - 1\right]$	P—the spread of the confidence level around λ compared to the true value of λ *max*—the higher of the two values
Concentration (copies/µL)	$C = \lambda / V * DF$	C—the sample concentration in copies per microliter V—the volume of each microchamber (432 pL)

Table 3 Computation of results *(continued)*

Quantity	Formula(e)	Description
Lower and upper 95% confidence intervals for Concentration (copies/µL)	$\Delta \text{lower} = \frac{\lambda}{V}\left[1 - e^{\frac{-1.96\sqrt{e^{\lambda}-1}}{\lambda\sqrt{n}}}\right] * DF$ $\Delta \text{upper} = \frac{\lambda}{V}\left[e^{\frac{1.96\sqrt{e^{\lambda}-1}}{\lambda\sqrt{n}}} - 1\right] * DF$	*DF*—the dilution factor specified during plate setup
Copy Number Variation	$CNV = Nr * \frac{\lambda_t}{\lambda_r}$	*CNV*—the estimated copy number N_r—the number of copies per genome of the reference target λ_t—the estimated average number of molecules per microchamber of the target λ_r—the estimated average number of molecules per microchamber of the reference
Lower and upper 95% confidence intervals for copy numbers	$\Delta \text{lower} = \left[e^{\ln\frac{\lambda_t}{\lambda_r} - e^{\ln\frac{\lambda_t}{\lambda_r} - 1.96\sigma_R}}\right] Nr$ $\Delta \text{upper} = \left[e^{\ln\frac{\lambda_t}{\lambda_r} + 1.96\sigma_R} - e^{\ln\frac{\lambda_t}{\lambda_r}}\right] Nr$ Where, $\sigma_R = \sqrt[2]{\frac{1-e^{-\lambda_t}}{n\lambda_t^2 e^{-\lambda_t}} + \frac{1-e^{-\lambda_r}}{n\lambda_r^2 e^{-\lambda_r}}}$	

QC failure and warning messages

The table that follows defines the QC failure and warning messages that can occur and recommended corrective actions.

Table 4 QC messages

QC Status	QC Message	Description	Recommendation
FAIL	FAILURE: Image registration error	The microchamber array cannot be located or identified with sufficient accuracy. This can be caused by excessive dust or debris on the plate or if the microchamber array was filled poorly.	• Ensure environmental cleanliness. • Ensure reaction mixture is made up properly. [1]

Table 4 QC messages *(continued)*

QC Status	QC Message	Description	Recommendation
FAIL	FAILURE: Array QC failure	QC problem or problems with the sample have been detected that prevent accurate analysis. Potential causes include loading issues or other failure modes.	Severe failures are rare but can occur. • Ensure environmental cleanliness. • Vortex and centrifuge reagents thoroughly in each step and prior to loading samples.[1] .
WARN	WARNING: Camera saturation in: [Dye name(s)]	Microchambers with high fluorescence that saturate the camera detector have been detected. This can be caused by a high concentration of probes.	Saturation can often be resolved by lowering probe concentrations.[1]
WARN	WARNING: Bridging between microchambers	A statistically improbable string of positive microchambers adjacent to each other that can indicate that amplifiable material is being shared between microchambers have been detected. This can be caused by pipetting errors or setting incorrect thresholds.	• Ensure that only 9 µL of reaction mixture is loaded into each sample well and pipettes are calibrated. • Ensure that thresholds for all dyes are set accurately.
WARN	WARNING: High rejection rate from QC bounds	A high number of microchambers rejected due to signal levels outside of QC channel bounds are detected. This could be caused by incorrect filling or incorrect bounds.	• Ensure that the minimum and maximum bounds in the QC Dye are set accurately. • Ensure that the correct master mix and correct dilution of master mix are used.
WARN	WARNING: Overloaded array in: [Dye name(s)]	No negative microchambers were detected. This impacts computation based on Poisson statistics. Possible causes: • Samples were run at a high concentration through a suboptimal dilution. • Incorrect thresholds were set in the affected dyes.	• Ensure that normal concentration is correct. • Ensure that thresholds for the overloaded dyes are set correctly.
PASS	PASS: No QC issues found	No widespread problems with the sample have been detected. The number of rejected microchambers (if any) are in acceptable bounds	Proceed with regular analysis workflow.

[1] If failures or warnings reoccur, save the data FSA files for use in troubleshooting.

C Modify Protocols

The **PROTOCOL** page in the **Runs** function lets you perform the following tasks when configuring the protocol.

- Modify the template protocol settings
- Set the optical channel *(optional)*
- Import an existing protocol
- Export the protocol

1. From the left pane click to open the **Runs** list page.
2. Use the search fields to find a run or select a run from the list, then go to the **Protocol** page.
3. Modify PCR parameters if needed.

Parameter	Actions
Temperature	Perform one of the following actions. • Enter a value in the temperature fields. • Drag the slider bars to adjust the temperature.
Dwell times	Enter in seconds or minutes and seconds in mm:ss format.
Cycles	Set the number of cycles by entering a value into the **Cycles** field.
RNA-RT	Select **RNA-RT** to add an extra temperature step for RNA reverse transcription to cDNA for RNA samples. Not required for DNA samples.
Preheat	Select **Preheat** to add a preheat step. Sometimes called hot start, preheating the samples before PCR helps to reduce non-specific binding at lower temperatures.

(continued)

Parameter	Actions
Two or three-step cycling	From the cycling option dropdown list, select **Two Step** or **Three Step** cycling.
Two-stage PCR cycle	Select **Two Stage PCR** to add a second PCR cycle stage.

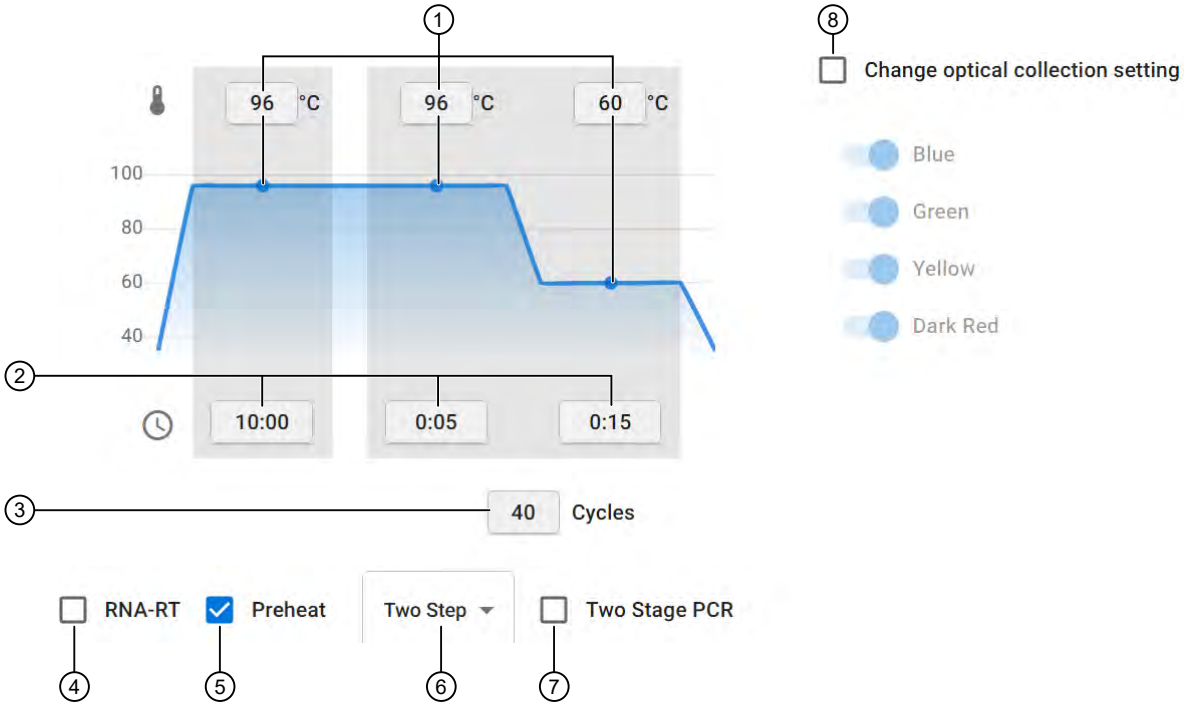


Figure 32 Protocol parameters

1. Temperature settings fields and slider bar
2. Time fields
3. Cycles field
4. RNA-RT setting
5. Preheat setting
6. Two Step or Three Step cycling option
7. Two Stage PCR setting
8. Change optical collection setting

4. *(Optional)* All optical channels are selected by default. Perform the following actions to change optical channel settings.

IMPORTANT! Disabling optical channels prevents data from being collected from those channels. Any existing sample groups using these channels are affected. To include a run in a study, all runs must have the same optical collection setting. For information about studies, see "Multi-plate analysis–Studies" on page 71.

a. Click **Change optical collection setting**.

b. When prompted to confirm changing the optical selection setting, click **CHANGE OPTICAL COLLECTION SETTING**.

c. Set the optical channels by toggling the channels on or off.

Note: If a channel is disabled, a message appears on the **PROTOCOL** page indicating that the protocol has disabled channels.

Note: If **Change optical collection setting** option is disabled, the software resets to the default setting of all channels in the on position.

5. *(Optional)* Import an existing protocol by performing the following actions.
   a. Click **IMPORT PROTOCOL**, then click **IMPORT FILE** to open the **File browser**, then navigate to the location of the AQUA file to import.
   b. Select the file, then select **Open**.
6. *(Optional)* Export the protocol by performing the following actions.
   a. Click **EXPORT PROTOCOL** to open the **File browser**, then navigate to the location where you want to save the exported protocol AQUA file.
   b. Click **Export**.
7. *(Optional)* To modify MAP plate setup, see “SETUP page (DRAFT runs)” on page 54.

D

Install, update, and move the QuantStudio™ Absolute Q™ Digital PCR System

Installation and environmental requirements

The room where the instrument is installed must be kept within the following operational environment conditions.

Condition	Acceptable range
Installation site	For indoor laboratory use only (Applicable pollution degree 2)
Operating temperature and humidity	15-30°C (60-85°F), 0-80% RH
Storage temperature and humidity	5-40°C (40-105°F), 0-80% RH
Vibration	Do not place the instrument adjacent to strong vibration sources. Excessive vibration during use can affect instrument performance.
Altitude	Up to 6,500 ft (2000 m)
Input voltage tolerance	+/-10%
Over voltage category	II

- Installation time: <10 minutes
- Required materials: scissors or a strap cutter
- Space requirement: The instrument is approximately 0.6 m (2 ft) cubed. The presentation drawer must not be obstructed and extends approximately 200 mm (8 in) from the front panel of the instrument when open. The power and USB connections are on the left side near the back of the instrument.
- Ensure that the fan vents on the back and bottom of the instrument are not obstructed.

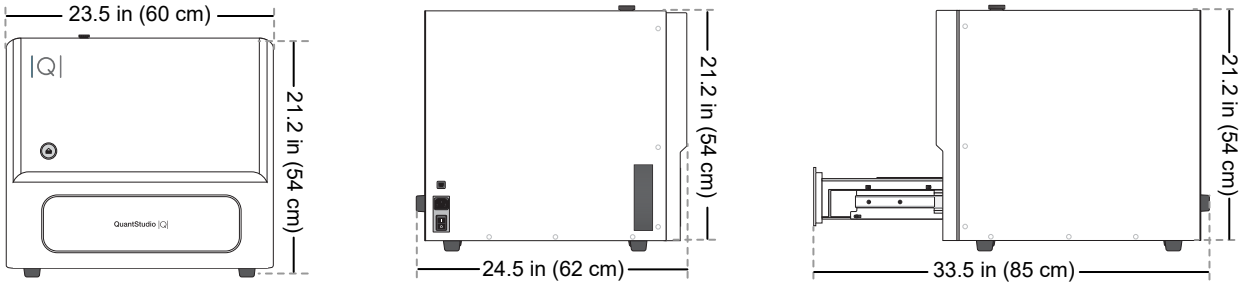


Figure 33 Instrument dimensions

IMPORTANT! Keep all packaging materials in good condition, as they are required if the instrument needs to be returned for any reason.

WARNING! The instrument requires 2–3 people for moving. Moving the system alone may result in serious injury.

AVERTISSEMENT ! Le déplacement de l'instrument nécessite 2 à 3 personnes. Si vous déplacez le système seul, vous risquez de vous blesser gravement.

Install the QuantStudio™ Absolute Q™ Digital PCR System

IMPORTANT! Ensure that the installation location meets the power and environmental requirements specified in "Installation and environmental requirements" on page 127.

1. With 2–3 people, carefully unbox the instrument by cutting the straps and lifting the top of the box off using the hand holes.

   Do not cut or damage any of the packaging. Keep all packaging as it is required for returns or service requests.

2. Carefully place the instrument on a flat, stable surface with no adjacent vibration sources.

3. Position the instrument so that there is access to the power and USB connectors on the left side of the system.

4. Once the instrument is in place, remove the shipping lock screw on the top of the instrument.
   a. With the power off, unscrew the shipping lock screw on the top of the instrument.

   b. Insert the provided white plastic cap into the screw hole.

      For more information on removing the shipping lock screw, see "Uninstall the shipping lock screw" on page 135.

      IMPORTANT! To prevent damage to the instrument, the shipping lock screw must be removed before powering on the instrument.

Keep the shipping lock screw in case the instrument needs to be moved or returned for service.

5. Confirm that the power switch is in the OFF, O, position and then connect the power cable to the instrument and a suitable power source.
6. Set up the dedicated computer and monitor near the instrument.
7. Use the power cable to connect the dedicated computer to a suitable power source.
8. Connect the keyboard and mouse to the back of the dedicated computer.
9. Power on the dedicated computer.

   IMPORTANT! To prevent damage to the instrument, only power on the dedicated computer at this step, do not power on the instrument.
10. Install the software onto the dedicated computer. See “Download and install the desktop software” on page 129.
11. When the software installation is complete, use the USB cable to connect the instrument to the dedicated computer.
12. Power on the instrument by moving the power switch located at the left side near the back to the I position.

    Wait approximately 30 seconds for the instrument to initialize.
13. Once connected to the software, check that there are no errors reported.

The system is ready for use.

Download and install the desktop software

Computer requirements for the desktop software

Install the QuantStudio™ Absolute Q™ Digital PCR Software on the computer provided by Thermo Fisher Scientific, and use it to control the instrument. Thermo Fisher Scientific does not support the use of customer-provided computers to control the instrument.

However, you can install the QuantStudio™ Absolute Q™ Digital PCR Software on a customer-provided computer to use the software to import run data for analysis. The following are the minimum requirements for a customer-provided computer.

- Operating system—Windows™ 10 (64-bit)
- Dell™ OptiPlex XE3 Tower computer

Download the desktop software

1. Go to **https://www.thermofisher.com/us/en/home/global/forms/life-science/quantstudio-absolute-q-software.html**.
2. Download the software package.

Install the desktop software

1. Sign in to the computer on which you are installing the desktop software.

   IMPORTANT! To install the software, you must have Computer Administrator privileges in the Windows™ 10 operating system.

2. From the downloaded software package, perform the following actions.
   a. Double-click **QuantStudio Absolute Q Digital PCR Software.exe**.
   b. Follow the prompts to install the software.
   c. Click **Finish**.

   By default, the software is installed to `C:\Program Files\Applied Biosystems\QuantStudio Absolute Q\Digital PCR Software 6`.
   The desktop software is added to the Windows™ start menu.

3. Start the QuantStudio™ Absolute Q™ Digital PCR Software.
4. When prompted, accept the End User License Agreement.

System settings

Systems settings are optional and are configured based on your implementation of the system.

1. Access the **System Settings** screen in the left pane by selecting ⚙ **System**.
2. Configure each setting as needed for your implementation.

Setting	Action
Enable security When set to on, this setting requires users to sign in with an ID and password.	Perform one of the following actions. • To enable, move the toggle switch to the on position. • To disable, move the toggle switch to the off position. If enabled, perform the following actions in the **SAE connection settings** dialog. 1. In the **IP address** field, enter the IP address of the SAE Administrator Console. 2. In the **Port** field, enter the port number of the SAE Administrator Console. 3. Click **TEST CONNECTION** to confirm successful communication to the SAE Administrator Console. 4. Enter a user name and password for an SAE system administrator. For implementations using theSecurity, Auditing, and E-signature (SAE) v2.2 software, use this setting to configure the connection to the SAE Administrator Console.
Remote API settings For implementations using automation, use this setting to configure access to the Remote API using an access code. The **Remote API settings** provides the following information. • **Port**: System assigned Port for the Remote API • **Fingerprint**: The hash of the SSL certificate. This is used as an identifier to ensure connection to a valid Remote API server For information about using automation with a third party system, see *QuantStudio™ Absolute Q™ Digital PCR System Remote API User Guide* (Pub No.MAN0028059)	Perform the following actions. 1. To enable, move the toggle switch to the on position. 2. To enter an access code, perform one of the following actions. • Select **GENERATE ACCESS CODE**, to create a system generated access code. • In the **Access Code** field, manually enter an access code. 3. Click **SAVE**. 4. Close the Absolute Q™ software and restart the computer for the settings to take affect. **Note:** Failure to restart the computer will prevent the instrument from connecting to the automation system.

(continued)

Setting	Action
Batch export location For implementations using automation, use this setting to set a file system location for export of batched runs.	Click **BROWSE**, then navigate to the location on the file system where batched run files are to be exported.
Run import location Use this setting to set the file system location for runs that are available for import.	Click **BROWSE**, then navigate to the location on the file system where run files for import are located.
Auto export completed runs Use this setting to set the data types and file system location for the export of information from manual runs. The following data files are available for export. • ZST files—Run information in ZST file format. • Run data CSV files—Run information in Excel™ spreadsheet file format. • Run results CSV files—Run results information in Excel™ spreadsheet file format.	Perform the following actions for each data type to be exported. 1. Select the checkbox associated with each data type to be exported. 2. Click **BROWSE**, then navigate to the location on the file system where the run files are to be exported.
Storage management Use the setting to determine the number of runs to save before the oldest run is deleted.	Perform the following actions to customize the number of runs to save before deletions. 1. To enable, move the toggle switch to the on position. 2. In the **Runs to retain** field, enter the number of runs to save.
Download system logs Use this option to download Field Service Archive (FSA) system logs for use in troubleshooting.	1. Click **DOWNLOAD SYSTEM FSA**. 2. In the **File explorer**, navigate to the location to save the files, then click **SAVE** 3. Send the files to your Thermo Fisher support representative for analysis using a file transfer program of your choosing. **Note:** For more information about Field Service Archive files, see "Field Service Archive files" on page 119.

3. When all settings needed for your implementation are configured, click **SAVE**.

Update the instrument software and firmware

If the software and/or firmware on the instrument is not compatible with the software of the desktop computer, you are automatically prompted to update the instrument software.

Note: Refer to the release notes for the update for details on the changes being implemented..

1. In the left pane of the QuantStudio™ Absolute Q™ Digital PCR Software, select to access the **Instrument** page.
   A dialog box appears with the following message, *Incompatible instrument configuration detected. Please update your instrument software and firmware.*.

2. Select the appropriate option or options from the following table.

Option	Action
UPDATE INSTRUMENT SOFTWARE	1. Select **UPDATE INSTRUMENT SOFTWARE**. 2. When prompted, select **UPDATE INSTRUMENT SOFTWARE**. 3. When the **Update successful** dialog appears, select **CLOSE**.
UPDATE FIRMWARE	1. Select **UPDATE FIRMWARE**. 2. When prompted, select **UPDATE FIRMWARE**. 3. When the **Update successful** dialog appears, select **CLOSE**.

Note: When the update is complete, the instrument automatically restarts.

IMPORTANT! Wait 5 minutes for the instrument to update. Manually power cycling the instrument prior to the completion of the update can result in damage to the instrument.

Moving the instrument

IMPORTANT! When moving the instrument the shipping lock screw must be manually installed before moving the unit, and manually removed after transport. Moving the instrument without the shipping lock screw in place can cause damage to the instrument.

IMPORTANT! When moving the instrument, make sure there is no plate in the instrument as it can become dislodged and jam mechanical parts during instrument transport.

Install the shipping lock screw

1. Power on the instrument.
2. Start the QuantStudio™ Absolute Q™ Digital PCR Software.
3. Open the plate door to ensure there is no plate loaded. If a plate is loaded, remove it.
4. Close the plate door.
5. In the left pane, select to access the **Instrument** page.
6. Click on the instrument and select **Prepare for Shipping**.
   Wait until a message stating *Ready for Shipping* appears before proceeding.
7. Remove the white plastic plug from the shipping screw hole and place it in the bag attached to the shipping screw.
8. Insert the shipping screw and screw it finger tight. Do not over tighten.
9. Close the software and power off the instrument.

1 Shipping lock screw

Uninstall the shipping lock screw

IMPORTANT! Perform this task before powering on the instrument.

1. Ensure that the power is off and the instrument is not plugged into a power source.
2. Unscrew the shipping lock screw from the top of the instrument.

① Shipping lock screw

3. Insert the white plastic cap in the shipping lock screw hole.
   The instrument is now ready for power-up and use.

Use the software with Security, Auditing, and E-signature (SAE) v2.2

The Security, Auditing, and E-signature (SAE) v2.2 software (SAE Administrator Console) is only compatible with the QuantStudio™ Absolute Q™ Digital PCR System.

For more information on Security, Auditing, and E-signature (SAE) v2.2, including definitions of accounts and roles, see the *SAE Administrator Console v2.0 or later User Guide for PCR systems* (Pub. No. MAN0017468).

Overview of the SAE Administrator Console components

The SAE Administrator Console includes three components.

- SAE Administrator Console that an administrator uses to configure the module.
- SAE server that stores settings, user accounts, and audit records.

  Note: The SAE server and SAE Administrator Console software are installed simultaneously on the same computer during installation.

- SAE screens in an application (sign in and audit that a user interacts with). QuantStudio™ Absolute Q™ Digital PCR Software is an application.

The SAE Administrator Console provides the following SAE functionality in the QuantStudio™ Absolute Q™ Digital PCR Software.

- **System security**—Controls user sign in and access to functions.
- **Auditing**—Tracks changes and actions performed by users.
- **E-signature**—Allows users to provide an electronic signature (user name and password) when performing certain functions.

Take the following into consideration based on the way that your SAE administrator configures the system.

- Some features and functions that are described in this guide may not be accessible to you.
- You may see dialog boxes and prompts when you use the software.

Overview of the QuantStudio™ Absolute Q™ Digital PCR Software functionality when SAE functions are enabled

The following features are active when SAE functions are enabled in the QuantStudio™ Absolute Q™ Digital PCR Software.

- Users must sign in with an SAE user account to use QuantStudio™ Absolute Q™ Digital PCR Software.
- Both audit objects and audit actions are tracked in the SAE Administrator Console. Audit actions are tracked automatically, audit objects are viewable when enabled.
- Run setup and software functions for a user are determined by the SAE application profile and user account settings.

Recommendations for SAE passwords

Thermo Fisher Scientific recommends enabling a password policy for SAE user accounts with the following minimum number of characters:

- Administrative users: 12 characters
- Non-administrative users: 8 characters

The use of a password manager is recommended in order to help to create secure passwords.

SAE functions not supported by the QuantStudio™ Absolute Q™ Digital PCR Software

The following SAE functions are not supported by the QuantStudio™ Absolute Q™ Digital PCR Software.

Function	Option not supported
System > Other Settings	• Open file from non-SAE system • Client offline sign in • Offline sign in threshold
Audit history	Instrument Run Records

(continued)

Function	Option not supported
e-Signature	• Ability to add e-Signature meanings • Ability to delete e-Signature meanings • Ability to configure actions that require e-Signature • Ability to control/configure e-Signature rights by user role • Ability to control reasons available for e-Signature • Ability to control/configure data to be signed for each e-Signature meaning Signed data in the e-Signature Records PDF report generated by the SAE Administrator Console does not contain any objects. To create a report with this information, print the result report from the QuantStudio™ Absolute Q™ Digital PCR Software. • Ability to control/configure number of signatures (by role) for each action requiring e-Signature

Enable SAE functions

Workflow

Enable SAE functions

Install the SAE Administrator Console and Absolute Q™ application profile (page 139)

Connect to the SAE server (page 139)

Enable SAE functions in QuantStudio™ Absolute Q™ Digital PCR Software (page 141)

Install the SAE Administrator Console and Absolute Q™ application profile

The following configurations of SAE server and SAE Administrator Console software are supported.

- SAE installed on a standalone computer that is connected to the Absolute Q™ dedicated computer and optional Absolute Q™ dedicated computers
- SAE and Absolute Q™ software that is installed on the Absolute Q™ dedicated computer and is connected to optional Absolute Q™ dedicated computers
- SAE and Absolute Q™ software that is installed on an Absolute Q™ dedicated computer that is connected to the Absolute Q™ dedicated computer and other optional Absolute Q™ dedicated computers

IMPORTANT! Before installing the application profile, see the release notes for compatibility information to ensure you are installing the Absolute Q™ application profile that is compatible with the version of Absolute Q™ software that you are using.

1. To download the SAE Administrator Console software and Absolute Q™ application profile go to **https://www.thermofisher.com/us/en/home/global/forms/life-science/quantstudio-absolute-q-software.html**.

2. Install the SAE server and SAE Administrator Console software on a computer with a static IP address (*recommended*) or a dynamic IP address.
   a. Unzip the downloaded software.
   b. Double-click **setup.exe**
   c. Follow the **InstallShield Wizard** prompts to install the software.
   d. Select **Typical** as the setup preference, then click **Next**.
   e. Click **Finish**.

   Note: The SAE server and SAE Administrator Console software are installed simultaneously during installation.

3. In the SAE Administrator Console, an SAE administrator must install the application profile for the Absolute Q™ software before SAE can be used.
   The application profile contains default settings for the Absolute Q™ software.
   For information on installing application profiles, see the *SAE Administrator Console v2.0 or later User Guide for PCR systems* (Pub. No. MAN0017468).

Connect to the SAE server

1. In the QuantStudio™ Absolute Q™ Digital PCR Software, select ⚙ **System ▸ SAE Connection Settings**.

2. Enter the IP address and port number of the SAE Administrator Console.
   If the SAE Administrator Console is installed on the same computer as the QuantStudio™ Absolute Q™ Digital PCR Software, enter ***localhost***.

If the SAE Administrator Console is installed on a different computer from the QuantStudio™ Absolute Q™ Digital PCR Software, enter the IP address of the computer on which the SAE Administrator Console is installed.

Note: If using a dynamic IP address, enter the hostname instead of the IP address to prevent the loss of a connection (see "Determine the hostname" on page 140).

The port number is the firewall port. See "Firewall ports that must be open" on page 140.

3. (*Optional*) Click **Test Connection** to confirm that the connection information is correct.
4. Click **Save**.

Determine the hostname

If the SAE Administrator Console is on a separate computer from the application and a dynamic IP address is used, the hostname is recommended instead of the IP address. This helps to prevent the loss of a connection between the SAE Administrator Console and the application

1. In the Windows™ search bar, enter ***cmd*** to open the **Command Prompt**.
2. Enter ***hostname***, then press **Enter**.

The hostname of the computer is displayed in the **Command Prompt**.

Firewall ports that must be open

The following ports must be open for the operating system on the computer that is running the SAE Administrator Console.

SAE Administrator Console version	Port	Condition
v2.0	8201	• Instrument-to-SAE Administrator Console server connection • Computer-to-SAE Administrator Console server connection[1]
v2.1 and later	8443	• Instrument-to-SAE Administrator Console server connection • Computer-to-SAE Administrator Console server connection[1]

[1] If the software is installed on a different computer than the SAE Administrator Console.

Firewall ports

To open a port for Microsoft™ Defender, add inbound rules for the port, and apply to all profiles.

To open a port for Norton Internet Security™, use the **Settings** menu to open the port.

No action is required to open a port for Symantec™ Endpoint Protection.

Enable SAE functions in QuantStudio™ Absolute Q™ Digital PCR Software

This procedure requires an SAE administrator account.

Before you enable SAE functions in the QuantStudio™ Absolute Q™ Digital PCR Software, you must complete the following tasks:

- Connect to the SAE server (see “Connect to the SAE server” on page 139).
- Close all protocol or analyzed run files.

1. In the QuantStudio™ Absolute Q™ Digital PCR Software, select **System ▸ Enable Security**.
2. Enter your SAE administrator account user name and password, then click **Sign In**.

The SAE administrator account is automatically signed into the software after SAE functions are enabled. The SAE user name is displayed in the upper-right corner of the software menu bar. All users must sign into the software while SAE functions are enabled.

To sign out of the SAE administrator account in the Absolute Q™ software, see “Sign out of the software using an SAE account” on page 141.

Note: Signing out of the SAE administrator account does not disable SAE functions in the Absolute Q™ software. To disable SAE functions in the Absolute Q™ software, see “Disable SAE functions in QuantStudio™ Absolute Q™ Digital PCR Software” on page 153.

Sign into QuantStudio™ Absolute Q™ Digital PCR Software using an SAE account

Sign in for the QuantStudio™ Absolute Q™ Digital PCR Software is only required if SAE functions are enabled by an SAE administrator (see “Enable SAE functions in QuantStudio™ Absolute Q™ Digital PCR Software” on page 141).

1. In the QuantStudio™ Absolute Q™ Digital PCR Software sign in screen, enter your SAE user name and password.
2. Click **Sign In**.

The user name of the SAE account that is signed in to the software appears in the menu bar.

Sign out of the software using an SAE account

1. In the lower-left corner of the left pane, click .
2. Click **Sign Out**.

Change your SAE account password

Note: External user account (External/Federated LDAP repository accounts) passwords cannot be changed in the QuantStudio™ Absolute Q™ Digital PCR Software, they can only be changed in their respective repository.

1. In the lower-left corner of the left pane, click .
2. Click **Change Password**.
3. Enter the password information, then click **OK**.

Default permissions and roles

The SAE Administrator Console provides the following default permissions and roles. You can use the default roles when you create SAE user accounts or create custom roles in the SAE Administrator Console v2.2 (see the *SAE Administrator Console v2.0 or later User Guide for PCR systems* (Pub. No. MAN0017468)).

- Administrator
- Scientist
- Technician
- Service

IMPORTANT! SAE permissions for a role apply to all user accounts that are assigned to the role.

The roles and associated user-configurable permissions are listed in the following table. You can also double-click the role in the **Roles** tab to display the list of permissions.

Note: The **No Privileges** role is used by the software when you set up user repositories. Do not assign this role to a user account.

Function	Description	Role			
		Administrator	Scientist	Technician	Service
Miscellaneous					
Service access	Access to the instrument service menu.	No	No	No	Yes
System settings	Access to the system menu.	Yes	No	No	Yes
Generate report	Create analysis reports.	Yes	Yes	Yes	Yes
E-SIGN run	Place an electronic signature on a run.	Yes	Yes	No	No
E-SIGN study	Place an electronic signature on a study.	Yes	Yes	No	No
Edit notes	Edit notes on plate setup.	Yes	Yes	Yes	Yes

(continued)

Function	Description	Role			
		Administrator	Scientist	Technician	Service
Accept or reject calibration results	Accept or reject the results provided with an instrument calibration.	No	No	No	Yes
Presets Management					
Create template	Create a template for a run.	Yes	Yes	Yes	Yes
Edit template	Edit an existing template.	Yes	Yes	Yes	Yes
Import template	Import a template from another system.	Yes	Yes	Yes	Yes
Export template	Export a template to another system.	Yes	Yes	Yes	Yes
Rename template	Rename an existing template.	Yes	Yes	Yes	Yes
Delete template	Delete a template from the system.	Yes	Yes	No	Yes
Save as template	Save a run as a template.	Yes	Yes	Yes	Yes
Create a batch run	Create multiple runs from the same template.	Yes	Yes	Yes	Yes
Edit protocol—templates	Change protocol settings in an existing template.	Yes	Yes	Yes	Yes
Assign samples and groups—templates	Assign samples and groups to wells on the plate in a template.	Yes	Yes	Yes	Yes
Edit groups and dye settings—templates	Modify groups and dye settings in an existing template.	Yes	Yes	Yes	Yes
Edit plate samples and rename samples—templates	Modify samples in an existing template.	Yes	Yes	Yes	Yes
Instrument Control					
Start run	Choose a protocol and start and stop instrument runs.	Yes	Yes	Yes	Yes
Stop run	Stop a run in progress.	No	Yes	Yes	Yes
Software or firmware update for instrument	Update the instrument software and firmware.	Yes	No	No	Yes
Pre-Run					
Edit protocol	Change protocol settings on a draft run.	Yes	Yes	Yes	Yes

(continued)

Function	Description	Role			
		Administrator	Scientist	Technician	Service
Assign samples and groups	Assign samples to set groups or load a group set in a run.	Yes	Yes	Yes	Yes
Edit groups and dye settings	Modify groups and dye settings on a draft run.	Yes	Yes	Yes	Yes
Edit plate samples and rename samples	Modify samples on a draft run.	Yes	Yes	Yes	Yes
Run analysis					
Change thresholds	Change channel thresholds in a run.	Yes	Yes	No	Yes
Edit groups and dye settings	Edit group definitions including dye settings in a run.	Yes	Yes	No	Yes
Edit and rename samples	Change sample names in a run.	Yes	Yes	Yes	Yes
Assign samples and groups	Assign samples to set groups or load a group set in a run.	Yes	Yes	Yes	Yes
Omit or include samples	Include or omit samples from an analysis in a run.	Yes	Yes	No	Yes
Run management					
Delete run	Delete a run from the database.	Yes	No	No	Yes
Import run	Import runs to and from ZST files.	Yes	Yes	Yes	Yes
Export run	Export runs to and from ZST files.	Yes	Yes	Yes	Yes
Rename run	Change the name of the run.	Yes	Yes	Yes	Yes
Study Analysis					
Change thresholds	Change sample and group thresholds during study analysis.	Yes	Yes	No	Yes
Edit groups	Edit groups contained in a study.	Yes	Yes	No	Yes
Edit plate samples and rename samples	Edit and rename samples in a study.	Yes	Yes	Yes	Yes
Omit or include samples	Include or omit samples from a study.	Yes	Yes	No	Yes
Create a study and add runs to a study	Create and add runs to studies.	No	Yes	Yes	Yes

(continued)

Function	Description	Role			
		Administrator	Scientist	Technician	Service
Study Management					
Import study	Import studies from other systems.	Yes	Yes	Yes	Yes
Export study	Export runs to and from ZST files.	Yes	Yes	Yes	Yes
Rename study	Change the name of a study.	Yes	Yes	No	Yes
Delete study	Delete a study.	Yes	Yes	No	Yes

Use audit functions

The following sections provide information on using SAE auditing functions.

Specify audit reason

Depending on how the audit settings are configured in the SAE Administrator Console, the **Enter Audit Reason** screen may appear when you make changes to a protocol or an analyzed run in the QuantStudio™ Absolute Q™ Digital PCR Software to prompt you to select an audit reason from the drop down list, or add a custom reason.

Note: Custom Reason is not displayed if audit settings are configured to require users to select a reason.

For more information on configuring audit settings, see the *SAE Administrator Console v2.0 or later User Guide for PCR systems* (Pub. No. MAN0017468).

View audit records

For instructions to view audit action records for a protocol or an analyzed run, see the *SAE Administrator Console v2.0 or later User Guide for PCR systems* (Pub. No. MAN0017468).

For a list of actions that are audited, see “Actions that are audited” on page 146.

For instructions to view audit object records of a specific run, see “View audit object records” on page 146.

View audit object records

Use the following steps to view the audit object record of a specific run by using the Run ID for the run.

1. In the QuantStudio™ Absolute Q™ Digital PCR Software, select the desired run.
2. In the upper-left corner of the run page, click next to the **Run ID** to copy the **Run ID** to the clipboard.
3. At the SAE Administrator Console perform the following steps.
   a. Select **Audit History > Application Object Records.**
   b. Select **Enable Application Objects Filtering**.
   c. In the **Object name** field, paste the **Run ID** that you copied in step 2.
   d. Click **Search**.

   The information regarding the run appears in results area of the **Audit History** screen.

Note: For assistance in interpreting audit history data, contact your Thermo Fisher representative.

Actions that are audited

The actions are audited and listed in the action records regardless of whether audits are enabled or disabled.

The following user actions are audited.

Function	Actions audited
Miscellaneous	• EULA accept or decline • Sign in • Sign out • Save system settings • Update instrument software/firmware version • Open and/or close instrument door (exact action with user name)

(continued)

Function	Actions audited
Templates	• Create, edit, or save a template • Save as template (when creating/editing a template) • Import or export protocol (when creating/editing a template) • Create a sample group (when creating/editing a template) • Edit dyes (when creating/editing a template) • Change optical settings (when creating/editing a template) • Add, edit, or delete notes in setup • Import template or templates • Export template or templates • Rename template • Delete template or templates • Generate batch runs
Runs	• Create, edit, and save a run • Save a run as a template (when creating/editing a run and viewing a completed run) • Import protocol (when creating/editing a run) • Export protocol (when creating/editing a run) • Update sample group assignment • Edit dyes (when creating/editing a run) • Add, edit, or delete notes in setup • Import run or runs • Export run or runs • Rename run • Delete run or runs • Start or stop run on the instrument • Start or stop a calibration run • Add a run or runs to a study

(continued)

Function	Actions audited
Runs—changes during analysis	• Update sample group assignment • Create or delete a sample group • Export protocol • Edit dyes • Add, edit, or delete notes in setup • Change threshold (both group threshold and threshold for a dye channel) • Omit sample • Pin or unpin threshold • Generate a report for a run • Download data for a run • Accept or reject calibration results • Generate a report for calibration run
Studies	• Create a study • Import study or studies • Export study or studies • Rename study • Delete study or studies • Add a run or runs to a study
Studies—changes during analysis	• Update sample group assignment • Create or delete a sample group • Export Protocol • Edit Dyes • Add, edit, or delete Notes in Setup • Change threshold (both group threshold and threshold for a dye channel) • Omit sample • Pin or unpin threshold • Generate a report for a run • Download data for a run • Accept or reject calibration results • Generate a report for calibration run

Export audit records

For information on exporting audit records for a protocol or an analyzed run, see the *SAE Administrator Console v2.0 or later User Guide for PCR systems* (Pub. No. MAN0017468).

Sign data in the software

An e-signature can optionally be added for plate setup and run results on the **Runs**, **Templates**, and **Studies** pages.

1. Chose from the following options to provide an e-signature for plate setup and run results.

Option	Actions
Runs page, **DRAFT**—Signing for plate protocol and setup.	1. From the left pane select to open the **Runs** list page. 2. Use the search field to find a run or select a run from the list.
Runs page, **COMPLETED**—Signing for protocol, setup, and results of the run.	1. From the left pane select to open the **Runs** list page. 2. Select the **COMPLETED** tab. 3. Use the search field to find a run or select a run from the list.
Studies page—Signing for protocol, setup, and results of the study.	1. From the left pane select to open the **Studies** list page. 2. Use the search field to find a study or select a study from the list.

2. Select **E-SIGN**, then select one of the following options from the dropdown list to indicate the meaning of the e-signature.
   - Reviewed & approved setup
   - Reviewed & approved results
3. Enter your user name and password.
4. Click **E-SIGN**.

If a run is signed and unmodified, the signature appears on reports that are created using **GENERATE REPORT**.

For information on how to view e-signature data in the SAE software, see *View and report audit and e-Signature records* in the *SAE Administrator Console v2.0 or later User Guide for PCR systems* (Pub. No. MAN0017468).

View and review e-Signatures

For information on how to view e-Signature data, see *View and report audit and e-Signature records* in the *SAE Administrator Console v2.0 or later User Guide for PCR systems* (Pub. No. MAN0017468).

The sections that follow provide detailed information for reviewing e-Signature data.

- For information on plate setup e-Signature data, see “Review plate setup e-Signature information” on page 150.
- For information on plate results e-Signature data, see “Review plate results e-Signature information” on page 152.

Review plate setup e-Signature information

The sections that follow provide descriptions of the information provided in the e-Signature plate setup record for draft runs and run templates. Optionally, this information can be printed.

Signature metadata

This section provides information regarding the signature metadata for each e-Signature plate setup record.

Table 5 Signature metadata

Object	Description
Meaning	The e-Signature option selected.
Signed Date	The date of e-Signature.
Signed By	The name of user.
Host ID	The instrument name.
Full Name	The user name.
Status	The status of the signature: • CURRENT: Valid • OBSOLETE: Invalid
Role	The role assigned to the user who performed the run.

Protocol information

This section provides information regarding the **protocol** section of the e-Signature plate setup record.

Table 6 Protocol details

Object	Description
ScanRed	Status of **True** indicates this optical channel was enabled. Status of **False** indicates this optical channel has been disabled.
ScanGreen	Status of **True** indicates this optical channel was enabled. Status of **False** indicates this optical channel has been disabled.
ScanYellow	Status of **True** indicates this optical channel was enabled. Status of **False** indicates this optical channel has been disabled.
ScanDarkRed	Status of **True** indicates this optical channel was enabled. Status of **False** indicates this optical channel has been disabled.
RNAStep_Duration	The duration of RNA-RT step *(optional)*.
RNAStep_Temperature	The temperature of RNA-RT step *(optional)*.

Table 6 Protocol details *(continued)*

Object	Description
PCRPreheat_Duration	The duration of pre-heat step *(optional)*.
PCRPreheat_Temperature	The temperature of pre-heat step *(optional)*.
PCR_Stage(1/2)_Step(1/2/3)_Duration	The duration of indicated stage and step.
PCR_Stage(1/2)_Step(1/2/3)_Temperature	The temperature of indicated stage and step.
Name	The name of protocol.

Plate channel information for each sample

This section provides information regarding the channels used in the **plate** section of the e-Signature plate setup record. If the channel was not used, the detail will reflect **None** in all data points. The figure that follows depicts a partial record.

Table 7 For each color — blue, green, yellow, red, and dark red

Object	Description
Channel	The name of the target.
Type	The analysis type selected.
Threshold	The predefined analysis threshold.
Selection	The dye selected for the channel.

Additional plate information

This section provides information regarding the additional information provided in the **plate** section of the e-Signature plate setup record.

Table 8 Other plate information

Object	Description
DilutionFactor	Total dilution from sample to reaction mix.
CNVRefNum	The number of copies of the reference genome.
Name	The sample name.
Group	The group name.
GroupType	the group analysis setting

Run metadata

The section provides information regarding the **run name** section of the e-Signature plate setup record.

Table 9 Run metadata

Object	Description
run name	The name given to the run at the instrument.
Columns	Columns enabled for the run.
LastEditedEPOCH	Epoch time stamp of the run.
Barcode	Plate barcode number.

Review plate results e-Signature information

The sections that follow provide descriptions of the information provided in the e-Signature plate results record for completed runs and studies. Optionally, this information can be printed.

Signature metadata

This section provides information regarding the signature metadata for each e-Signature plate results record.

Table 10 Signature metadata

Object	Description
Meaning	E-Signature option selected.
Signed Date	Date of e-Signature.
Signed By	Name of user.
Host ID	Instrument name.
Status	Status of the signature: • CURRENT: Valid • OBSOLETE: Invalid

Results by group

This section provides information regarding the **groups** section of the e-Signature plate results record. A column is included for each dye used.

Table 11 For each group, for each dye

Object	Description
Total	One of the following options: • If replicates, this is the group average of microchambers. • If pooled, this is the total pooled microchambers.

Table 11 For each group, for each dye *(continued)*

Object	Description
Positive	Group positive microchambers.
Conc.(cp./uL)	Group concentration in copies per microliter.

Results for samples

This section provides information regarding the **samples** section of the e-Signature plate results record. A column is included for each dye used.

Table 12 For each sample, for each dye

Object	Description
Total	One of the following options: • If replicates, this is the group average of microchambers • If pooled, this is the total pooled microchambers
Positive	Group positive microchambers.
PosThresh	Analysis threshold input by user.

Run metadata

The section provides information regarding the **run name** section of the e-Signature plates result record.

Table 13 Run metadata

Object	Description
run name	The name given to the run at the instrument.
Columns	Columns enabled for run.
LastEditedEPOCH	Epoch time stamp of the run.
Barcode	Plate barcode number.

Disable SAE functions in QuantStudio™ Absolute Q™ Digital PCR Software

This procedure requires an SAE administrator account.

Close all plate files and data files.

1. In QuantStudio™ Absolute Q™ Digital PCR Software, select ⚙ **System** ▸ **Disable Security**.
2. Enter the password of the SAE administrator account, then click **Sign In**.

Maintain the instrument

Clean the Absolute Q™ instrument and plate nest

All surfaces should be dry and free of dust and lint before operation.

Clean the outside of the instrument with a damp, lint-free cloth using one of the following solutions:

- Mild soap
- 70% ethanol in water

Clean the plate nest surface gently with a lint-free cloth (microfiber cloth or optical lens cleaning cloth) using 70% ethanol in water. Do not wipe the grooves that surround the plate nest.

IMPORTANT! The plate nest is covered in a thin graphite sheet. This sheet is susceptible to scratches and may impact results if it is damaged. It is important to only wipe the graphite surface with lint-free wipes or use air-dusters. Contact technical support if this surface becomes damaged (see Appendix I, “Documentation and support”).

Maintenance

For best results when using the instrument, the following practices are recommended:

- The plate nest must be inspected and cleaned before each run.
- Ensure that the fan vents on the back and bottom of the Absolute Q™ instrument are not obstructed.
- Ensure that system dyes are calibrated on a yearly basis.

Note: A warning message appears on the **Instrument** page 45 days prior to dye calibration expiration. If the dyes are not calibrated within that time frame, a warning message appears indicating that the calibration has expired and remains on the **Instrument** page until the dyes are calibrated.

IMPORTANT! System dye calibration must only be performed by qualified field service engineers. Attempting to calibrate dyes without the assistance of a field service engineer may compromise run data for analysis.

For information about maintenance and service plans, contact technical support (see Appendix I, “Documentation and support”).

Product Specifications

QuantStudio™ Absolute Q™ Digital PCR Instrument specifications

Dimensions (unpacked)	620 mm (l) x 600 mm (w) x 540 mm (h) 24.5 in (l) x 23.5 in (w) x 21.2 in (h)
Dimensions (packaged)	860 mm (l) x 860 mm (w) x 790 mm (h) 33.5 in (l) x 34 in (w) x30 (h)
Weight	Approximately 60 kg, 132 lbs
Connections	Power, USB 3.0 (to dedicated computer)
Cooling mode	Forced convection
Illumination	Rax, Blue, Phosphor Green high-power LED
Optical channels	5 (fixed configuration)
Power input	100-240 V, 50-60Hz
Power rating	1200-1600 W
Rated current	12 A (110V), 8.5 A (230 V)
Maximum noise level	70 dB

Dedicated computer requirements

Operating system	Windows™ 10 (64-bit) or later
Computer	Dell™ Tower

QuantStudio™ Absolute Q™ Digital PCR Instrument Optical Configuration

The QuantStudio™ Absolute Q™ Digital PCR Instrument comes in a single optical configuration and is pre-calibrated during manufacturing. It can be field calibrated for enhanced spectral compensation.

Note: For information about HEX™ dye support, contact a Thermo Fisher service and support representative.

Note: A warning message appears on the **Instrument** page 45 days prior to dye calibration expiration. If the dyes are not calibrated within that time frame, a warning message appears indicating that the calibration has expired and remains on the **Instrument** page until the dyes are calibrated.

#	Color	Excitation filter peak	Emission filter peak	System dyes
1	Blue	466	520	FAM™ dye
2	Green	514	560	VIC™ dye *(recommended)* HEX™ dye[1,2]
3	Yellow	549	589	ABY™ dye
4	Red	589	625	ROX™ dye
5	Dark Red	630	684	CY™5 dye *(recommended)* JUN™ dye

[1] For information about HEX™ dye support, contact a Thermo Fisher service and support representative.

[2] HEX™ data from two instruments cannot be combined into a study, even if the systems are co-calibrated. For more information, see "Multi-plate analysis–Studies" on page 71.

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, visit **thermofisher.com/support**.

AVERTISSEMENT ! SÉCURITÉ GÉNÉRALE. L'utilisation de ce produit d'une manière non spécifiée dans le manuel d'utilisation peut entraîner des blessures ou endommager l'instrument ou l'appareil. Assurez-vous que toute personne utilisant ce produit est formée aux pratiques générales de sécurité pour les laboratoires et aux informations de sécurité fournies dans le présent document.

- Avant d'utiliser un instrument ou un dispositif, lisez et assimilez les informations de sécurité figurant dans le manuel d'utilisation fourni par le fabricant de l'instrument ou du dispositif.
- Avant de manipuler des produits chimiques, lisez et assimilez toutes les fiches de données de sécurité (FDS) applicables et utilisez les équipements de protection individuelle appropriés (gants, blouses, lunettes de protection, etc.). Pour consulter les fiches de données de sécurité, rendez-vous sur le site **thermofisher.com/support**.

Symbols on this instrument

Symbols may be found on the instrument to warn against potential hazards or convey important safety information. In this document, the hazard symbol is used along with one of the following user attention words.

- **CAUTION!**—Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.
- **WARNING!**—Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.
- **DANGER!**—Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury.

Standard safety symbols

Symbol and description
CAUTION! Risk of danger. Consult the manual for further safety information.
CAUTION! Caution, air inlet.
CAUTION! Hot surface.
CAUTION! Potential biohazard.

Symbole et description
MISE EN GARDE ! Risque de danger. Consulter le manuel pour d'autres renseignements de sécurité.
MISE EN GARDE ! Risque de choc électrique.
MISE EN GARDE ! Surface chaude.
MISE EN GARDE ! Danger biologique potentiel.

Control and connection symbols

Symbols and descriptions	
	On (Power)
	Off (Power)
	Protective conductor terminal (main ground)

Conformity symbols

Conformity mark	Description
	Indicates conformity with the WEEE Directive 2012/19/EU. **CAUTION!** To minimize negative environmental impact from disposal of electronic waste, do not dispose of electronic waste in unsorted municipal waste. Follow local municipal waste ordinances for proper disposal provision and contact customer service for information about responsible disposal options. **MISE EN GARDE !** Pour réduire l'empreinte écologique résultant de l'élimination des composants électroniques, ne les jetez pas dans les déchets municipaux non triées. Respectez les réglementations locales en matière de déchets pour un traitement approprié et contactez le service clientèle pour en savoir plus sur les solutions responsables.

Safety information for instruments not manufactured by Thermo Fisher Scientific

Some of the accessories provided as part of the instrument system are not designed or built by Thermo Fisher Scientific. Consult the manufacturer's documentation for the information needed for the safe use of these products.

Instrument safety

General

CAUTION! **Do not remove instrument protective covers.** If you remove the protective instrument panels or disable interlock devices, you may be exposed to serious hazards including, but not limited to, severe electrical shock, laser exposure, crushing, or chemical exposure.

MISE EN GARDE ! **Ne retirez pas les couvercles de protection de l'instrument.** Si vous retirez les panneaux de protection des instruments ou si vous désactivez les dispositifs de verrouillage, vous risquez de courir de graves dangers, comme, par exemple, un choc électrique, une exposition au laser, un écrasement ou une exposition à des produits chimiques.

Hot Surface

CAUTION! **Hot surface.** During instrument operation, the temperature of the plate nest can be as high as 100°C. The instrument has a software interlock to prevent the door from opening if the plate nest temperature is over 45°C, but if the system appears to be malfunctioning use caution when operating near the plate nest.

MISE EN GARDE ! **Surface chaude.** En cours de fonctionnement, la température des plaques peut atteindre 100°C. L'instrument est doté d'un logiciel de verrouillage qui empêche l'ouverture de la porte si la température des plaques est supérieure à 45°C. Toutefois, si le système semble présenter un dysfonctionnement, soyez prudent lorsque vous travaillez à proximité des plaques.

Air inlet

CAUTION! **Air inlet.** Air inlet is only suitable for atmospheric air and not pressurized gas. Do not connect flammable gas to the air inlet port. Do not restrict air inlet port.

MISE EN GARDE ! **Arrivée d'air.** L'arrivée d'air ne convient qu'à l'air atmosphérique et non aux gaz sous pression. Ne raccordez pas de gaz inflammable à l'orifice d'arrivée d'air. Veillez à ne pas obstruer l'orifice d'arrivée d'air.

Physical injury

CAUTION! Moving and Lifting Injury. Improper lifting can cause painful and permanent back injury.

Things to consider before lifting or moving the instrument or accessories:

- Depending on the weight, moving or lifting may require two or more persons.
- If you decide to lift or move the instrument after it has been installed, do not attempt to do so without the assistance of others, the use of appropriate moving equipment, and proper lifting techniques.
- Ensure you have a secure, comfortable grip on the instrument or accessory.
- Make sure that the path from where the object is to where it is being moved is clear of obstructions.
- Do not lift an object and twist your torso at the same time. Keep your spine in a good neutral position while lifting with your legs.
- Participants should coordinate lift and move intentions with each other before lifting and carrying.
- For smaller packages, rather than lifting the object from the packing box, carefully tilt the box on its side and hold it stationary while someone else slides the contents out of the box.

MISE EN GARDE ! Blessures causées par le déplacement et le soulèvement. Soulever de manière inappropriée peut provoquer des lésions dorsales douloureuses et permanentes.

Éléments à prendre en compte avant de soulever ou de déplacer l'instrument ou ses accessoires:

- Selon le poids, deux personnes ou plus peuvent être nécessaires pour déplacer ou soulever l'instrument.
- Si vous décidez de soulever ou de déplacer l'instrument après son installation, n'essayez pas de le faire seul, sans un équipement approprié et sans avoir recours à des techniques appropriées.
- Assurez-vous d'avoir une prise sûre et confortable sur l'instrument ou l'accessoire.
- Assurez-vous que le chemin entre l'endroit où se trouve l'objet et l'endroit où il est déplacé est libre de tout obstacle.
- Ne soulevez pas un objet et ne pivotez pas votre torse en même temps. Tenez votre colonne vertébrale dans une position bien droite en vous relevant.
- Les participants doivent coordonner leurs mouvements avant de soulever et de porter.
- Pour les petits colis, au lieu de soulever l'objet de son emballage, inclinez soigneusement le carton sur le côté et maintenez-le immobile pendant que quelqu'un d'autre fait glisser le contenu hors du carton.

Electrical safety

WARNING! Ensure appropriate electrical supply. For safe operation of the instrument:

- Plug the system into a properly grounded receptacle with adequate current capacity.
- Ensure the electrical supply is of suitable voltage.
- Never operate the instrument with the ground disconnected. Grounding continuity is required for safe operation of the instrument.

AVERTISSEMENT ! Veiller à utiliser une alimentation électrique appropriée. Pour garantir le fonctionnement de l'instrument en toute sécurité :

- Brancher le système sur une prise électrique correctement mise à la terre et de puissance adéquate.
- S'assurer que la tension électrique est convenable.
- Ne jamais utiliser l'instrument alors que le dispositif de mise à la terre est déconnecté. La continuité de la mise à la terre est impérative pour le fonctionnement de l'instrument en toute sécurité.

WARNING! Power Supply Line Cords. Use properly configured and approved line cords for the power supply in your facility.

AVERTISSEMENT ! Cordons d'alimentation électrique. Utiliser des cordons d'alimentation adaptés et approuvés pour raccorder l'instrument au circuit électrique du site.

WARNING! Disconnecting Power. To fully disconnect power either detach or unplug the power cord, positioning the instrument such that the power cord is accessible.

AVERTISSEMENT ! Déconnecter l'alimentation. Pour déconnecter entièrement l'alimentation, détacher ou débrancher le cordon d'alimentation. Placer l'instrument de manière à ce que le cordon d'alimentation soit accessible.

Cleaning and decontamination

CAUTION! Cleaning and Decontamination. Use only the cleaning and decontamination methods that are specified in the manufacturer user documentation. It is the responsibility of the operator (or other responsible person) to ensure that the following requirements are met:

- No decontamination or cleaning agents are used that can react with parts of the equipment or with material that is contained in the equipment. Use of such agents could cause a HAZARD condition.
- The instrument is properly decontaminated a) if hazardous material is spilled onto or into the equipment, and/or b) before the instrument is serviced at your facility or is sent for repair, maintenance, trade-in, disposal, or termination of a loan. Request decontamination forms from customer service.
- Before using any cleaning or decontamination methods (except methods that are recommended by the manufacturer), confirm with the manufacturer that the proposed method will not damage the equipment.

MISE EN GARDE ! Nettoyage et décontamination. Utiliser uniquement les méthodes de nettoyage et de décontamination indiquées dans la documentation du fabricant destinée aux utilisateurs. L'opérateur (ou toute autre personne responsable) est tenu d'assurer le respect des exigences suivantes:

- Ne pas utiliser d'agents de nettoyage ou de décontamination susceptibles de réagir avec certaines parties de l'appareil ou avec les matières qu'il contient et de constituer, de ce fait, un DANGER.
- L'instrument doit être correctement décontaminé a) si des substances dangereuses sont renversées sur ou à l'intérieur de l'équipement, et/ou b) avant de le faire réviser sur site ou de l'envoyer à des fins de réparation, de maintenance, de revente, d'élimination ou à l'expiration d'une période de prêt (des informations sur les formes de décontamination peuvent être demandées auprès du Service clientèle).
- Avant d'utiliser une méthode de nettoyage ou de décontamination (autre que celles recommandées par le fabricant), les utilisateurs doivent vérifier auprès de celui-ci qu'elle ne risque pas d'endommager l'appareil.

Instrument component and accessory disposal

CAUTION! To minimize negative environmental impact from disposal of electronic waste, do not dispose of electronic waste in unsorted municipal waste. Follow local municipal waste ordinances for proper disposal provision and contact customer service for information about responsible disposal options.

MISE EN GARDE ! Pour réduire l'empreinte écologique résultant de l'élimination des composants électroniques, ne les jetez pas dans les déchets municipaux non triées. Respectez les réglementations locales en matière de déchets pour un traitement approprié et contactez le service clientèle pour en savoir plus sur les solutions responsables.

Safety and electromagnetic compatibility (EMC) standards

The instrument design and manufacture complies with the following standards and requirements for safety and electromagnetic compatibility.

Safety standards

Reference	Description
EU Directive 2011/65/EU & Commission Delegated Directive (EU) 2015/863	European Union "RoHS Directive" – Restriction of hazardous substances in electrical and electronic equipment
IEC 61010-1	*Safety requirements for electrical equipment for measurement, control, and laboratory use – Part 1: General requirements*
IEC 61010-2-010	*Safety requirements for electrical equipment for measurement, control and laboratory use – Part 2-010: Particular requirements for laboratory equipment for the heating of materials*
IEC 61010-2-081	*Safety requirements for electrical equipment for measurement, control and laboratory use – Part 2-081: Particular requirements for automatic and semi-automatic laboratory equipment for analysis and other purposes*

EMC standards

Reference	Description
EMC EN 61326-1	*Electrical Equipment for Measurement, Control and Laboratory Use – EMC Requirements – Part 1: General Requirements*
FCC Class A equipment Caution	This device complies with Part 15 of the FCC rules. Operation is subject to the following two conditions: **1.** This device may not cause harmful interference, and **2.** This device must accept any interference received, including interference that may cause undesired operation.
FCC Part 15 Subpart B (47 CFR)	*U.S. Standard Radio Frequency Devices* This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his own expense.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if needed) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

AVERTISSEMENT ! PRÉCAUTIONS GÉNÉRALES EN CAS DE MANIPULATION DE PRODUITS CHIMIQUES. Pour minimiser les risques, veiller à ce que le personnel du laboratoire lise attentivement et mette en œuvre les consignes de sécurité générales relatives à l'utilisation et au stockage des produits chimiques et à la gestion des déchets qui en découlent, décrites ci-dessous. Consulter également la FDS appropriée pour connaître les précautions et instructions particulières à respecter :

- Lire et comprendre les fiches de données de sécurité (FDS) fournies par le fabricant avant de stocker, de manipuler ou d'utiliser les matériaux dangereux ou les produits chimiques. Pour obtenir les FDS, se reporter à la section « Documentation et support » du présent document.
- Limiter les contacts avec les produits chimiques. Porter des équipements de protection appropriés lors de la manipulation des produits chimiques (par exemple : lunettes de sûreté, gants ou vêtements de protection).
- Limiter l'inhalation des produits chimiques. Ne pas laisser les récipients de produits chimiques ouverts. Ils ne doivent être utilisés qu'avec une ventilation adéquate (par exemple, sorbonne).
- Vérifier régulièrement l'absence de fuite ou d'écoulement des produits chimiques. En cas de fuite ou d'écoulement d'un produit, respecter les directives de nettoyage du fabricant recommandées dans la FDS.
- Manipuler les déchets chimiques dans une sorbonne.

- Veiller à utiliser des récipients à déchets primaire et secondaire. (Le récipient primaire contient les déchets immédiats, le récipient secondaire contient les fuites et les écoulements du récipient primaire. Les deux récipients doivent être compatibles avec les matériaux mis au rebut et conformes aux exigences locales, nationales et communautaires en matière de confinement des récipients.)
- Une fois le récipient à déchets vidé, il doit être refermé hermétiquement avec le couvercle fourni.
- Caractériser (par une analyse si nécessaire) les déchets générés par les applications, les réactifs et les substrats particuliers utilisés dans le laboratoire.
- Vérifier que les déchets sont convenablement stockés, transférés, transportés et éliminés en respectant toutes les réglementations locales, nationales et/ou communautaires en vigueur.
- **IMPORTANT !** Les matériaux représentant un danger biologique ou radioactif exigent parfois une manipulation spéciale, et des limitations peuvent s'appliquer à leur élimination.

Biological hazard safety

WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

AVERTISSEMENT ! Risque biologique potentiel. En fonction des échantillons utilisés sur cet instrument, la surface peut être considérée comme présentant un risque biologique. Utilisez des méthodes de décontamination appropriées lorsque vous travaillez en présence de risques biologiques.

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020
  www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2020-P.pdf
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition, and associated monographs)
  www.who.int/publications/i/item/9789240011311

AVERTISSEMENT ! RISQUE BIOLOGIQUE. Les échantillons biologiques tels que les tissus, les fluides corporels, les agents infectieux et le sang de l'homme et d'autres animaux sont susceptibles de transmettre des maladies infectieuses. Effectuez tous vos travaux dans des installations correctement équipées et dotées du matériel de sécurité approprié (par exemple, des dispositifs de confinement physique). L'équipement de sécurité peut également inclure des articles de protection personnelle, tels que des gants, des manteaux, des blouses, des couvre-chaussures, des bottes, des respirateurs, des masques faciaux, des lunettes de sécurité ou des lunettes de protection. Les personnes doivent être formées conformément aux exigences réglementaires applicables et aux exigences de l'entreprise ou de l'institution avant de travailler avec des matières potentiellement dangereuses. Respectez toutes les réglementations locales, nationales et/ou provinciales applicables. Les références suivantes proposent des recommandations générales pour la manipulation d'échantillons biologiques dans un environnement de laboratoire.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 6th Edition, HHS Publication No. (CDC) 300859, révision de juin 2020
  www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2020-P.pdf
- Laboratory biosafety manual, fourth edition. Genève: Organisation mondiale de la santé; 2020 (Laboratory biosafety manual, fourth edition, et monographies associées)
  www.who.int/publications/i/item/9789240011311

Documentation and support

Related documentation

Document	Publication number	Description
QuantStudio™ Absolute Q™ Digital PCR Starter Kit User Guide	MAN0025653	Describes the setup, use, and analysis of runs using the QuantStudio™ Absolute Q™ Digital PCR Starter Kit assay. (Catalog No. A52732)
QuantStudio™ Absolute Q™ Digital PCR System Site Preparation Guide	MAN0026431	Describes the site preparation required for installing the QuantStudio™ Absolute Q™ dPCR System.
QuantStudio™ Absolute Q™ Digital PCR System IT Checklist	MAN0028309	Describes the IT setup requirements for successful installation of the QuantStudio™ Absolute Q™ dPCR System, and for the effective support of the instrument by Thermo Fisher Scientific.
QuantStudio™ Absolute Q™ Digital PCR System Remote API User Guide	MAN0028059	Describes the Remote API for the automation of the QuantStudio™ Absolute Q™ dPCR System.
SAE Administrator Console v2.0 or later User Guide for PCR systems	MAN0017468	Describes the setup and use of the Security, Auditing, and E-signature (SAE) module.

Note: For additional documentation, see “Customer and technical support” on page 168.

Customer and technical support

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- 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

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