

QuantStudio™ 3D Digital PCR System

USER GUIDE

for use with:

QuantStudio™ 3D Digital PCR Instrument

ProFlex™ 2 × Flat PCR System or Dual Flat Block GeneAmp™ PCR System 9700

QuantStudio™ 3D Digital PCR 20K Chip v2

QuantStudio™ 3D Digital PCR Master Mix v2

QuantStudio™ 3D Digital PCR Chip Loader

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

**Manufacturer:**

Life Technologies Holdings Pte Ltd |
Block 33 |
Marsiling Industrial Estate Road 3 |
#07-06, Singapore 739256

Products:

QuantStudio™ 3D Digital PCR Instrument
ProFlex™ 2 × Flat PCR System
Dual Flat Block GeneAmp™ PCR System 9700
QuantStudio™ 3D Digital PCR Chip Loader

**Manufacturer:**

Life Technologies Corporation |
6055 Sunol Blvd |
Pleasanton, CA 94566

Products:

QuantStudio™ 3D Digital PCR 20K Chip v2



Life Technologies Corporation |
7335 Executive Way | Frederick, MD
21704 | USA

Products:

QuantStudio™ 3D Digital PCR Master Mix v2

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

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C.0	February 2015	Updated for firmware and software revisions, including changes to instrument networking, thermal cycling, chip analysis, maintenance, software access, and computer requirements. Updated to latest corporate boilerplate. Updated general sample and chip preparation, system installation, troubleshooting, and parts and materials.
B.0	April 2014	Added support for the ProFlex™ PCR System and revised the chip loading instructions.
A.0	December 2013	Updated the manual chip preparation and added procedures for chip preparation using the QuantStudio™ 3D Digital PCR Chip Loader and wireless network installation.
02	June 2013	Updated general chip preparation and instrument networking.
01	March 2013	Initial version

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About the QuantStudio™ 3D Digital PCR System

The QuantStudio™ 3D Digital PCR System provides quantitative and qualitative detection of target nucleic acid sequences (targets) using post-PCR (endpoint) analysis.

Use the QuantStudio™ 3D Digital PCR Instrument to perform imaging and preliminary analysis of a QuantStudio™ 3D Digital PCR 20K Chip v2. The chip must have been loaded with fluorescent-labeled quantitative PCR reagents (TaqMan™ probe-based assays or SYBR™ Green primer-based assays) and thermal cycled using a ProFlex™ 2 × Flat PCR System (or Dual Flat Block GeneAmp™ PCR System 9700). The QuantStudio™ 3D AnalysisSuite™ Software performs subsequent analysis and post-processing to yield relative or absolute quantification results from the raw imaging data.

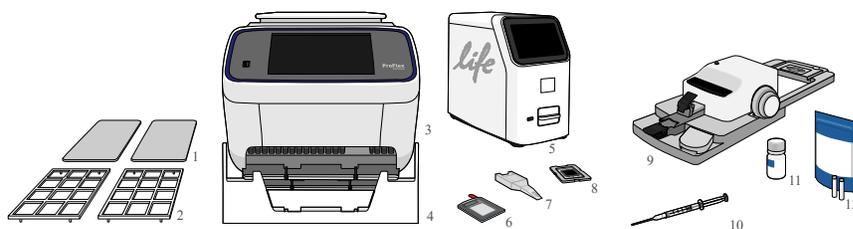


Figure 1 Components of the QuantStudio™ 3D Digital PCR System

- ① QuantStudio™ 3D Digital PCR Thermal Pads
- ② QuantStudio™ 3D Digital PCR Chip Adapters
- ③ ProFlex™ 2 × Flat PCR System (or Dual Flat Block GeneAmp™ PCR System 9700)
- ④ QuantStudio™ 3D Tilt Base
- ⑤ QuantStudio™ 3D Digital PCR Instrument
- ⑥ QuantStudio™ 3D Digital PCR Chip Lid v2
- ⑦ QuantStudio™ 3D Digital PCR Sample Loading Blade
- ⑧ QuantStudio™ 3D Digital PCR 20K Chip v2
- ⑨ (Optional) QuantStudio™ 3D Digital PCR Chip Loader
- ⑩ Immersion Fluid syringe
- ⑪ QuantStudio™ 3D Digital PCR Master Mix v2
- ⑫ Fluorescent-labeled quantitative PCR reagents (TaqMan™ Assays or SYBR™ Green primers)
- ⑬ (Not shown) QuantStudio™ 3D AnalysisSuite™ Software

Instruments, kits, consumables, and accessories

The following tables describe the products covered in this user guide.

Instrument system		
Item	Catalog No.	Amount
QuantStudio™ 3D Digital PCR System package with v2 chips and master mix, includes: <ul style="list-style-type: none"> QuantStudio™ 3D Digital PCR Instrument QuantStudio™ 3D Digital PCR Chip Loader QuantStudio™ 3D Digital PCR 20K Chips v2 QuantStudio™ 3D Digital PCR Master Mix v2 ProFlex™ 2 × Flat PCR System 	A29154	1 system
QuantStudio™ 3D Digital PCR Instrument with power cord	4489084	1 instrument
QuantStudio™ 3D Digital PCR Tilt Base for ProFlex™ Thermal Cycler	A24898	1 base
QuantStudio™ 3D Digital PCR Tilt Base for GeneAmp™ PCR System 9700	4486414	1 base
QuantStudio™ 3D Digital PCR Chip Adapter Kit for Flat Block Thermal Cycler	4485513	1 adapter
QuantStudio™ 3D Extended Arm WiFi Adapter	A28598	1 wireless adapter
QuantStudio™ 3D Digital PCR 20K Chip Kit v2 and Master Mix v2, includes: <ul style="list-style-type: none"> 8 × 12 packs of v2 chips, chip lids, and QuantStudio™ 3D Digital PCR Sample Loading Blades 24 syringes of Immersion Fluid, plus tips 1.5 mL of QuantStudio™ 3D Digital PCR Master Mix v2 	A26317	1 kit (96 chips)
QuantStudio™ 3D Digital PCR 20K Chip Kit v2, includes: <ul style="list-style-type: none"> 12 v2 chips, chip lids, and QuantStudio™ 3D Digital PCR Sample Loading Blades 3 syringes of Immersion Fluid, plus tips 	A26316	1 kit (12 chips)
QuantStudio™ 3D Digital PCR Immersion Fluid, 1.6 mL per syringe	A27322	3 syringes

Instrument system		
Item	Catalog No.	Amount
QuantStudio™ 3D Digital PCR Chip Loader	4482592	1 loader
QuantStudio™ 3D Digital PCR Chip Lids v2 and QuantStudio™ 3D Digital PCR Sample Loading Blades	A29247	12 each
QuantStudio™ 3D Digital PCR Master Mix v2	A26358 A26359	1.5 mL 5.0 mL
QuantStudio™ 3D Digital PCR Reagent Kit, includes: <ul style="list-style-type: none"> Assays and reagents for system installation and verification, or training of operators on the use of the system 	A26360	1 kit
QuantStudio™ 3D Digital PCR Starter Kit, includes: <ul style="list-style-type: none"> QuantStudio™ 3D Digital PCR Reagent Kit QuantStudio™ 3D Digital PCR 20K Chip Kit v2 (12 chips) 	A26361	1 kit

Required materials not supplied

This system uses common molecular biology equipment, supplies, and reagents. 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
Clean room-grade, low-lint polyester wipes	Fisher Scientific (Cat. No. NC0375992) or MLS
Gloves, powder-free, nitrile	MLS
Microcentrifuge	MLS
Pipette tips, 10 to 1000 µL	MLS
Pipettes, P10 to P1000	MLS
Reaction tubes, DNase/RNase-free, non-stick or low-binding: 0.5-mL or 1.5-mL	Fisher Scientific (Cat. No. 13-864-253 or 13-864-254) or MLS
Vortexer	MLS
Water, DNase-free, sterile-filtered	MLS
Water, deionized	MLS

(continued)

Item	Source
Isopropanol	MLS
<i>(Required for manual chip loading only)</i> Heated block (capable of maintaining $40\pm 1^{\circ}\text{C}$)	MLS

Power line regulator

Use a 1.5-kVA power line regulator in areas where the supplied power fluctuates in excess of $\pm 10\%$ of the normal voltage. Power fluctuations can adversely affect the function of the systems.

Note: A power line regulator monitors the input current and adjusts the power supplied to the systems. It does not protect against a power surge or failure.

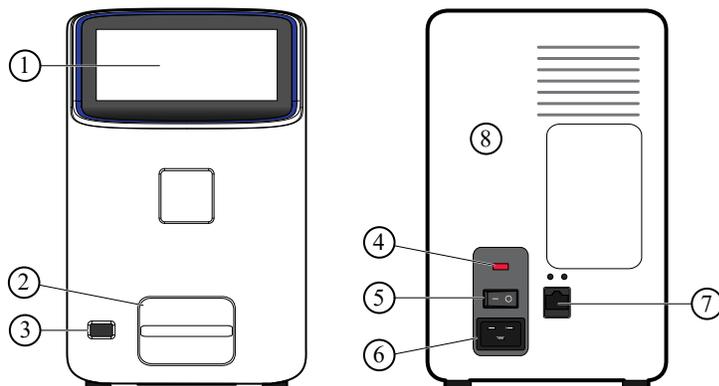
Surge protector

Use a 10-kVA surge protector (line conditioner) in areas with frequent electrical storms or near devices that are electrically noisy, such as refrigerators, air conditioners, or centrifuges. Short-duration, high-voltage power fluctuations can abruptly terminate the function of, and thereby damage the components of, the systems.

Note: A dedicated line and ground between the systems and the building's main electrical service can also prevent problems caused by power fluctuations.

About the instrument

The QuantStudio™ 3D Digital PCR Instrument consists of the components shown in the following figure.



- ① **Touchscreen**—Provides access to the instrument functions, such as data transfer and instrument operation.
- ② **Chip tray**—Conveys the QuantStudio™ 3D Digital PCR 20K Chip v2 to and from the imaging stage in the interior of the instrument.
- ③ **USB port**—Provides USB communication with the instrument. Can be used to transfer data to and from the instrument.
- ④ **Fuse cover**—Dual 1.6A, Time Lag T, 250 VAC, 5 × 20-mm electrical fuses that protect the instrument from excessive electrical current.
- ⑤ **Power switch**—Power switch for the instrument, where the states are on (|) or off (O).
- ⑥ **Power port**—A 100–240 VAC port that provides power to the instrument.
- ⑦ **Ethernet port**—An RJ45 port that provides Ethernet (gigabit) communication with the instrument.

Data collection

The QuantStudio™ 3D Digital PCR Instrument collects raw fluorescence data from the QuantStudio™ 3D Digital PCR 20K Chip v2 following PCR amplification.

The instrument performs multiple image captures of the chip in the following three phases:

1. **Excitation** – The instrument illuminates all wells of the chip, exciting the fluorophores in each reaction.
2. **Emission Collection** – The instrument optics collect the residual fluorescence emitted from the wells. The resulting image consists of light corresponding to the range of emission wavelengths for the filter(s) in use.
3. **Interpretation** – The instrument assembles a digital representation of the residual fluorescence collected over a fixed time interval.

After a run, the instrument determines the location and intensity of the fluorescent signals in each image, the dye associated with each fluorescent signal, and the significance of the signal.

Supported dyes

The QuantStudio™ 3D Digital PCR System features a filter set that is optimized for the Applied Biosystems™ FAM™, ROX™, and VIC™ dyes.

About the software

The QuantStudio™ 3D Digital PCR System includes a software suite used to analyze and manage digital PCR data generated by the QuantStudio™ 3D Digital PCR Instrument. The QuantStudio™ 3D AnalysisSuite™ Software includes a server and Connect cloud-based platform deployment options that can satisfy a broad range of laboratory requirements.

System requirements

The QuantStudio™ 3D AnalysisSuite™ Software is a web-based application that is recommended for use with the following Internet browsers.

- Google™ Chrome™ v37 or later
- Microsoft™ Internet Explorer™ v10 or later
- Mozilla™ Firefox™ v32 or later
- Apple™ Safari™ v6 or later

Note: The software performance may vary based on your system configuration, and requires an internet connection capable of 300kbps/300kbps (upload/download) or better. If your network employs a firewall that restricts outbound traffic, it must be configured to allow outbound access to apps.lifetechnologies.com on HTTPS-443.

About the chip

The QuantStudio™ 3D Digital PCR 20K Chip v2 is a 10-mm² high-density reaction plate that has a single array of 20,000 reaction wells. Hydrophobic coatings on the chip surface enable loading and isolation of independent PCR reactions within the wells.

Once loaded with a reaction, the chip must be sealed using the QuantStudio™ 3D Digital PCR Chip Lid v2 and filled with Immersion Fluid. Figure 2 shows the components of the chip assembly.

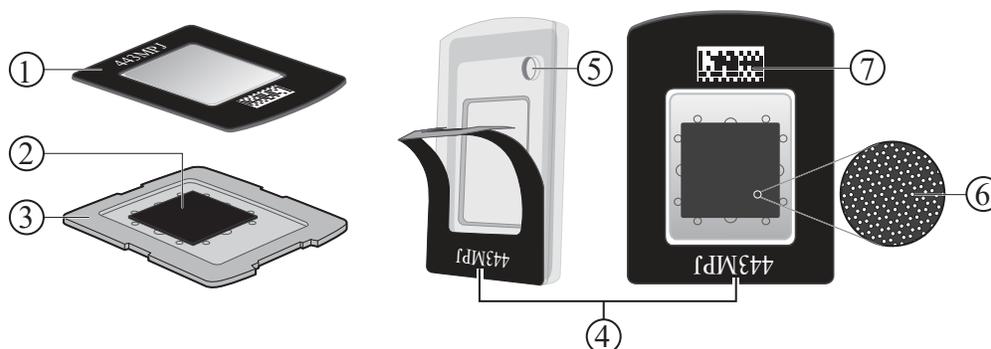


Figure 2 QuantStudio™ 3D Digital PCR 20K Chip v2

- ① **QuantStudio™ 3D Digital PCR Chip Lid v2**—The lid used to seal the chip for thermal cycling and imaging.
- ② **QuantStudio™ 3D Digital PCR 20K Chip v2**—A 10-mm² chip with 20,000 wells that contain the individual PCR reactions.
- ③ **Chip case**—The thermal-conductive base that secures and protects the chip.
- ④ **Chip ID**—A unique alphanumeric ID on the chip lid.
- ⑤ **Fill port**—The hole in the chip case lid through which Immersion Fluid is injected onto the chip.
- ⑥ **Reaction wells**—The 20,000 physical holes on the surface of the chip that contain the individual PCR reactions.
- ⑦ **2D barcode**—A barcode that encodes the same alphanumeric string as the Chip ID, scanned by the QuantStudio™ 3D Digital PCR Instrument for chip tracking.

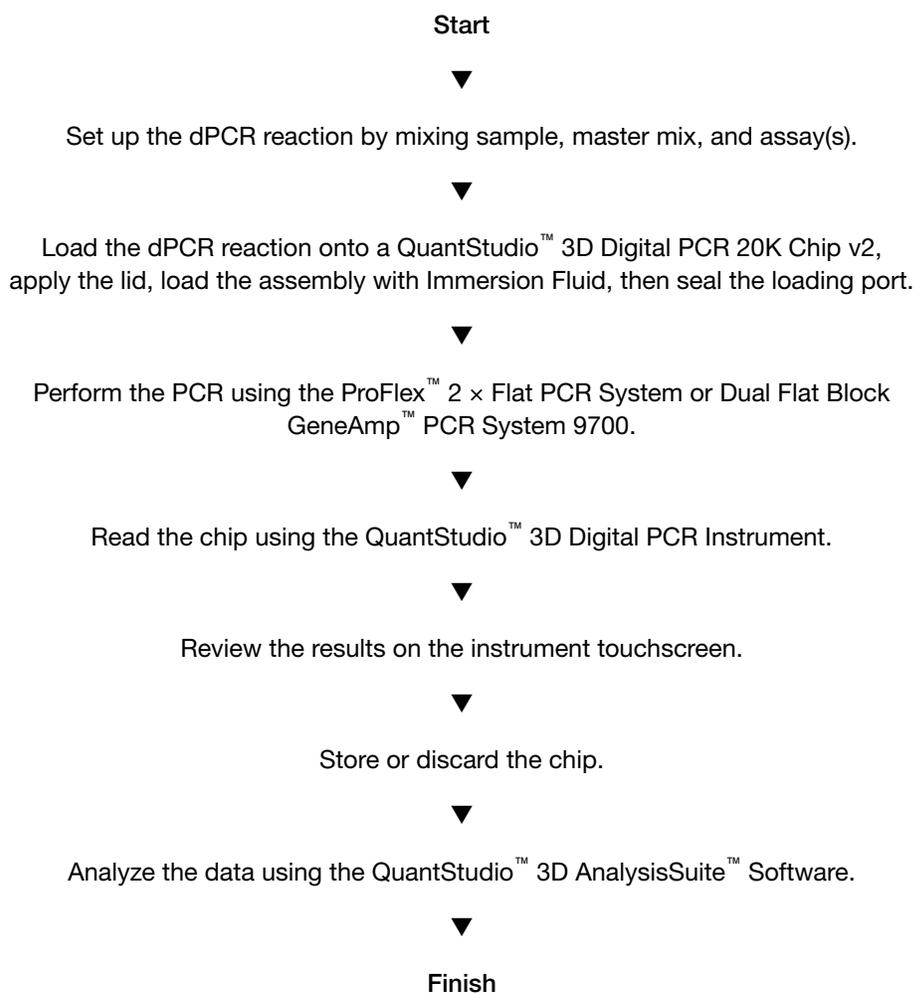
2

Getting Started

For system installation instructions, see Appendix A, “Install the QuantStudio™ 3D Digital PCR System”.

Operational workflow

The following shows a single experiment workflow on the QuantStudio™ 3D Digital PCR System. The procedures for sample and/or consumable preparation and result analysis can vary depending on the specific experiment that you are performing.



Note: See the user documentation for the QuantStudio™ 3D AnalysisSuite™ Software for detailed information on data analysis.

Connect the QuantStudio™ 3D Digital PCR Instrument to a network

If you choose not to connect the QuantStudio™ 3D Instrument using the setup wizard during installation, you can connect it to a network at any time afterwards to streamline data transfer to the QuantStudio™ 3D AnalysisSuite™ Software. See Appendix D, “Networking” for the complete installation procedure, including guidelines for integrating the instrument into your laboratory network.

IMPORTANT! This document *does not* provide adequate detail to integrate the QuantStudio™ 3D Instrument into all possible network architectures. Because your laboratory network can contain advanced features (such as firewalls), we recommend that you consult a network administrator before connecting your instrument.

Connect the QuantStudio™ 3D AnalysisSuite™ Software

You can connect to the QuantStudio™ 3D AnalysisSuite™ Software before or after you install the QuantStudio™ 3D Digital PCR Instrument. The software supports two methods of deployment (server and Connect cloud-based platform), so the method that you follow will depend upon the deployment you purchased. Regardless of the deployment, you can use the AnalysisSuite™ Software web application to analyze data generated by your QuantStudio™ 3D Instrument. For more information, see the user documentation for the QuantStudio™ 3D AnalysisSuite™ Software.

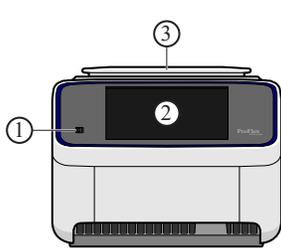
Choosing a thermal cycler

IMPORTANT! Due to the unique physical characteristics of the QuantStudio™ 3D Digital PCR 20K Chip v2, the ProFlex™ 2 × Flat PCR System and the GeneAmp™ PCR System 9700 are the only thermal cyclers approved to run prepared chips.

Compatible thermal cyclers

The QuantStudio™ 3D Digital PCR Instrument can image the QuantStudio™ 3D Digital PCR 20K Chip v2 prepared using the ProFlex™ 2 × Flat PCR System or the Dual Flat Block GeneAmp™ PCR System 9700. Both automated thermal cyclers perform nucleic acid amplification using the polymerase chain reaction (PCR) and are designed to run the chip.

The ProFlex™ 2 × Flat PCR System interface consists of a touchscreen that is used for both data entry and display. The GeneAmp™ PCR System 9700 interface consists of a control panel with a full numeric keypad, soft keys, and an LCD display screen that shows run information. Both the ProFlex™ PCR System and the GeneAmp™ PCR System 9700 use flat sample block modules that install to the tops of the instrument base modules. The sample block modules include two flat aluminum sample blocks that are designed to perform thermal cycling of up to 24 sealed chips simultaneously. Both sample block modules include heated covers that are manually positioned over the sample blocks before each PCR run. The covers apply pressure to the chips to seat them firmly and precisely on the sample blocks, ensuring efficient heat transfer.



ProFlex™ Instrument



GeneAmp™ Instrument

- ① USB port
- ② Touchscreen
- ③ Heated cover
- ④ LCD display
- ⑤ Keypad
- ⑥ Power button

3

Prepare the digital PCR reaction and load the chips

- Guidelines for loading and sealing chips 18
- Prepare the DNA samples 19
- Prepare the digital PCR reactions 21
- Load the chips using the QuantStudio™ 3D Digital PCR Chip Loader 23

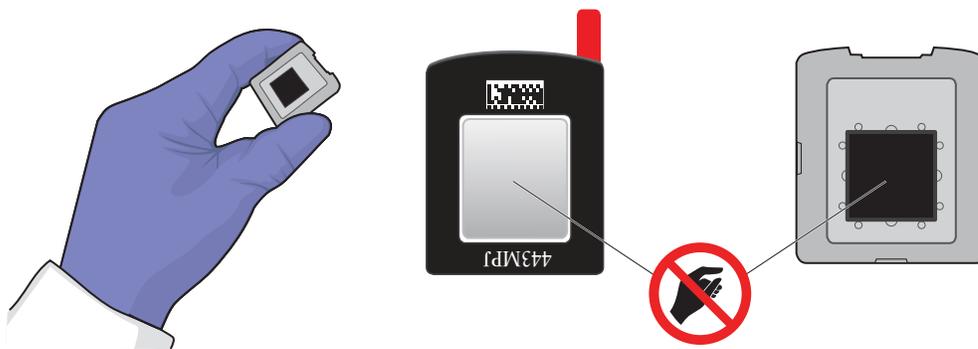
Guidelines for loading and sealing chips

To ensure proper processing and analysis of loaded chips, handle them according to the following guidelines:

- Always wear powder-free gloves when loading and sealing chips.

IMPORTANT! Never handle chips or chip lids without gloves. Oils from your hands can contaminate the components and interfere with thermal cycling and imaging.

- Hold the chip and lid gently by their sides.
 - If you accidentally touch the chip surface, discard the chip.
 - If you accidentally touch the chip lid window, clean it using a laboratory wipe that has been sprayed with isopropanol, then dry by wiping in one direction using a clean low-lint wipe.



- Use a permanent pen or marker to label the back of each loaded chip for sample tracking.
- Load each chip within 2 hours after opening it.
- Load chips in alphanumeric order (according to the chip ID printed on the lid) to avoid data entry errors.
- Apply the chip lid and add Immersion Fluid immediately after loading each chip to avoid evaporation.

- Load and seal chips in batches of up to 24 chips (the maximum number that can be loaded onto one thermal cycler).
- If you do not intend to load an opened chip immediately, cover the chip using the plastic plate included in the chip package to prevent contamination. If you plan to keep the opened chip for longer than a day, the chip should be stored with desiccant.
- Use all of the Immersion Fluid within 60 minutes of uncapping the syringe. After a syringe is opened, you cannot recap it for later use.
- Thermal cycle the chips within 2 hours after loading them.

Prepare the DNA samples

We recommend the following best practices for the preparation of DNA template, genomic DNA (gDNA) or 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.

Quality of DNA

Use gDNA or cDNA template that:

- Is extracted from the raw material that you are testing with an optimized protocol; 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 absorbance 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 absorbance 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:

- 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

Quantitation methods

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

We recommend the following methods of quantitation:

- Quant-iT™ assay nucleic acid quantitation using the Qubit™ Quantitation Platform
or
- Use spectrophotometer to determine nucleic acid concentration

Sample dilution

Should a target be present at a sufficiently high concentration in the sample of interest, it is possible that all reaction replicates will be positive, thus preventing the determination of the target concentration. In this case, the sample must first be diluted prior to running the digital PCR experiment.

Determine the optimal dilution when the target is known

In a dPCR experiment, gDNA samples are diluted down to a limiting quantity, such that most individual PCR reactions contain either zero or one target molecule. The procedure for determining the optimal dilution for a sample differs depending on whether or not the target copy number per genome is known.

If the target copy number per genome of your samples is known, dilute the samples so that each reaction well on a chip will contain approximately 0.6 to 1.6 copies of the target sequence. For example:

1. Assuming 3.3 pg/copy of a given gene in the human genome, and a reaction-well volume of 755 pL
2. Dilute the stock gDNA in a given sample to ~1,045 copies/μL or 3.45 ng/μL in the final reaction, resulting in ~0.73 copies per reaction well.

Determine the target copy number per genome

To determine the copy number per genome:

1. If the source or species of the gDNA is known, refer to <http://www.cbs.dtu.dk/databases/DOGS/index.html> to determine the size of the genome.
2. Once the size of the genome is known, calculate the mass using the following formula:

$$m = (n) (1.096 \times 10^{-21} \text{ g/bp})$$
 where m is the genome mass in grams, and n is the genome size in base pairs.

The following example calculates the mass of the human genome using the Celera Genomics estimate of 3.0×10^9 bp (haploid):

$$m = (3.0 \times 10^9 \text{ bp}) (1.096 \times 10^{-21} \text{ g/bp})$$

$$m = 3.3 \times 10^{-12} \text{ g or } 3.3 \text{ pg}$$

The example is relevant to any gene that is present at the “normal” rate of two copies per diploid genome, such as RNase P, and provides a basis to perform a digital screening experiment to determine the optimal digital range.

Determine the optimal dilution when the target is unknown

If the target copy number per genome is unknown (e.g., 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 each sample that includes three to four data points above and below the expected digital range. This will ensure that one of the data points is within the optimal digital range. If real-time data is available for the assay and sample, use it to determine the start and end points of the dilution series.

Prepare the digital PCR reactions

Required materials

- Genomic or complementary DNA sample(s)
- Fluorescent-labeled quantitative PCR reagents (TaqMan™ Assays or SYBR™ Green primers) for your experiment
- QuantStudio™ 3D Digital PCR Master Mix v2
- Gloves, powder-free, nitrile
- Low-lint polyester wipes (clean-room grade)
- Microcentrifuge
- Permanent marker or pen
- Calibrated pipettes and barrier tips, P10 to P1000
- Reaction tubes, DNase/RNase-free, non-stick or low-binding, 0.5-mL or 1.5-mL
- TE Buffer, 1X Molecular Biology Grade
- Vortex mixer
- Water, DNase-free, sterile-filtered

Guidelines for dPCR sample preparation

When preparing samples for dPCR amplification:

- Use a positive-displacement pipette or aerosol-resistant pipette tips, and follow proper pipette-dispensing techniques to prevent aerosols.
- Wear clean gloves and a clean lab coat (not previously worn while handling amplified PCR products or used during sample preparation).
- Change gloves whenever you suspect that they are contaminated.
- Maintain separate areas and dedicated equipment and supplies for pre- and post-PCR activities.
- Never bring amplified products into the PCR setup area.
- Open and close all sample tubes carefully. Centrifuge tubes before opening.
- Keep reactions and components capped as much as possible.
- Clean lab benches and equipment periodically with 10% bleach solution. Use DNAZap™ PCR DNA Degradation Solutions (Cat. No. AM9890).

Recommended DNA volume and concentration

The recommended DNA volume in the following reaction is based on a human gDNA sample at 10 ng/μL concentration (final concentration in the reaction is ~3.45 ng/μL), with the target sequence present at two copies per diploid genome. This recommended volume will vary depending upon species, sample type, and sample concentration. For best results, we recommend adding sufficient DNA such that the concentration of target sequence in the final reaction is between 200 and 2,000 copies/μL.

Prepare the dPCR reaction mix for each sample

Prepare the PCR reactions for loading on the QuantStudio™ 3D Digital PCR 20K Chip v2.

1. Thaw the following at room temperature, and ensure that the tubes are at room temperature before using:
 - QuantStudio™ 3D Digital PCR Master Mix v2
 - TaqMan™ Assay(s)
2. Prepare the genomic DNA sample:
 - a. Dilute the DNA sample as needed so that the concentration of target sequence in the final reaction is between 200 and 2,000 copies/μL.
 - b. Vortex, then briefly centrifuge the DNA sample.
 - c. Using a permanent marker, label a 0.5- or 1.5-mL reaction tube with the sample name.
3. When the master mix has warmed to room temperature, gently invert the tube 10 times (or gently vortex on low-medium speed).
4. In a 0.5- or 1.5-mL low-bind tube, prepare the following dPCR reaction mix at room temperature. Scale the volumes appropriately, depending on the number of samples.

Table 1 dPCR reaction mix—example amounts

The example amounts assume that you are running two chips per human gDNA sample at 10 ng/μL.

Item	Amount		
	1 sample/ 2 chips ^[1]	Stock	Final
Master mix	17.4 μL	2X	1X
TaqMan™ Assay(s), 20X (primer/probe mix)	1.7 μL	20X	1X
Diluted genomic DNA ^[2]	3.5 μL	10 ng/μL	1 ng/μL
Water	12.2 μL	—	—
Total volume	34.8 μL	—	—
Volume to load per chip	14.5 μL	—	—

^[1] Volumes include 20% excess to compensate for volume loss from pipetting.

^[2] Depending on sample source, required input DNA amount may vary.

5. Mix well by gently pipetting up and down (or gently vortex on low-medium speed).
6. Transfer 34.8 µL of the dPCR reaction mix to the labeled reaction tube.
7. Cap the reaction tube, briefly centrifuge, then immediately proceed to load the chips.

IMPORTANT! For optimal results, load the chips as soon as possible after setting up the reactions. If you placed the reactions on ice, warm them to room temperature before loading.

Load the chips using the QuantStudio™ 3D Digital PCR Chip Loader

For instructions on loading chips manually, see Appendix E, “Load chips manually”.

The following procedure describes how to load the QuantStudio™ 3D Digital PCR 20K Chip v2 automatically using a QuantStudio™ 3D Digital PCR Chip Loader. This procedure assumes that you have prepared your PCR reactions as explained in “Prepare the dPCR reaction mix for each sample” on page 22. Before proceeding, see “Guidelines for loading and sealing chips” on page 18.

Materials required

The following materials are required to load and seal chips.

- Prepared digital PCR reactions
- QuantStudio™ 3D Digital PCR 20K Chip v2
- QuantStudio™ 3D Digital PCR Chip Lid v2
- QuantStudio™ 3D Digital PCR Sample Loading Blades
- Immersion Fluid syringe
- Immersion Fluid syringe tip
- QuantStudio™ 3D Digital PCR Chip Loader
- Isopropanol
- Gloves, powder-free, nitrile
- Scissors
- Clean room-grade, low-lint polyester wipes
- Microcentrifuge
- Pipettes and tips, P10 to P1000
- Vortexer

Chip loader status light

The LED on top of the chip loader indicates the instrument status. The light is illuminated at all times when the loader is powered on and changes color according to instrument readiness.

Color	State	Status
Blue	Solid	The chip loader is booting.
	Flashing	The chip nest is not at temperature or the lever arm is not fully open.
Green	Solid	The chip loader is at the correct temperature and ready to load a chip.
	Flashing	The chip loader is loading a chip.
Red	Flashing	The chip loader has encountered an error.

Recovering from an error

Perform the following steps if the chip loader status light flashes red, indicating that it has encountered an error.

1. Power off the chip loader, wait 30 seconds, then power it on.
2. If the status light is still flashing red, press the load button and record the sequence of colors displayed.
3. Contact technical support (see “Customer and technical support” on page 123).

Prepare the chip loading workspace

IMPORTANT! Wear powder-free gloves while preparing chips.

IMPORTANT! The QuantStudio™ 3D Digital PCR Chip Loader must be placed on a level surface to ensure consistent loading across the chips.

1. Plug in and power on the chip loader.
2. Fold back the chip loader arm, then wait until the status light illuminates solid green, indicating that the chip nest has reached operating temperature (≤ 20 minutes depending on room temperature).
3. Remove the following consumables from their packaging and place them on a clean, dry, lint-free surface:
 - Chip lid
 - QuantStudio™ 3D Digital PCR Sample Loading Blade

Prepare the syringe containing the Immersion Fluid

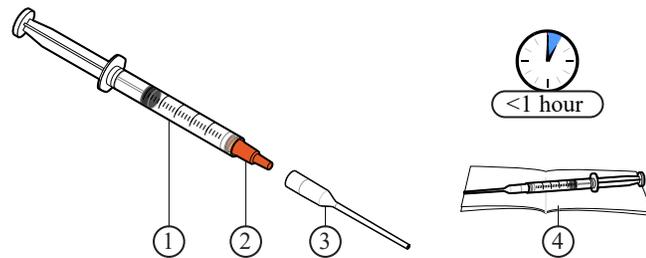
1. Remove the Immersion Fluid syringe, plunger, and tip from the packaging.
2. Before uncapping the syringe, gently pull back the plunger 1-2 mm and release it to break any resistance that may have formed during storage.
3. Remove the cap from the syringe, then attach the syringe tip by pushing it into place.

IMPORTANT! No twisting or screwing is required to attach the tip.

4. Carefully depress the plunger until Immersion Fluid flows from the tip of the assembled syringe. Place it on a clean surface and proceed to the next steps.

IMPORTANT! Confirm that the tip is locked firmly in place before proceeding.

IMPORTANT! Open only one syringe at a time. Use all of the Immersion Fluid within 60 minutes of uncapping the syringe. After a syringe is opened, you cannot recap it for later use.



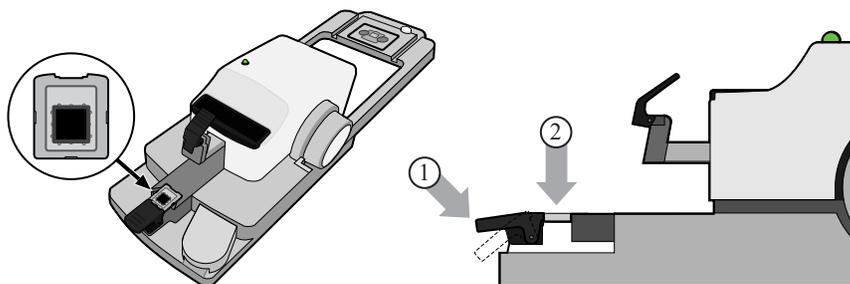
- ① Immersion Fluid syringe
- ② Cap (remove)
- ③ Syringe tip (push to attach)
- ④ Low-lint wipe

Load and seal the chips

Before using the QuantStudio™ 3D Digital PCR Chip Loader, wait until the status light illuminates solid green, indicating that the chip nest has reached operating temperature.

1. Open the package containing a QuantStudio™ 3D Digital PCR 20K Chip v2. Gently grasp the chip by its sides and load it face-up into the chip nest.
2. Press down on the chip nest lever to open the clamp, and place the chip in the nest. Release the lever to lock the chip into place.

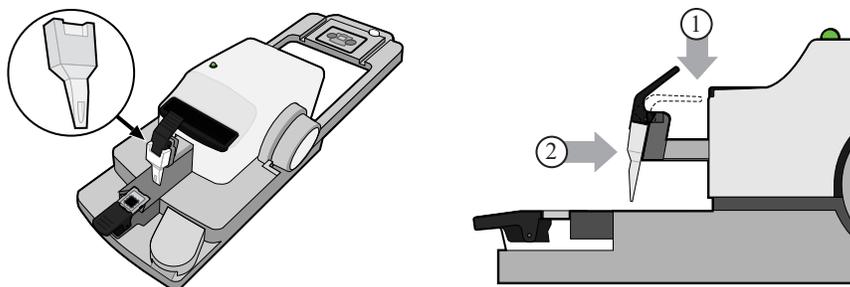
IMPORTANT! Use the image next to the chip nest to correctly orient the chip within the chip loader.



- ① Chip nest lever
- ② Chip nest

3. Press the sample loading blade lever, then install the QuantStudio™ 3D Digital PCR Sample Loading Blade into the loader head.

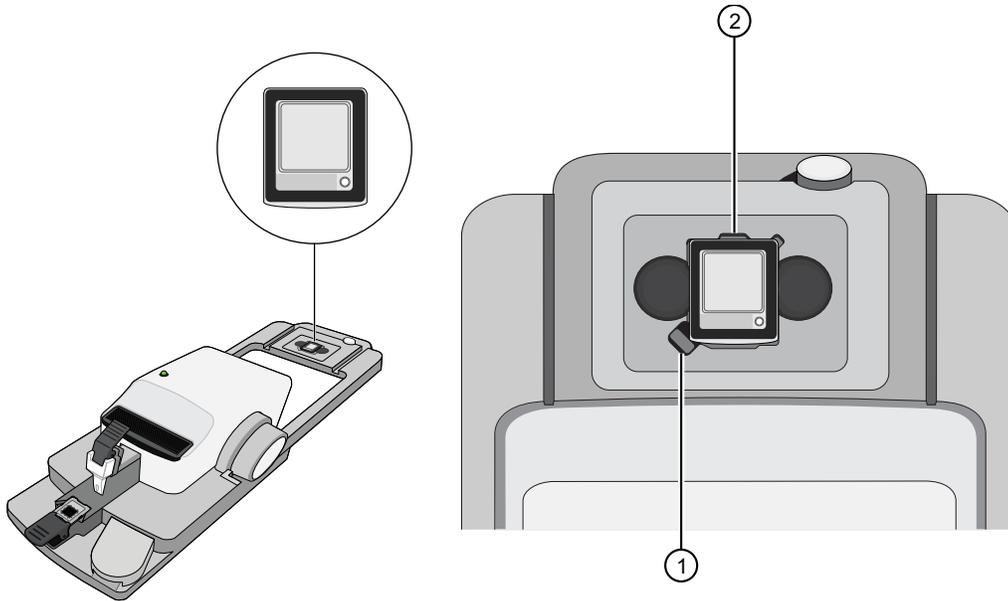
IMPORTANT! Press the blade firmly against the loader head to confirm that the blade is properly seated.



- ① Loading blade lever
- ② Loading blade

4. Grasping the lid by its sides, peel away the red protective film from the back of the chip lid. Avoid contact with the exposed sticky surface.
5. Press the lid nest button, and carefully place the lid with the sticky side up into the nest in the correct orientation. Slowly release the button to lock the lid in place.

IMPORTANT! Ensure that the lid is oriented correctly, as shown in the figure below. Do not release the lid nest button too quickly or the lid may pop out.



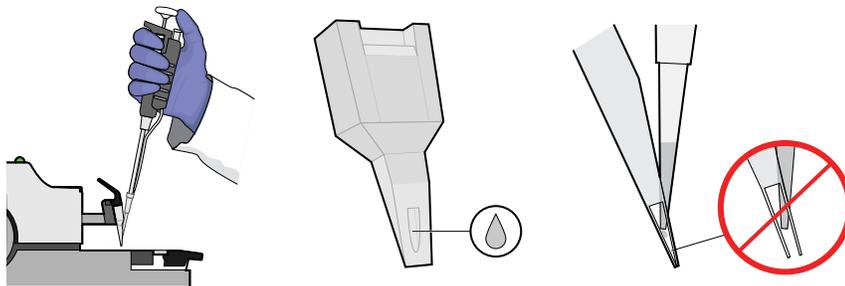
- ① Lid nest button
- ② Lid nest, with the lid in the correct orientation

6. Briefly vortex and centrifuge the prepared dPCR reaction (from “Prepare the dPCR reaction mix for each sample” on page 22), then carefully transfer 14.5 μL of the solution into the sample loading port of the loading blade. If the reactions were placed on ice, allow them to warm prior to loading.

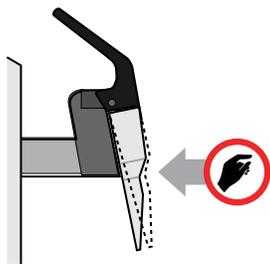
While filling the blade:

- Hold the pipette so that the tip meets the loading port at a sharp angle and so it does not deflect either blade (forward or rear).
- Do *not* depress the pipette to the second stop. Doing so increases the chance of introducing an air bubble to the blade.
- Avoid creating air bubbles.

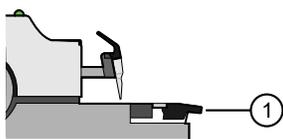
Note: Although the master mix may not distribute evenly in the loading blade immediately after loading, the fluid will spread across the blade prior to loading.



7. Ensure that the loading blade is firmly seated on the loader arm.



8. Press the dark grey/black loading button to load the chip.



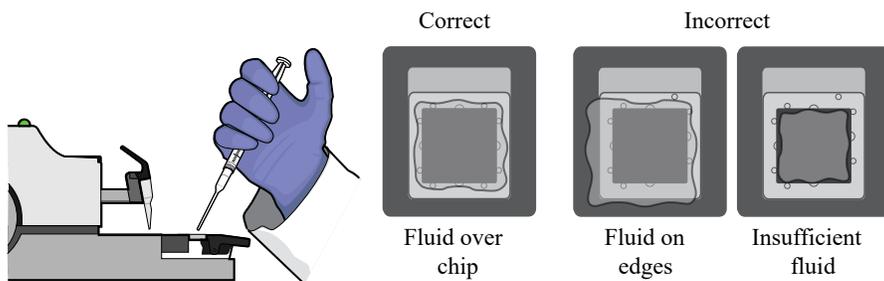
① Loading button

The status light flashes green during the loading sequence, and displays solid green when finished.

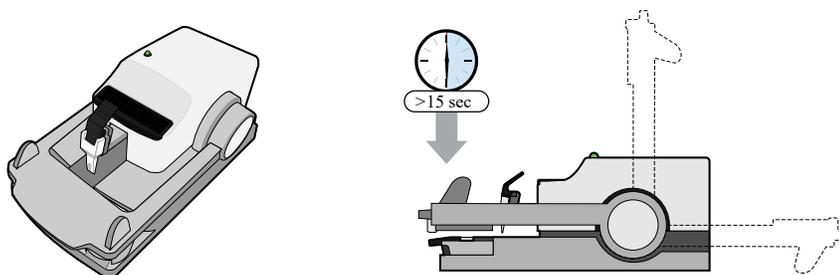
9. After loading, hold the Immersion Fluid syringe at an angle over the chip surface without touching the surface, and *slowly* add several drops of fluid directly onto the chip so that the fluid covers the entire surface. After dispensing, remove any fluid from the edges of the chip case with a low-lint wipe that has been sprayed with isopropanol.

IMPORTANT!

- Expel the fluid slowly to avoid flushing out the reactions in the chip wells.
- Do not allow the syringe tip to touch the chip surface.
- Cover the entire surface of the chip, or reactions in the exposed wells may evaporate.
- Do *not* fill the entire chip case with fluid. A small amount of fluid running off the surface of the chip is acceptable.



10. Rotate the loader arm so that the chip lid solidly contacts the chip. Firmly press down on the arm for 15 seconds to ensure a tight seal (you can count each flash of the status light, which flashes at 1-second intervals).



IMPORTANT! The loading arm requires >20 lbs of force to seal the lid. If you suspect that you are applying insufficient pressure, press down longer (>15 seconds).

11. Press the lid nest button to release the chip lid, then lift and return the loader arm to its original position.

IMPORTANT! Lift the loader arm gently and do not press down again to avoid creating a vacuum.

Proceed to fill the chip case with Immersion Fluid.

Fill the chip case with Immersion Fluid

1. Hold the chip and lid assembly by its edges at a 45° angle so that air can escape from the fill port as you fill it.
2. Hold back the top half of the chip lid label to expose the fill port.

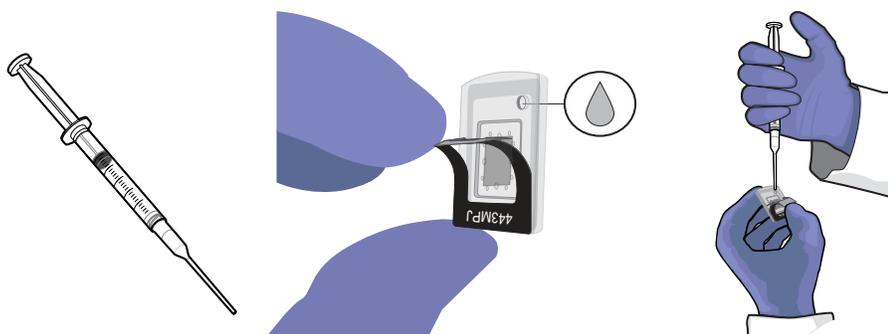


Figure 3 QuantStudio™ 3D Digital PCR Chip Lid v2

3. Carefully dispense Immersion Fluid into the port until the chip case contains an air bubble slightly larger than the fill port (<2–3 mm in diameter). Rotate the chip slightly to expose any hidden bubble. If a bubble larger than 2–3 mm is present, add additional fluid.
4. Using a low-lint wipe, remove any excess Immersion Fluid from the chip case to ensure optimal imaging.

Proceed to sealing the chip case.

Seal the chip case with the label

1. Gently pull back the top half of the label on the chip lid.
2. Remove the label backing and press the label firmly over the fill port for 5 seconds to ensure a tight seal.



IMPORTANT! Press only on the label region of the chip lid. Pressure placed on the window of the chip lid can expel the PCR reactions out of the chip wells.

3. Gently run your fingers over the entire label to seal the remainder of the label.
4. Inspect the sealed chip for potential problems:
 - **Leaks** – Confirm that no fluid is leaking from the fill port or from the seal between the chip and lid.
 - **Bubbles** – Check for large or multiple bubbles inside the chip case. One small air bubble is acceptable.
 - **Correct lid orientation** – Confirm that the lid is correctly aligned on the chip.

If the sealed chip fails any of the criteria above, discard it and prepare another chip. The sealed chip cannot be opened and resealed.

5. Store the prepared chip in a clean, dry, dark location until you are ready to load it onto the thermal cycler.

Thermal cycle prepared chips within 2 hours after loading them.

Note: You can label the back of the chip with a permanent marker. This will not affect the imaging data.

4

Thermal cycle the chips

■ Materials required	31
■ Prepare the thermal cycler	31
■ Guidelines for handling loaded chips	34
■ Perform the PCR	34
■ Unload the thermal cycler	37

Materials required

- Loaded and sealed QuantStudio™ 3D Digital PCR 20K Chip v2
- ProFlex™ 2 × Flat PCR System (or Dual Flat Block GeneAmp™ PCR System 9700)
- QuantStudio™ 3D Digital PCR Chip Adapters
- QuantStudio™ 3D Digital PCR Thermal Pads
- QuantStudio™ 3D Digital PCR Tilt Base
- Isopropanol
- Gloves, powder-free, nitrile
- Low-lint polyester wipes, clean-room grade

Prepare the thermal cycler

Before running each batch of chips, perform the following to ensure efficient thermal cycling:

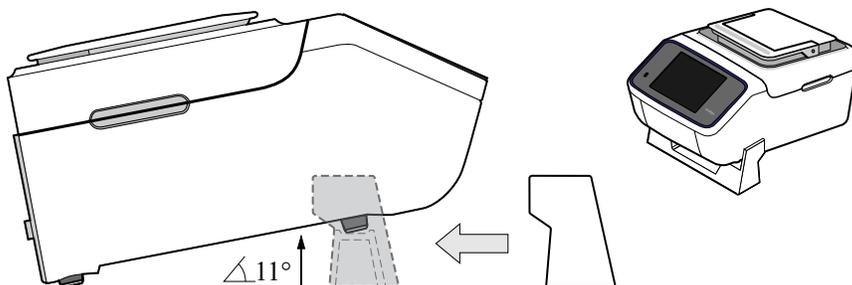
- Confirm that clean QuantStudio™ 3D Digital PCR Thermal Pads are available for use.

IMPORTANT! If a Thermal Pad becomes dirty, clean the pad using a low-lint wipe sprayed with isopropanol, then let it dry. Fluids, debris, and other contaminants that adhere to the pad can transfer to the chips during thermal cycling and fluoresce during imaging.

IMPORTANT! Use only Thermal Pads that are approved for use with chips. Thermal compression pads from other PCR applications may not apply uniform pressure or heat distribution to all chips on the sample block.

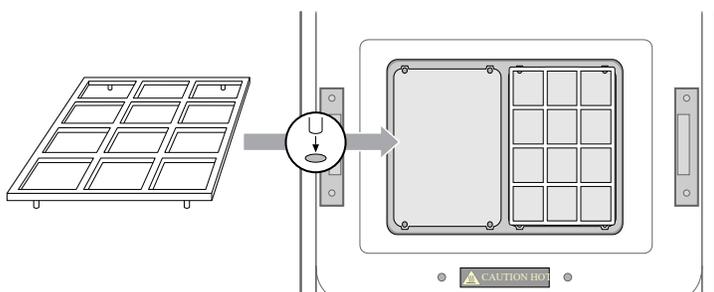
- Confirm that a QuantStudio™ 3D Digital PCR Tilt Base is installed beneath the thermal cycler.

IMPORTANT! The Tilt Base inclines the sample block by 11°, the angle required for the optimal performance of the chips.

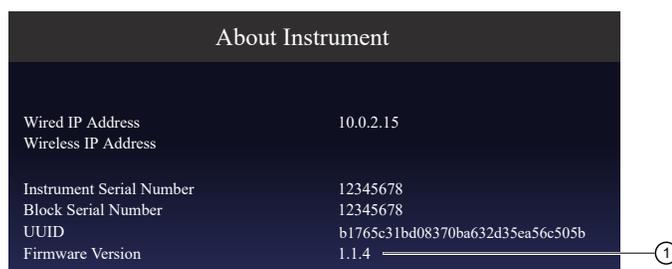


- Open the heated cover and confirm that QuantStudio™ 3D Digital PCR Chip Adapters are installed to both sample blocks.

Note: Each Chip Adapter includes a set of four alignment pegs that fit into the holes of a flat sample block. The Chip Adapter can fit onto either side of the sample block (right or left).



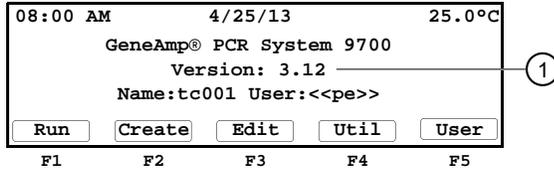
- Confirm the thermal cycler is running the correct firmware version number to thermal cycle chips:
 - ProFlex™ 2 × Flat PCR System—The version number displayed in the About Instrument screen must be **1.1.4** or later.



① v1.1.4 or later

Note: If the ProFlex™ 2 × Flat PCR System version is earlier than 1.1.4, see the *ProFlex™ PCR System User Guide* (Pub. No. MAN0007697) for information on upgrading the instrument firmware before thermal cycling chips.

- GeneAmp™ PCR System 9700—The version number displayed in the main menu must be **3.12** or later.



① v3.12 or later

Note: If the GeneAmp™ PCR System 9700 version is earlier than 3.12, contact technical support to upgrade the instrument firmware before thermal cycling chips (see “Customer and technical support” on page 123).

- Confirm that the thermal cycler is programmed with the method for thermal cycling chips. If missing, create the PCR method before proceeding.

Note: See the *ProFlex™ PCR System User Guide* (Pub. No. MAN0007697) or the *GeneAmp™ PCR System 9700 Base Module User Manual* (Pub. No. 4303481) for more information on programming methods.

IMPORTANT! The heated cover MUST be at 70°C before loading the chips. Excessive heat from the heated cover may cause the chips to leak during thermal cycling.

Table 2 ProFlex™ 2 x Flat PCR System PCR Method

PCR protocol					Cover temp.	Reaction volume
Stage 1	Stage 2		Stage 3			
96.0°C	60.0°C	98.0°C	60.0°C	10.0°C	70.0°C	1 nL ^[1]
1.6°C/sec	1.6°C/sec	1.6°C/sec	1.6°C/sec	1.6°C/sec		
0:10:00	0:02:00	0:00:30	0:02:00	∞		
1x (Hold)	39x (Cycles)		1x (Hold)			

^[1] 33 nL for firmware earlier than v1.1.4. The reaction volume on the instrument display does not refer to the reaction volume on the chip and should not be changed.

Table 3 GeneAmp™ PCR System 9700 PCR Method

PCR protocol					Run speed	Reaction volume
Stage 1	Stage 2		Stage 3			
96.0°C	56.0°C	98.0°C	60.0°C	10.0°C	Standard	20 µL ^[1]
10 min	2 min	30 sec	2 min	∞		
1x (Hold)	39x (Cycles)		1x (Hold)			

^[1] The reaction volume on the instrument display does not refer to the reaction volume on the chip and should not be changed.

Guidelines for handling loaded chips

To ensure the proper function of your loaded chips, handle them according to the following guidelines:

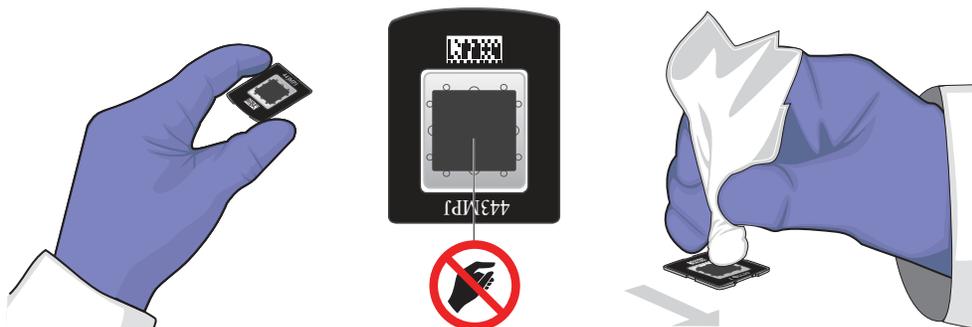
- Always wear powder-free gloves.

IMPORTANT! Oils from your hands can contaminate the chips and interfere with imaging.

- Handle the loaded chips by gently gripping the sides of each chip case, being careful to not apply pressure to the chip within it.
- Do not touch the window of the chip lid. If you accidentally touch the window, clean it using a laboratory wipe that has been sprayed with isopropanol, then dry by wiping in one direction using a clean low-lint wipe.

Note: Debris or excess sample on the lid can interfere with thermal uniformity during thermal cycling, or fluoresce during imaging.

- Thermal cycle chips within 2 hours after loading them.



Perform the PCR



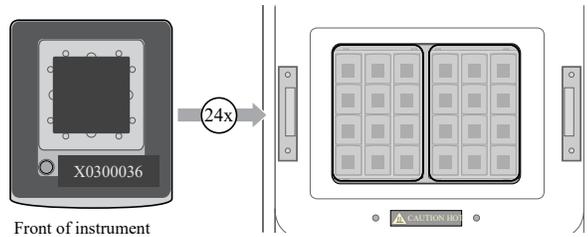
CAUTION! PHYSICAL INJURY HAZARD. During operation, the sample block can be heated to 100°C. Before performing the following procedure, be sure to wait until the sample block reaches room temperature.

IMPORTANT! Thermal cycle prepared chips within 2 hours after loading them.

IMPORTANT! Thermal cycling must be performed on a ProFlex™ 2 × Flat PCR System or a Dual Flat Block GeneAmp™ PCR System 9700 as described in this guide. No other thermal cyclers can be substituted at the time of the release of this manual.

1. Open the heated cover of the thermal cycler and wipe the surface of both sample blocks using a low-lint wipe to ensure that they are clean and dry.
2. Confirm that the Tilt Base is installed and Chip Adapters are installed in *both* sample blocks (even if you are using only one block).

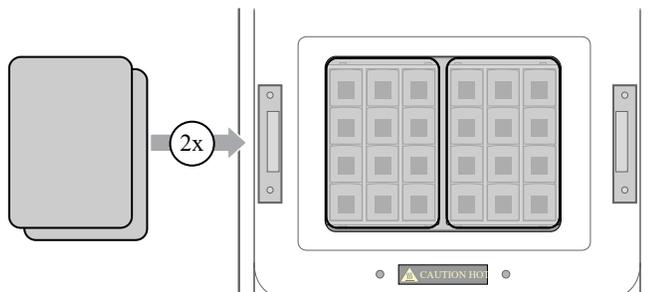
- Place the chips onto the sample block so that the fill ports on the chips are positioned toward the *front* of the thermal cycler. The fill port must be elevated during thermal cycling to ensure that any bubbles float to the top of the case.



IMPORTANT! If you are thermal cycling less than 24 chips, load according to the following guidelines:

- Load the right sample block first, placing at least 1 chip on the right sample block.
 - Balance the load between the left and right blocks so that the pressure applied by the heated cover and thermal pads is uniform across all of the loaded chips.
-

- Lay the Thermal Pads over the chips.



IMPORTANT! Make sure that each thermal pad is centered on the sample block and it completely covers the chips.

Do not allow the pads to become dirty. Fluids, debris, and other contaminants that adhere to the pad can transfer to the chips during thermal cycling and fluoresce during imaging.

- Close and engage the heated cover of the thermal cycler.

- Use the thermal cycler to select and start the pre-programmed run for the chips. See the user guide for your thermal cycler for more information on running methods.



When cycling is complete, proceed to unload the thermal cycler.

Unload the thermal cycler

You can remove the chips from the thermal cycler immediately after the final extension step at 60°C is complete and the temperature of the block is $\leq 25^{\circ}\text{C}$. Alternatively, the chips can remain on the block for up to 24 hours at 10°C.

IMPORTANT! We recommend that you read chips within 1 hour after you remove them from the sample block.



CAUTION! PHYSICAL INJURY HAZARD. During operation, the sample block may reach temperatures of 100°C. Before removing chips, wait until the block reaches room temperature.

1. If you programmed the thermal cycler to perform a 10°C hold after cycling, do the following to prevent condensation:
 - a. Confirm the final extension step at 60°C is complete, then stop the run.
 - ProFlex™ PCR System – Press **Stop Run**, then press **OK**.
 - GeneAmp™ PCR System 9700 – Press **STOP** twice, then press **Exit**.
 - b. Allow the thermal cycler to sit for at least 10 minutes with the heated cover closed.
2. Open the heated cover to expose the chips.
3. Remove the thermal pads from the sample block and set them on a clean, dry surface.

Note: Do not discard the pads. They can be reused multiple times.

4. Remove the Chip Adapters from the sample block and place them on a clean, dry surface. Remove the chips from the adapters and allow them to equilibrate to room temperature.

Note: To remove a chip from a adapter, push the chip from beneath and grasp the edges of the case, then remove it.

IMPORTANT! Be careful to not apply pressure to the chips.

5. Inspect each chip for leaks or potential problems. Using a low-lint wipe, remove any condensation or Immersion Fluid from the chip surface by wiping in one direction. If necessary, use a low-lint wipe sprayed with isopropanol to remove any dried residue. Make sure the surface is thoroughly clean.



The chip is now ready to be imaged.

IMPORTANT! Store chips in a clean, dry, dark location until you are ready to image them. Prolonged exposure to light can diminish the fluorescence of the photosensitive dyes in the chips.

Note: If a chip is sealed improperly, it may leak and contaminate the sample block. Remove any fluid from the sample block using a low-lint wipe sprayed with isopropanol.

5

Image and analyze the chips

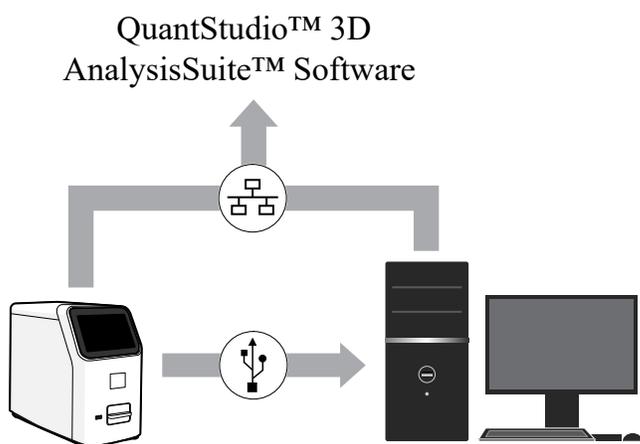
- About imaging and primary analysis 39
- About the instrument interface 40
- Chip imaging 41
- Primary analysis 45
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About imaging and primary analysis

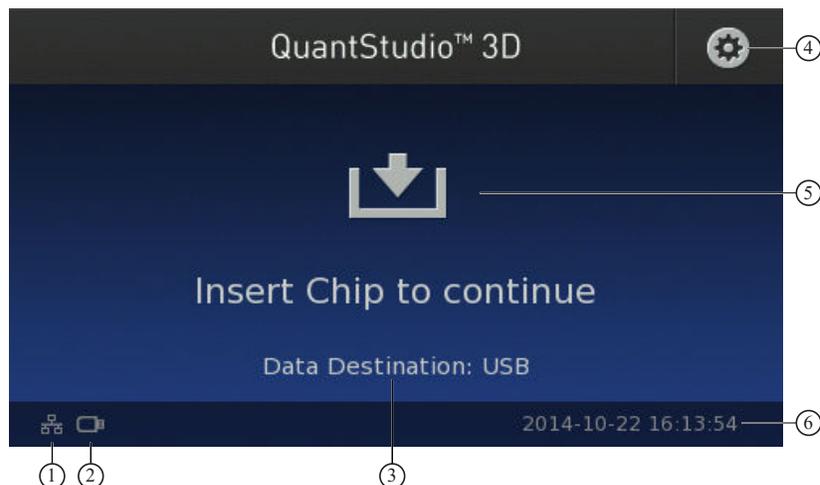
The QuantStudio™ 3D Digital PCR Instrument performs image capture and subsequent preliminary analysis of chips following thermal cycling. The instrument processes chips individually, where each read lasts approximately one minute. After reading a chip, the instrument performs an initial evaluation of the imaging data collected during the run and displays the results on the instrument touchscreen.

QuantStudio™ 3D System data flow

For each imaged chip, the QuantStudio™ 3D Instrument generates and saves a single experiment (.eds) file that contains the processed imaging data and the results from the preliminary analysis on the instrument. Depending on the chosen data destination, the instrument transfers the saved imaging data directly to the Connect cloud-based platform for immediate access by the AnalysisSuite™ Software, or to a network file server or local USB drive for later manual import.



About the instrument interface



① **Network status icon**—Represents the status of the QuantStudio™ 3D Digital PCR Instrument network connection:

- —Wired (Ethernet) connection selected; connected to network.
- —Wired (Ethernet) connection selected; no connection available.
- —Wireless connection selected; connected to network.
- —Wireless connection selected; no connection available.

② **Data destination icon**—Represents the selected data destination:

- —Connect cloud-based platform destination selected.
- —USB drive destination selected.
- —Network share destination selected.

③ **Data Destination**—The name of the location where the instrument will save data. Touch , then touch **Data Destinations** to select a location.

④ **Settings button**—Touch to open the Instrument Settings menu that allows you to set the system date and time, the network connection, the data destination, and perform other maintenance and configuration tasks.

⑤ **Chip presence indicator**—Indicates whether the instrument is ready to load a chip. The instrument immediately begins processing when a chip is loaded.

Note: If the instrument can read the chip ID on the chip lid, the ID is displayed on the touchscreen. If the instrument cannot read the ID, it is listed as unknown.

⑥ **Date/time stamp**—Displays the date and time of the system in <yyyy-mm-dd>_<hh:mm:ss> format. Touch , then touch **Instrument Settings** to set the system date and time.

Chip imaging

Materials required

- Prepared and thermal cycled chips
- Isopropanol
- Gloves, powder-free, nitrile
- Low-lint polyester wipes, clean-room grade

Guidelines for imaging multiple chips

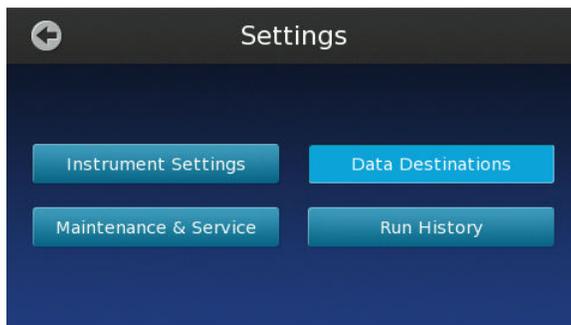
For optimal efficiency when running large numbers (>6) of chips, follow the guidelines below:

- Run the chips in alphanumeric order (according to chip ID) to help avoid mistakes when entering sample information.
- If you are running multiple chips, you can run the next chip in the series when the touchscreen displays the Analyzing Chip screen, as described in the following procedure.

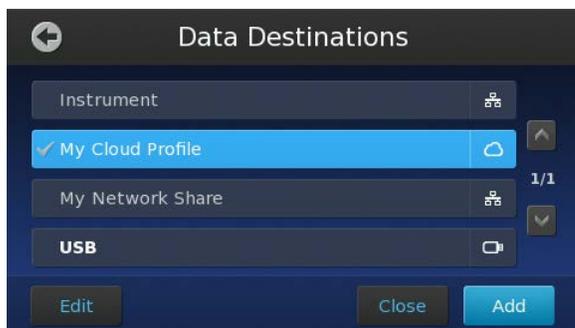
Specify the data destination

Imaging data is always saved on the instrument, but you can also automatically transfer the data from a run to a USB, the Connect cloud-based platform, or a network destination, as described below.

1. From the Main Menu of the touchscreen, touch  to open the Settings menu, then touch **Instrument Settings** followed by **Data Destinations**.



2. In the Data Destinations screen, touch the desired option:
 - Select **Instrument** to only save data on the instrument.
 - Select **My Cloud Profile** (the Connect cloud-based platform) or **My Network Share** to save to destinations that you can set up as described in “Create a network destination to receive run data” on page 103.
 - Select **USB** to save to a USB key that you insert into the slot on the instrument before imaging.



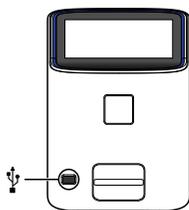
Note: Whichever destination you select, imaging data is also saved on the instrument, up to a limit of ~600 experiment files, after which the oldest files will be purged from memory and replaced with newer files.

Image the chips

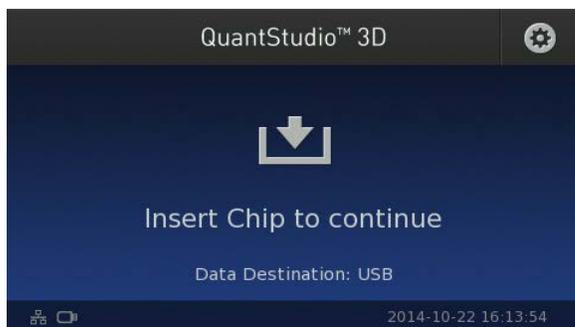
IMPORTANT! Before imaging, confirm that the latest firmware is installed on the QuantStudio™ 3D Digital PCR Instrument.

Note: If you are unsure of the firmware version, see “Check the instrument firmware” on page 87.

1. If you selected **USB** in the Data Destinations screen (as described above), insert a USB device into the USB port on the front of the instrument.

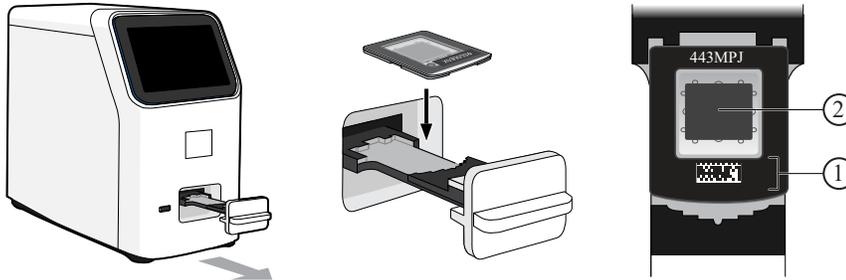


The touchscreen displays the USB drive icon () when the QuantStudio™ 3D Instrument has mounted the drive.



- Open the chip tray and load the chip face-up into the tray, with the chip ID and fill port toward the front of the instrument.

Note: The chip will only fit in the tray in one orientation.



- Orient the chip barcode toward the front of the instrument.
- Confirm that the chip window is clean and correctly aligned to the chip.

- Confirm that the chip is correctly aligned, then close the tray to begin chip detection and imaging. You can monitor the progress of the run in the touchscreen, which counts down the time remaining.

IMPORTANT! Do not open the chip tray or remove the USB drive (if present) while the instrument displays the countdown. Doing so will invalidate the data and require you to repeat the run.

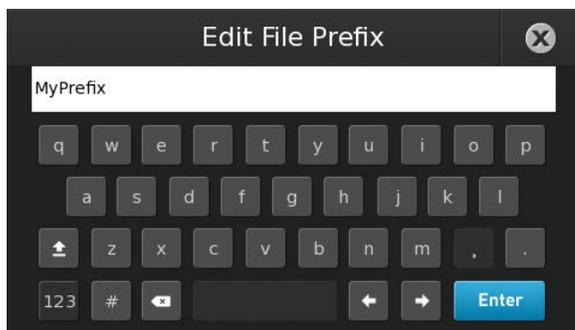


Note: You can stop the run at any time by touching **Cancel**.

- (Optional) During the run, touch the **Add Prefix** field to enter a prefix for each experiment (.eds) file name.

Note: The file prefix is an alphanumeric string that can be used to further identify and group related experiments after transfer to AnalysisSuite™ Software. The file prefix:

- Can be up to 174 characters long.
- Must consist of alphabetic and numeric characters.
- Can contain hyphens, but not as the first or last character.
- Cannot include spaces or special characters (; : " < > * + = \ | ? ,).



Using the prefix example shown above, the resulting file name would be `MyPrefix_date_time_chipID.eds`.

5. The instrument is done imaging when the touchscreen displays the Analyzing Chip screen. At this point, you can either wait for the instrument to complete the analysis and display the results, or you can open the chip tray and remove the chip.



Note: If the run fails because the instrument is unable to read the chip, inspect the chip for problems and read the chip again. If the run fails repeatedly, discard the chip and prepare a replacement.

6. (Optional) You can read another chip without waiting for analysis to complete. To begin the next run, remove the imaged chip and load another chip.

7. After chip analysis on the instrument is complete, the results are stored on the instrument.
 - If you have not removed the chip from the tray, the touchscreen will display the results after analysis (see “About the results screen” on page 46).



- If you have already removed the chip, the touchscreen will return to the Main Menu. To view the saved results, touch the  button in the Main Menu, then touch **Run History** and select from the list of results files.

Note: If analysis results in a red flag () for either the FAM™ or VIC™ dye, inspect the chip for problems and read the chip again.

After a chip has been read:

- You can reread imaged chips, but we recommend storing them in the dark if you plan to reread them later. We recommend rereading them within 1 hour after the initial reading, though storing them in the dark may prolong their read life.
- If you read multiple chips in succession, touch the scroll buttons ( / ) to scroll through the results screens.
- The instrument retains a copy of the imaged chip data that you can access from the Run History screen.

Note: The instrument can store approximately 600 experiment files before it begins purging the files from memory. When the instrument reaches the limit, it replaces the oldest file exported from the cache with the data from the current run.

Primary analysis

About imaging data and primary analysis

After the QuantStudio™ 3D Digital PCR Instrument images a chip, the instrument performs an initial evaluation of the raw data to determine its viability. The instrument identifies the wells within the captured images and performs a quality assessment of the composite results to determine whether the instrument has collected usable data. If the data pass the initial quality assessment, the instrument calculates the concentration of the nucleic acid sequence targeted by the FAM™ and VIC™ dye-labeled probes. The results are reported in copies per μL along with the results of the quality assessment displayed using colored flags.

About the results screen

After chip imaging and analysis, the results are stored on the QuantStudio™ 3D Digital PCR Instrument.

- If you have not removed the chip from the tray, the instrument touchscreen will display the results after analysis.
- If you have already removed the chip, the touchscreen will return to the Main Menu. To view the saved results for each run, touch the  button in the Main Menu, then touch **Run History** and select from the list of results files.

In the results screen, the instrument displays the following information:



- ① **Chip ID**—Displays the ID number of the chip. If the instrument cannot read the chip ID, the touchscreen displays the date and time of the run.
- ② **File name**—Name of the experiment (.eds) file created for the imaged chip.
Note: After the analysis, the instrument copies the file to the selected destination and stores the file locally.
- ③ **FAM/VIC concentration estimate**—Displays the estimated concentration in copies per μL calculated from the fluorescence signal generated by amplification of the nucleic acid sequence targeted by the FAM™ and/or VIC™ dye-labeled probes.
The instrument also displays a flag next to each concentration estimate, color-coded to indicate the data quality.
- ④ **Network status icon**—Represents the status of the instrument connection to the network:
 - —Wired (Ethernet) connection selected; connected to network.
 - —Wired (Ethernet) connection selected; no connection available.
 - —Wireless connection selected; connected to network.
 - —Wireless connection selected; no connection available.
- ⑤ **Data destination icon**—Represents the selected data destination:
 - —Connect cloud-based platform destination selected.
 - —USB drive destination selected.
 - —Network share destination selected.
- ⑥ **Scroll buttons**—Allow you to display the results of previously imaged chips when running them in a series.

Data quality flags

Data quality flags generated and displayed by the software are a measure of the overall chip quality, based on well quality thresholds and other data analysis characteristics.

Note: For more information on quality assessment in the software, see the user documentation for the QuantStudio™ 3D AnalysisSuite™ Software.

In order, from high quality to low quality:

-  (green) – Review of the analysis results is suggested, but not required. This flag is shown if the data meets all quality thresholds.
-  (yellow) – Review of the analysis results is suggested. This flag is shown if the:
 - Instrument cannot clearly identify the population of unamplified wells.
 - Distribution of unamplified wells on the chip is not uniform.
 - Sample concentration is outside the optimal range (200-2000 copies/μL) for the system as currently defined by the chip quality metrics.
-  (red) – Review of the analysis results is strongly suggested. If necessary, reimage the chip or rerun the sample. This flag is shown if the total number of filled wells is < 5000 or the percentage of low quality wells (those with well quality < 0.5) is > 25%.

About data files

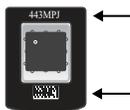
For each chip, the QuantStudio™ 3D Digital PCR Instrument stores the processed imaging data and the results of the preliminary analysis in a single experiment (.eds) file.

The instrument names each experiment file uniquely by combining the ID of the chip and the date/time of the run according to the following formula:

`prefix_130531_195817_BO498N.eds`

① ② ③ ④

- ① **File prefix**—The optional user-specified text.
- ② **Date stamp**—The date at which the chip was run, expressed in the "yymmdd" format where *yy* is the last two digits of the year, *mm* is the numerical order of the month, and *dd* is the day of the run.
- ③ **Time stamp**—The time at which the chip was run, expressed in the "hhmmss" format where *hh* is the hour (in 24-hour format), *mm* is the minutes, and *ss* is the seconds.
- ④ **Chip ID**—The number on the chip lid and encoded in the 2D barcode that uniquely identifies the chip.



IMPORTANT! If the instrument cannot read the ID of a loaded chip, then it uses the date/time of the run instead to name the file.

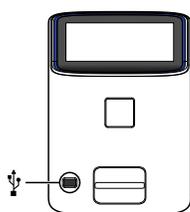
Note: In the example shown above, the file name indicates that the file contains data for chip *BO498N* which was run at 7:58 pm on May 31, 2013.

Following an image analysis, the QuantStudio™ 3D Digital PCR Instrument transfers the experiment (.eds) file for the chip to the selected destination *and* saves a copy of the file locally to the instrument. You can access and/or download the experiment (.eds) files of previous runs from the Run History screen.

Transfer the results

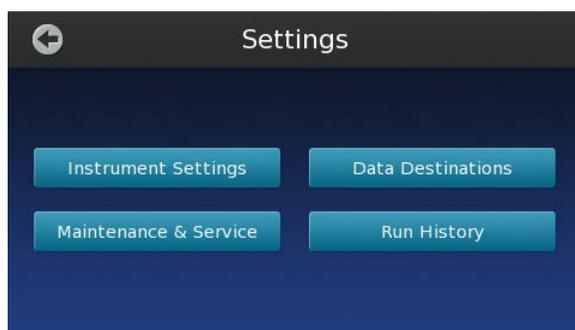
If you did not transfer data from the instrument to a USB, the Connect cloud-based platform, or a network location at the time of the run (as described in “Specify the data destination” on page 41), you can use the Run History screen to copy experiment files to a USB drive for storage or manual transfer. This may be necessary if the network connection failed or the USB drive was full during an run.

1. Insert a USB drive into the port on the front of the instrument.



2. In the touchscreen, touch  in the Main Menu.
3. In the Settings Menu, touch **Run History** to view the results for the past experiments.

Note: The QuantStudio™ 3D Digital PCR Instrument can store approximately 600 experiment files before it begins purging the files from memory. When the instrument reaches the limit, it replaces the oldest file exported from the cache with the data from the current run.



- In the Run History screen, touch the entry of the experiment that you want to transfer or view.



The Run History screen displays the data transfer status next to each file in the instrument memory:

- Pass** – The instrument successfully transferred the file to the selected destination.
 - In Progress** – The instrument is either processing the imaging data for the associated file, or transferring the file to the selected destination.
 - Not exported** – File transfer not attempted, or the selected destination is the instrument (file transfer not required).
 - Export Failed** – The instrument was unable to transfer the file to the selected destination.
 - Run Failed** – The instrument was unable to process the imaging data for the chip.
- In the Results Details screen, touch **Export**, then touch **Export** again to transfer the file to the USB drive.



- When finished, touch  several times to return to the Main Menu, then remove the USB drive from the instrument.

After exporting experiment (.eds) files to the USB drive, you can upload them to the Connect cloud-based platform for analysis using the QuantStudio™ 3D AnalysisSuite™ Software.

Using the QuantStudio™ 3D AnalysisSuite™ Software

After the analyzed imaging data has been transferred to the QuantStudio™ 3D AnalysisSuite™ Software, you can log into the software to perform the secondary analysis as described below.

Access the software from a Connect cloud-based platform account

When deployed in the Connect cloud-based platform configuration, the AnalysisSuite™ Software is accessed as a web-based application that can be run using any compatible web browser (see “System requirements” on page 13).

To access the software from a Connect platform account:

1. Confirm that your computer has a network connection.

Note: If your computer does not have a network connection, contact customer support for information on available AnalysisSuite™ Software deployment options (see “Customer and technical support” on page 123).

2. Open a browser window and go to thermofisher.com/analysissuite.

Note: Ensure that your browser is set to accept cookies and JavaScript is turned on. If you cannot access the website, contact customer support.

3. Sign into the software using your Connect platform user name and password, or follow the instructions to create a new user account.

Note: If you cannot sign in to the software, contact customer support.

Access the software from the server

System requirements

The QuantStudio™ 3D AnalysisSuite™ Software is a web-based application that is recommended for use with the following Internet browsers.

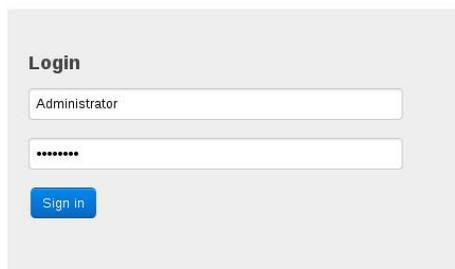
- Google™ Chrome™ v37 or later
- Microsoft™ Internet Explorer™ v10 or later
- Mozilla™ Firefox™ v32 or later
- Apple™ Safari™ v6 or later

Note: The software performance may vary based on your system configuration, and requires an internet connection capable of 300kbps/300kbps (upload/download) or better. If your network employs a firewall that restricts outbound traffic, it must be configured to allow outbound access to apps.lifetechnologies.com on HTTPS-443.

Access the software from the server desktop

1. Double-click **QuantStudio™ 3D AnalysisSuite™** on the server desktop.
By default, the software opens in the Google™ Chrome™ browser.
2. At first login to the software as the administrator, enter **Administrator** as the username and **Password** as the password.

QuantStudio™ 3D AnalysisSuite™



Login

Administrator

Sign in



Note: If you cannot sign in to the software, contact Thermo Fisher Scientific customer support.

Note: Thermo Fisher Scientific recommends that you change the administrator password after initial login to the software. For information about managing your profile, see the *QuantStudio™ 3D AnalysisSuite™ Software Help*.

Access the software from another computer

1. Confirm that your computer has a network connection and that it is connected to the same network as the server.

Note: If your computer does not have a network connection, contact a Thermo Fisher Scientific sales representative for information about available AnalysisSuite™ Software deployment options.

2. Open a browser window and go to `http://<server IP address>/quantstudio3d`, using the <server IP address> you received from your server administrator (see *QuantStudio™ 3D AnalysisSuite™ Server System Quick Reference* (Pub. No. 4486248)).

Note: Using your browser settings, confirm that cookies and JavaScript™ are turned on for the website to function correctly. If you cannot access the website, then contact Thermo Fisher Scientific customer support.

3. Sign in to the software as one of the following users.

- **Administrator**—Enter the user name and password (see “Access the software from the server desktop” on page 51).
- **End-user**—Enter the user name and password you received from your server administrator.

Note: If you cannot sign in to the software, contact Thermo Fisher Scientific customer support.

Obtain information from the Help system

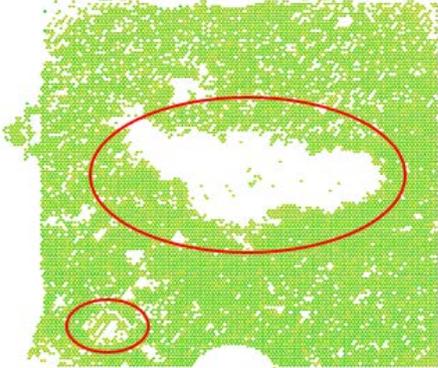
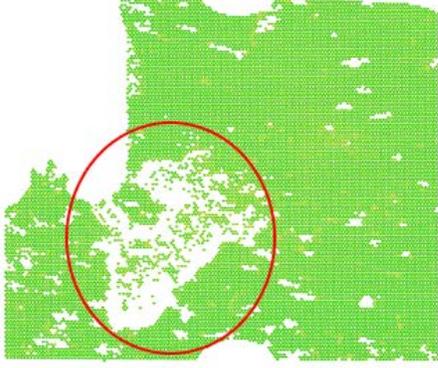
The QuantStudio™ 3D AnalysisSuite™ Software features a Help system that describes how to use each feature of the user interface. You can use the Help system to find topics of interest by:

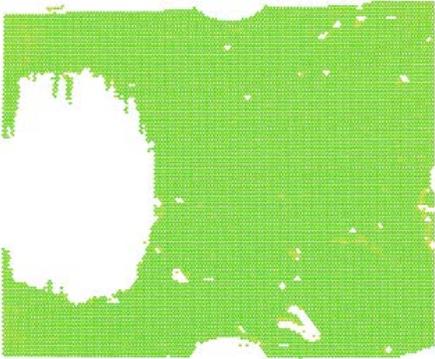
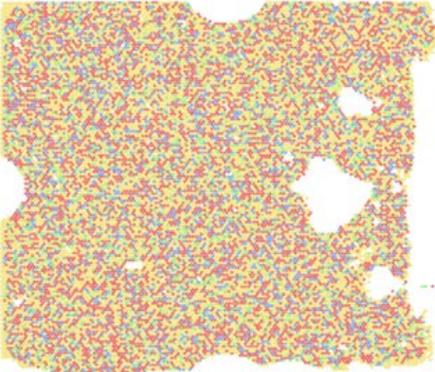
- Reviewing the table of **Contents** in the sidebar.
- Searching for a specific topic – Enter your search term in the **Search** field in the upper right, then click **Enter**. The search results will appear under the **Search** tab in the sidebar.

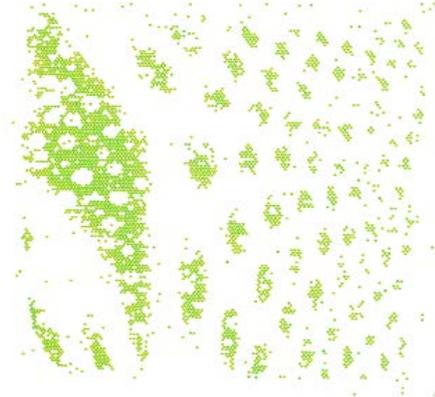
To access the Help system click  from any screen.

Troubleshooting chip images using the Chip View

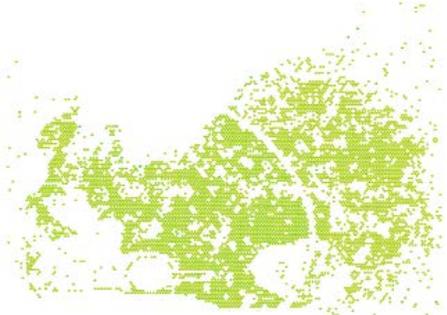
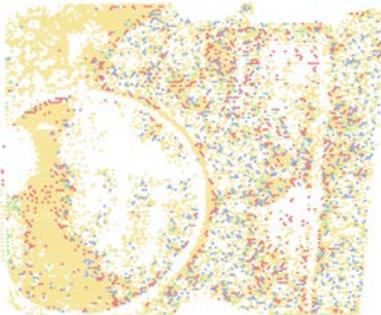
The following table summarizes some of the more common problems that can affect chip imaging. In the examples shown below, the conditions triggered a data flag and were diagnosed using the Chip View of the QuantStudio™ 3D AnalysisSuite™ Software.

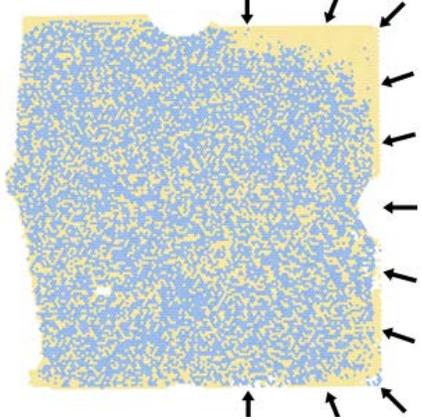
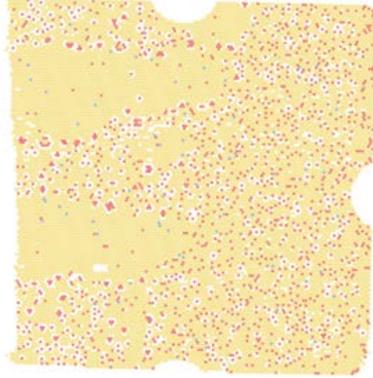
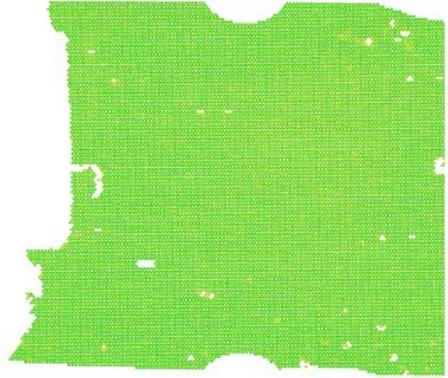
Observation	Possible Cause	Action
<p>Chip view</p>  <p>Chip view</p> 	<ul style="list-style-type: none"> Excess PCR reaction was present on the chip after loading it with the loading blade. <i>(Manual load only)</i> The loading blade was drawn across the chip too quickly or at an angle shallower than 70–80°. 	<p>If possible, use the AnalysisSuite™ Software to filter the low-quality data points, or discard the chip and run the sample again.</p> <p>To prevent leaving excess PCR reaction on the chips:</p> <ul style="list-style-type: none"> Install the Tilt Base on the thermal cycler. You must thermal cycle the chips at an 11° angle. <i>(Manual load only)</i> Ensure that the heated block used to load the chips is set to 40±1°C. <i>(Manual load only)</i> Wait 20 seconds after loading a chip before pre-wetting it with Immersion Fluid. <i>(Manual load only)</i> Draw the loading blade across the chip slowly (>10 seconds) and at a 70–80° angle.

Observation	Possible Cause	Action
<p>Chip view</p>  <p>Chip view</p> 	<p>A bubble was present in the loading blade when it was used to apply the PCR reaction to the chip.</p>	<p>If possible, use the AnalysisSuite™ Software to filter the low quality data points, or discard the chip and run the sample again.</p> <p>When loading the loading blades:</p> <ul style="list-style-type: none"> • If you are using a manual pipette, pipette to the first stop. • Decrease your pipetting speed.

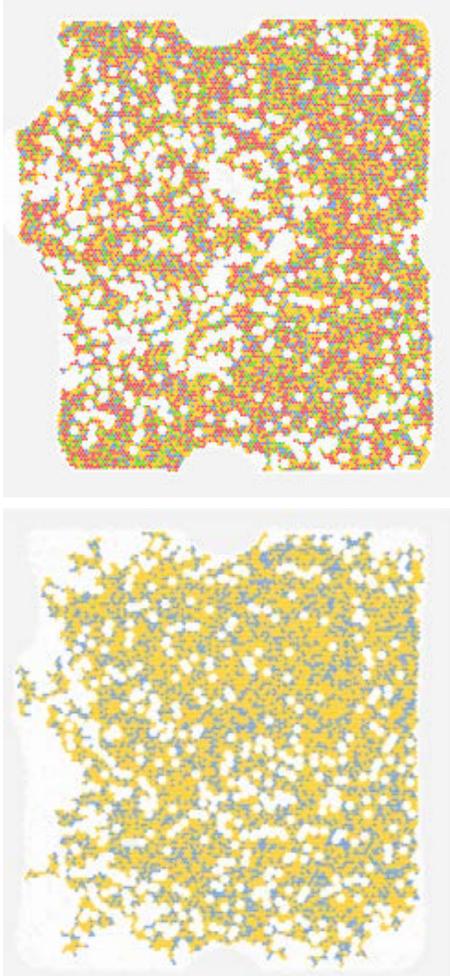
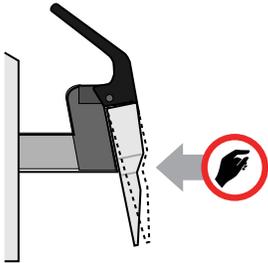
Observation	Possible Cause	Action
<p>Chip view</p> 	<ul style="list-style-type: none"> • The chip leaked during thermal cycling or imaging. • A large bubble was present in the chip (insufficient Immersion Fluid). • Excess Immersion Fluid is present on the chip lid. 	<p>If present, remove excess Immersion Fluid from the chip lid and run the chip again.</p> <p>If possible, use the AnalysisSuite™ Software to filter the low-quality data points.</p> <p>To minimize leakage:</p> <ul style="list-style-type: none"> • Leave a small bubble when filling chips with Immersion Fluid. • When covering chips with Immersion Fluid, do not get fluid on the sides of the chip base.

(continued)

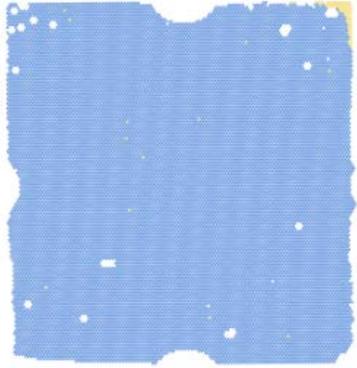
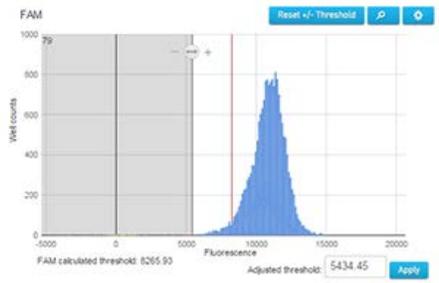
Observation	Possible Cause	Action
<p>Chip view</p> 	<ul style="list-style-type: none"> The chip leaked during thermal cycling or imaging. A large bubble was present in the chip (insufficient Immersion Fluid). Excess Immersion Fluid is present on the chip lid. 	<p>If you use the chip loader to seal the chips, ensure that you are using at least 20 lbs of pressure when applying the chip lid. If you are not sure that you are applying sufficient force, increase the duration of the press.</p> <p>Ensure that the ramp rate of the thermal cycler is set to 1.6°C/second.</p> <p>Ensure that the heated cover is set to 70°C (not to the default setting of 104°C).</p>
<p>Chip view</p> 		<p>If the steps above do not resolve leaking, it may be beneficial to degas the Immersion Fluid immediately before use:</p> <ol style="list-style-type: none"> Ensure that the cap is on securely. Pull the syringe plunger to 3 mL to create a partial vacuum in the syringe. Hold the syringe plunger at the 3-mL mark and shake vigorously for ~30 seconds. Hold the syringe vertically (tip up), remove the cap, then use the plunger to push any air out of the syringe. Use the Immersion Fluid immediately. <p>If you are sealing chips <i>manually</i>, also do the following to minimize leakage:</p> <ul style="list-style-type: none"> Wear correctly fitted gloves to prevent the material from snagging during lid application. Ensure that the chip lid is correctly aligned on the chip. Firmly press all four corners when applying the lid. When pressing the chip, firmly hold the sides of the chip and wiggle it back and forth.

Observation	Possible Cause	Action
<p>Chip view</p> 	<p>The chip was not completely covered with Immersion Fluid immediately after loading (while still on the chip loader or heated block)</p> <p>Note: (<i>QuantStudio™ 3D Instrument firmware version 1.1 or earlier</i>) Yellow around the edges is a strong indication of evaporation.</p>	<p>Add Immersion Fluid as soon as the loading blade is off of the chip.</p> <p>Ensure that the entire chip is covered with Immersion Fluid, even the edges.</p> <p>Note: When adding Immersion Fluid, the fluid can overflow into the chip base, but do not allow it to spread onto the sides of the chip lid.</p>
<p>Chip view</p> 	<p>Immersion Fluid was not applied to the chip immediately after loading (evaporation of the PCR reaction).</p>	
<p>Chip view</p> 	<p>Debris or other contaminants are present on the chip lid during imaging.</p>	<p>No action required.</p> <p>The AnalysisSuite™ Software can compensate for small quantities of debris or other contaminants on the chip.</p>

(continued)

Observation	Possible Cause	Action
	<p>Condensation may have been present on the chip during imaging.</p>	<p>IMPORTANT! Condensation may not be visible on the chip.</p> <ul style="list-style-type: none"> • If condensation is <i>not</i> visible – If possible, read the chip again after it has warmed to room temperature. Once equilibrated, the data quality, flag, and separation of amplified versus non-amplified peaks should improve noticeably. • If condensation is visible – Using a low-lint wipe, remove any condensation or Immersion Fluid from the surface of the chip by wiping in one direction. If needed, use a low-lint wipe sprayed with isopropanol to remove any dried residue.
<p>Chip view</p> 	<p>The loading blade was not seated correctly within the loader arm.</p>	<p>Before pressing the loading button, confirm that the loading blade is firmly seated and flush against the loader arm.</p> 

(continued)

Observation	Possible Cause	Action
<p>Chip view</p>  <p>■ FAM ■ No Amp</p> <p>Histogram view</p> 	<p>Excess template was loaded onto the chip.</p>	<p>In the AnalysisSuite™ Software, check the copies per reaction and dilute the sample accordingly so that it is within the digital range.</p>

(continued)

Observation	Possible Cause	Action
<p>Chip view</p>  <p>Chip view</p>	<p>Too much sample was left in loading blade.</p> <p>Note: The top image on the left was generated by QuantStudio™ 3D Instrument firmware v1.1 or earlier. The bottom image was generated by firmware v2.0 or later. In v2.0 or later, the overloaded sections of the chip appear in white and these regions are filtered out and removed from analysis.</p>	<p>Ensure that you are loading only 14.5 µL of reaction mix into the loading blade.</p>



Install the QuantStudio™ 3D Digital PCR System

Time required for installation

You can install the QuantStudio™ 3D Digital PCR System in approximately 1 hour.

Note: The time can vary depending on the type of networking solution that you choose. See Appendix D, “Networking” for a complete description of the networking options supported by the system.

Materials required

- Safety glasses
- Scissors or pocketknife

Before you begin

- Confirm that the installation site meets the environmental and electrical requirements explained in Appendix B, “Specifications and Layout”.
- If you intend to connect the QuantStudio™ 3D Digital PCR Instrument to your network, confirm that the site contains a viable connection that meets the networking requirements explained in Appendix D, “Networking”.

IMPORTANT! Confirm that the QuantStudio™ 3D Digital PCR Instrument is unloaded before you power off and move the instrument. If the instrument is moved while loaded, the chip inside may become dislodged from the chip tray and obstruct the chip tray mechanism.

- **IMPORTANT!** If you are moving the instrument from a previous installation, confirm that the chip tray is empty before you power off and move the instrument. If the instrument is moved with a chip inside, the chip may become dislodged and obstruct the chip tray mechanism.
-

Plan the laboratory layout

When planning the placement of the QuantStudio™ 3D Digital PCR System components, follow good laboratory practices for PCR. When preparing samples for dPCR amplification, maintain separate areas and dedicated equipment and supplies for pre-PCR (sample preparation, reaction setup, chip loading and sealing) and PCR/post-PCR (amplification and chip reading). Do not bring amplified products into the reaction setup area.

Room	Activities	Equipment
dPCR Setup Work Area (pre-dPCR room)	<ul style="list-style-type: none"> • Sample preparation and DNA extraction • Extracted DNA sample storage • dPCR reaction preparation and DNA sample additions • Chip loading and sealing 	<ul style="list-style-type: none"> • General laboratory supplies (such as gloves and wipes) • Microcentrifuge • Vortexer • Pipettes, P10 to P1000 with tips • Chips, chip lids, and loading blades • QuantStudio™ 3D Digital PCR Chip Loader (if used) • Immersion Fluid
Amplified DNA Work Area (PCR/post-PCR room)	<ul style="list-style-type: none"> • PCR amplification • Chip reading and storage 	<ul style="list-style-type: none"> • ProFlex™ 2 × Flat PCR System (or Dual Flat Block GeneAmp™ PCR System 9700) • QuantStudio™ 3D Digital PCR Instrument

Choose the instrument locations

Instrument location can affect performance. To prevent vibration, place instruments on a solid, stable, level surface that allows free airflow overhead and around the sides and backs. To ensure optimal heat dissipation, keep all instrument ventilation slots free of obstructions. For specific environmental requirements, see the following references.

Instrument	For environmental requirements, see...
QuantStudio™ 3D Digital PCR Instrument	"Environmental requirements" on page 81
ProFlex™ 2 × Flat PCR System	<i>ProFlex™ PCR System User Guide</i> (Pub. No. MAN0007697)
Dual Flat Block GeneAmp™ PCR System 9700	<i>GeneAmp™ PCR System 9700 Base Module User Manual</i> (Pub. No. 4303481)

Install the QuantStudio™ 3D Digital PCR Instrument

1. Plan the organization of your laboratory. Identify the locations of all stations in the QuantStudio™ 3D Instrument workflow, including:
 - Reagent and consumable storage
 - PCR reaction preparation
 - Chip loading and sealing
 - PCR amplification on the ProFlex™ 2 × Flat PCR System or Dual Flat Block GeneAmp™ PCR System 9700
 - Chip reading on the QuantStudio™ 3D Instrument

Note: See “Plan the laboratory layout” on page 61 for more information on planning the instrument placement.

2. Confirm that you have the following materials:
 - QuantStudio™ 3D Digital PCR Instrument (with power cord)
 - QuantStudio™ 3D Digital PCR Chip Adapters
 - QuantStudio™ 3D Digital PCR Thermal Pads
 - QuantStudio™ 3D Digital PCR 20K Chip v2 (12-pack)
 - Base Module for the ProFlex™ PCR System or the GeneAmp™ PCR System 9700
 - ProFlex™ 2 × Flat PCR System or GeneAmp™ PCR System 9700 Dual Flat Block
 - QuantStudio™ 3D Digital PCR Tilt Base (appropriate for your thermal cycler)
 - QuantStudio™ 3D Digital PCR Chip Loader

If you have not received any of the items listed above, contact customer support (see “Customer and technical support” on page 123).

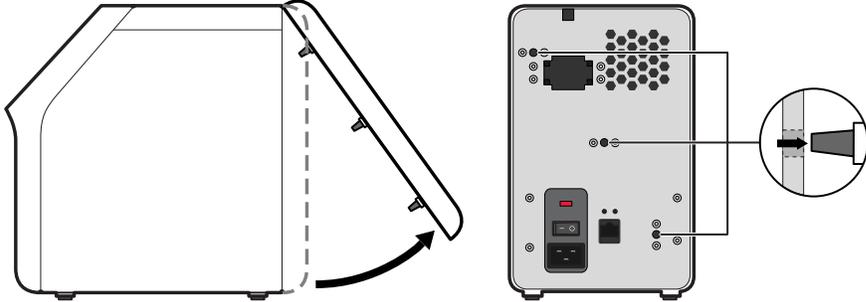
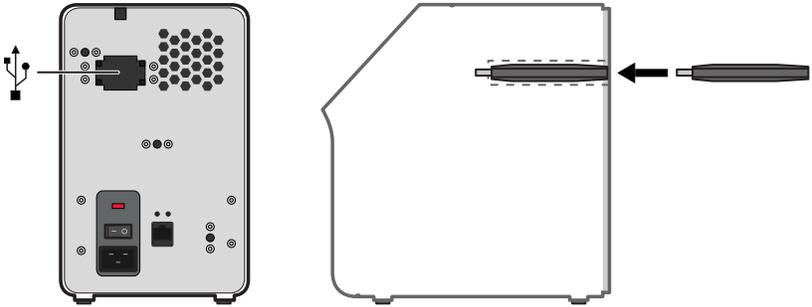
3. If you plan to connect your instrument to a network, confirm that you have one of the following:
 - (Wired network) Category-6 Ethernet cable
 - (Wireless network) QuantStudio™ 3D Extended Arm WiFi Adapter (Cat. No. A28598)

Note: Connecting the instrument to a network is optional and can be done later.

4. Confirm that all chemistry kits and accessories have been stored at the correct temperature.
5. Unpack the QuantStudio™ 3D Instrument:
 - a. Open the instrument package and confirm that you have received all parts listed on the shipping manifest. If you are missing parts, contact customer support.
 - b. Remove the instrument and set it on a clean level surface, then remove the protective cover.
 - c. Inspect the instrument for damage caused during transportation. If the instrument is damaged, note the location and appearance of the damage, then contact customer support or your service representative for assistance.

6. If you intend to connect the QuantStudio™ 3D Instrument to a network, prepare the physical network connection:

Note: Connecting the instrument to a network is optional and can be done later.

Connection	Action
Wired	Connect one end of a standard category-6 Ethernet cable to the RJ-45 port on the back panel of the instrument and the other end to an open network port.
Wireless	<ol style="list-style-type: none"> <li data-bbox="581 520 1414 611">1. If required for network setup, record the MAC address of the QuantStudio™ 3D Extended Arm WiFi Adapter, which is located on the underside of the adapter. <li data-bbox="581 625 878 653">2. Unplug the instrument. <li data-bbox="581 667 1474 730">3. Turn the instrument over, then use your fingers to carefully pry open the rear panel. <p data-bbox="618 737 1446 863">IMPORTANT! The rear panel is secured to the instrument by three pegs that require significant force to decouple. If necessary, insert a flathead screwdriver into the slot at the base of the panel to pry it from the instrument chassis.</p>  <li data-bbox="581 1207 1446 1304">4. Uncap the WiFi Adapter and insert it into the internal USB port <i>inside</i> the instrument. When the device is seated in the USB port, firmly push on the adapter to ensure that it is plugged in completely. <p data-bbox="618 1318 1430 1381">Note: The internal USB port is accessed through a small opening in the instrument chassis.</p>  <li data-bbox="581 1724 1182 1751">5. Reattach the rear panel to the instrument chassis.

7. Connect the power cord to the instrument, press the power switch to the ON position, then wait for it to start (~30 seconds).

The instrument is ready to configure when the touchscreen displays the End User License Agreement.

IMPORTANT! If the instrument does not start, contact customer support or your service representative for assistance.

8. In the End User License Agreement screen, touch **Accept** to accept the agreement.
9. In the Network Connection screen, select the appropriate action:

IMPORTANT! The QuantStudio™ 3D Instrument is pre-configured for DHCP network operation. If connecting your instrument to a network that requires static IP assignment or advanced network configuration, touch **Skip Network Connection** and connect your instrument to the network following the installation.

- Touch **Connect to Network** to connect the instrument to a wired or wireless network that supports DHCP. Go to the next step.
- Touch **Skip Network Connection** if you do not want to connect to a network, if you want to connect the instrument at a later time, or if your network requires static IP assignment or advanced network settings.



10. In the Choose Connection Type screen, select the appropriate connection:

Connection	Action
Wired	Do one of the following: <ul style="list-style-type: none"> • LAN connection—Touch  LAN, then wait for the instrument to connect to the network. • Server connection—See the <i>QuantStudio™ 3D AnalysisSuite™ Server System Quick Reference</i> (Pub. No. 4486248) for instructions on setting up a wired server connection.

(continued)

Connection	Action
Wireless	<ol style="list-style-type: none"> 1. Touch  Wireless. 2. In the Available Wireless Network screen, touch the desired wireless hotspot. Note: The signal strength of a wireless hotspot is indicated by the number of bars present in the wireless icon (). The presence of a lock icon () indicates that the hotspot is secure (WPA, WEP, or WPA2).  <ol style="list-style-type: none"> 3. If your selected wireless hotspot is secure, enter the required authentication information (user name/password), then touch OK. 
None (no network connection)	 Offline to configure the instrument to save data to a local USB drive.

The instrument is connected to the network when the touchscreen displays the Network Complete screen.

11. In the Network Complete screen, touch **OK**.



12. In the Configuration screen, touch **Edit** to change the instrument settings.



13. In the Edit Configuration screen, modify the fields as needed, then touch **Save**.

- Touch the **Instrument Name** field, enter up to a 16-character name for the instrument, then touch  to save the setting.

IMPORTANT! The instrument name is an alphanumeric string used to identify the instrument on the network. The instrument name:

- Can be up to 16 characters long.
 - Must consist of alphabetic and numeric characters.
 - Can contain hyphens, but not as the first or last characters in the name.
 - Cannot include spaces or special characters (; : " < > * + = \ | ? ,).
-

Note: By default, the Instrument Name is set as the serial number of the instrument.

- Touch the **Time Zone** field, then select the correct continent and region from the list.
- Touch the **Date** field, enter the current date, then touch **Enter**.
- Touch the **Time** field, enter the appropriate time units, then touch **Enter**.

14. In the Configuration screen, touch **OK** to accept the instrument configuration.

The QuantStudio™ 3D Instrument is ready to use when the touchscreen displays the Start Run screen.



15. Open the QuantStudio™ 3D Digital PCR Chip Loader package and place the loader on the surface where you will load chips. Attach the power cord to the chip loader and connect it to an outlet.

IMPORTANT! Do not power on the chip loader until you are ready to use it.

IMPORTANT! Do not install the chip loader in an area that may damage the enclosure.

IMPORTANT! When loading chips, the chip loader must be placed on a level surface.

The instrument is now ready to use.

Install the ProFlex™ 2 × Flat PCR System

This section includes the essential procedures for preparing the ProFlex™ 2 × Flat PCR System for use. For complete instructions, see the *ProFlex™ PCR System User Guide* (Pub. No. MAN0007697).

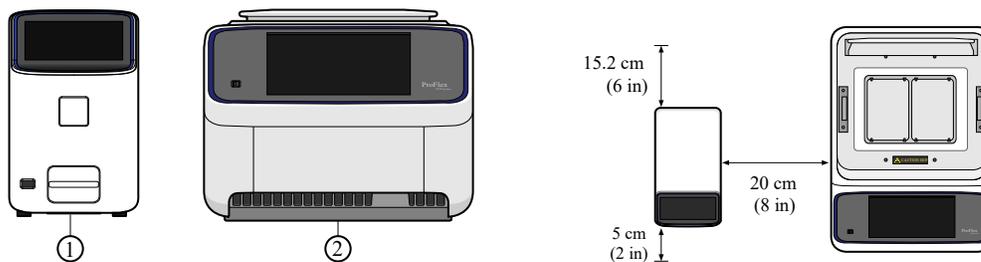
Note: For information on preparing the Dual Flat Block GeneAmp™ PCR System 9700, see “Set up the GeneAmp™ PCR System 9700” on page 75.

Unpack and place the ProFlex™ PCR System Base Module

1. Open the package containing the Base Module.
2. Remove the packing crate from the package, and verify that it contains all of the parts listed on the shipping manifest.

If you have not received one or more of the parts, contact customer support for replacement (see “Customer and technical support” on page 123).

3. Remove the Base Module from the package and set it on a clean, level surface, then remove the protective cover.



- ① QuantStudio™ 3D Instrument
- ② ProFlex™ PCR System

IMPORTANT! The ProFlex™ PCR System must be installed on a *level* surface.

Note: If not located next to the thermal cycler, the QuantStudio™ 3D Instrument requires only 15.2 cm (6 in) of clearance on either side of the instrument.

4. Inspect the Base Module for damage caused during transportation.

If the instrument is damaged, note the location and appearance of the damage, then contact customer support for assistance.

IMPORTANT! Save the packing materials and box in case you need to ship the instrument for service.

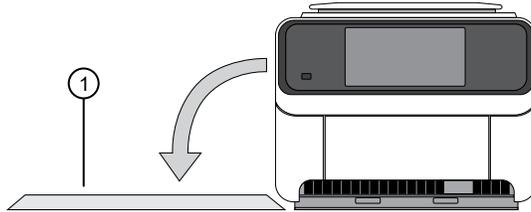
Prepare the workspace and instrument

1. Clear a space to the right or left of the instrument, then place the drop cloth over the area to provide padding for the instrument during the installation of the risers.
2. Unpack the Tilt Base Kit packages, then place the contents near the instrument.
3. Load an empty consumable onto the sample block of the instrument, then close the lid.
4. Power off and unplug the instrument.

Install the Tilt Base

IMPORTANT! Before rolling the instrument onto its side, complete the tasks described in “Prepare the workspace and instrument” above.

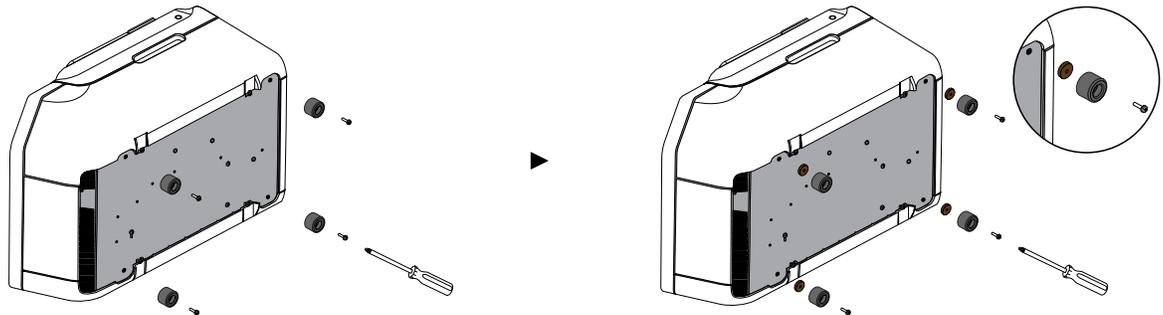
1. Carefully roll the instrument onto the workspace protected by the drop cloth.



- ① Drop cloth (either on right or left side of the instrument)

2. Use the screwdriver to remove the four footings from the instrument.
3. Fit the four risers between the base and the footings, then reattach the footings using the screwdriver.

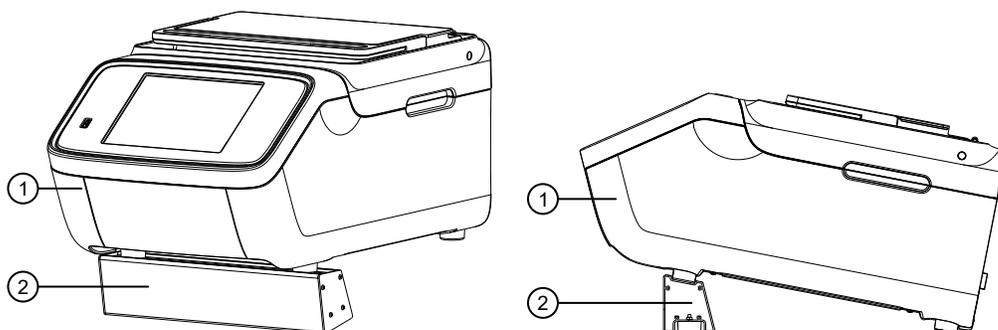
Ensure that the risers are firmly seated between the instrument base and the footings.



4. Carefully roll the instrument back onto its footings.

- Lift the front end of the instrument, then slide the Tilt Base underneath the front footings of the instrument.

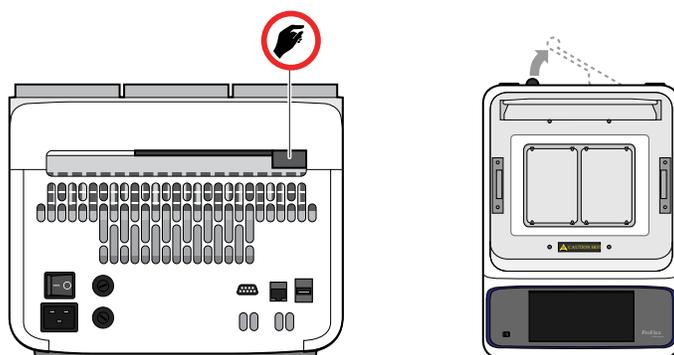
IMPORTANT! Before removing your hands from the instrument, ensure that the front footings are securely seated within the Tilt Base as shown in the following figures.



- Instrument at optimal angle
- Properly installed Tilt Base

Unpack and install the sample block module

- Open the package containing the sample block module and inspect it for damage caused during transportation.
If the module is damaged, note the location and appearance of the damage, then contact customer support for assistance.
- Pull the lever on the rear panel of the ProFlex™ PCR System Base Module away from the unit to its maximum extension.



- Place the sample block module onto the Base Module so that the latching mechanism aligns with the base, then push the lever back into the instrument to secure it.

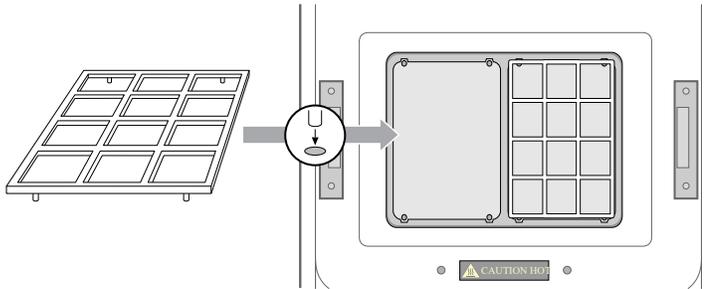
IMPORTANT! After the sample block is installed, you must completely insert the lever into the instrument to ensure that the module is locked into place. If not, the base module may fail to detect the sample block module and generate an error when powered on for the first time.

4. Use compressed air to remove any particles from the sample blocks that may have collected on them during transportation.
5. Close the heated cover.

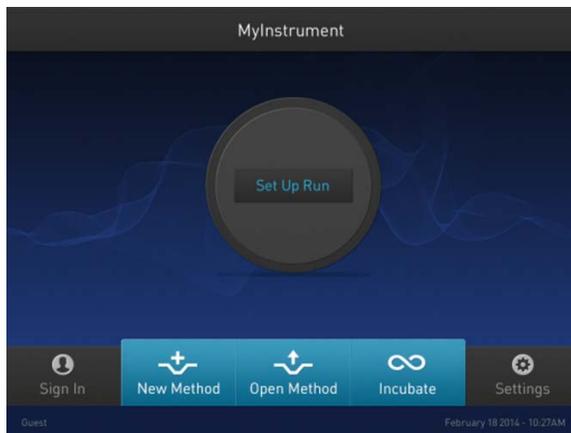
Set up the ProFlex™ PCR System

1. Open the package containing the Chip Adapters and install them.

Note: Each Chip Adapter includes a set of four alignment pegs that fit into the holes of a flat sample block. The Chip Adapter can fit onto either side of the sample block (right or left).



2. Connect the power cord, power on the ProFlex™ PCR System, then wait for it to start (about 45 seconds). Follow the onscreen instructions to complete the installation. The ProFlex™ PCR System is ready to use when the Main Menu displays.



IMPORTANT! If the instrument does not start, check the position of the sample block module. The instrument cannot be powered on if the module is seated incorrectly or if the lever is not completely closed. If the instrument does not start even after adjusting the sample block module, or if the screen contains any permanent patterns of lines or bars, contact technical support for assistance.

3. Configure the ProFlex™ PCR System settings as described in the *ProFlex™ PCR System User Guide* (Pub. No. MAN0007697).

4. For optimal results, create a new method from the existing **3D** template supplied with the ProFlex™ PCR System and verify the pre-defined method for thermal cycling the chips:

Table 4 ProFlex™ 2 × Flat PCR System PCR Method

PCR protocol					Cover temp.	Reaction volume
Stage 1	Stage 2		Stage 3			
96.0°C	60.0°C	98.0°C	60.0°C	10.0°C	70.0°C	1 nL ^[1]
1.6°C/sec	1.6°C/sec	1.6°C/sec	1.6°C/sec	1.6°C/sec		
0:10:00	0:02:00	0:00:30	0:02:00	∞		
1x (Hold)	39x (Cycles)		1x (Hold)			

[1] 33 nL for firmware earlier than v1.1.4. The reaction volume on the instrument display does not refer to the reaction volume on the chip and should not be changed.



Note: For information on opening templates and programming the thermal cycler, see the *ProFlex™ PCR System User Guide* (Pub. No. MAN0007697). If you cannot locate the **3D** template on your ProFlex™ PCR System, you may need to upgrade the firmware (see “Prepare the thermal cycler” on page 31).

The installation of the ProFlex™ PCR System is complete and the thermal cycler is ready for use.

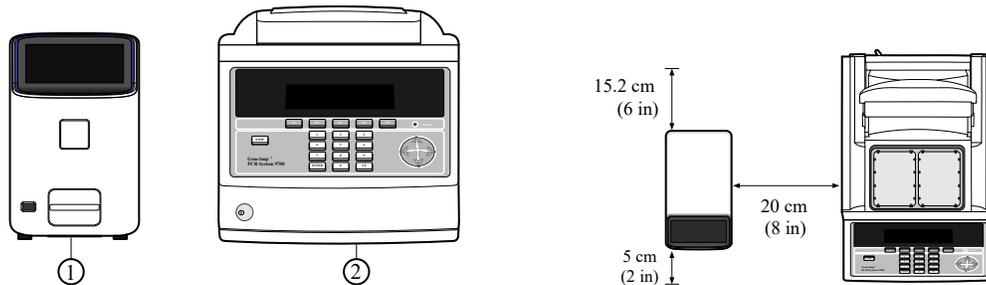
Install the GeneAmp™ PCR System 9700

This section includes the essential procedures for preparing the GeneAmp™ PCR System 9700 for use. For complete instructions, see the *GeneAmp™ PCR System 9700 Base Module User Manual* (Pub. No. 4303481) or the *GeneAmp™ PCR System 9700 Dual Flat Block User Manual* (Pub. No. 4307808).

Note: For information on preparing the **ProFlex™ 2 × Flat PCR System**, see “Install the ProFlex™ 2 × Flat PCR System” on page 67.

Unpack and place the GeneAmp™ PCR System 9700 Base Module

1. Open the package containing the Base Module.
2. Remove the packing crate from the package, and verify that it contains all of the parts listed on the shipping manifest.
If you have not received one or more of the parts, contact customer support for replacement (see “Customer and technical support” on page 123).
3. Remove the Base Module from the package and set it on a clean level surface, then remove the protective cover.



- ① QuantStudio™ 3D Instrument
- ② GeneAmp™ PCR System 9700

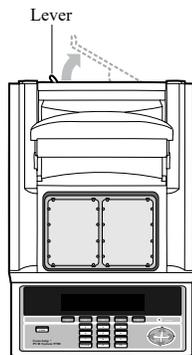
IMPORTANT! The GeneAmp™ PCR System 9700 must be installed on a *level* surface.

Note: If not located next to the GeneAmp™ PCR System 9700, the QuantStudio™ 3D Instrument requires only 15.2 cm (6 in) of clearance on either side of the instrument.

4. Inspect the GeneAmp™ PCR System 9700 Base Module for damage caused during transportation.
If the instrument is damaged, note the location and appearance of the damage, then contact customer support for assistance.

Unpack and install the Dual Flat Block Module

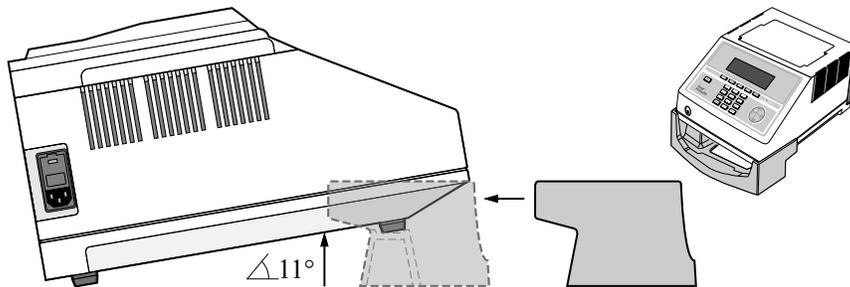
1. Open the package containing the Dual Flat Block Module.
2. Pull the lever out from the GeneAmp™ PCR System 9700 Base Module.



3. Remove the Dual Flat Block Module from the package, remove the protective cover, place it onto the Base Module, then push the Dual Flat Block Module back to seat the electrical connections.
4. Push the lever into the Base Module to secure the Dual Flat Block Module.
5. Inspect the Dual Flat Block Module for damage caused during transportation.
If the instrument is damaged, note the location and appearance of the damage, then contact customer support for assistance.

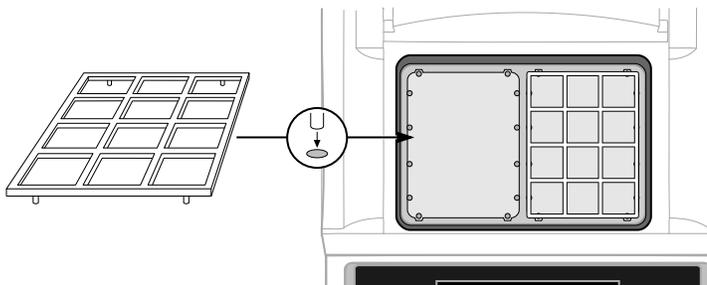
Install the Tilt Base and Chip Adapter

1. Open the package containing the QuantStudio™ 3D Digital PCR Tilt Base for GeneAmp™ PCR System 9700 and install it beneath the thermal cycler.



- Open the packages containing the QuantStudio™ 3D Digital PCR Chip Adapter Kit for Flat Block Thermal Cycler and install them.

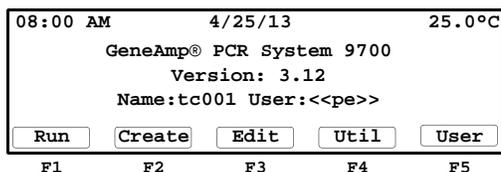
Note: Each Chip Adapter includes a set of four alignment pegs that fit into the holes of a flat sample block. The Chip Adapter can fit onto either side of the sample block (right or left).



Set up the GeneAmp™ PCR System 9700

- Connect the GeneAmp™ PCR System 9700 power cord, power on the thermal cycler, then wait for it to start (about 30 seconds).

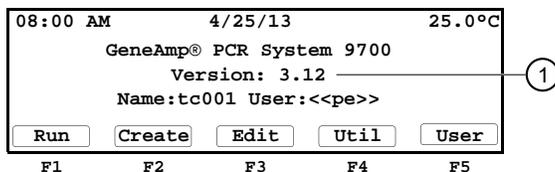
The cooling fan powers up and the start-up screens appear. The thermal cycler is ready to use when the Main Menu displays.



IMPORTANT! If the thermal cycler does not start, check the position of the Dual Flat Block Module. The instrument cannot be powered on if the module is seated incorrectly. If the thermal cycler does not start even after adjusting the Dual Flat Block Module, or if the screen contains any permanent patterns of lines or bars, contact technical support for assistance.

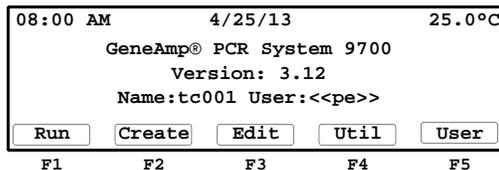
- When the thermal cycler displays the main menu, confirm that the version number displayed on the screen is **3.12** or later. If the version number is earlier than 3.12, contact technical support to upgrade the instrument firmware before continuing.

IMPORTANT! The GeneAmp™ PCR System 9700 must be running firmware version 3.12 or later to thermal cycle the chips.

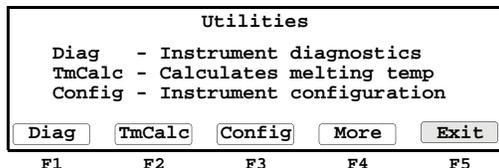


① v3.12 or later

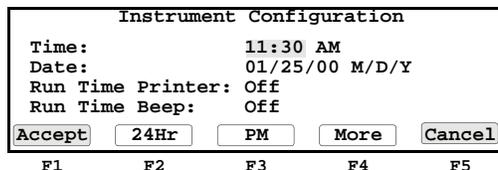
3. From the Main menu, press **Util** (F4).



4. From the Utilities screen, press **Config** (F3).



5. From the Instrument Configuration screen, set the instrument time:
- Use the Circular Key Pad to select the **Time** field.
 - Press the **24 Hr** (F2) or **PM (AM)** (F3) soft keys until the format you want for the current time displays in the Time field.
 - Use the numeric keys to type in the hours followed by minutes.
 - Press **Accept** (F1) when your entries are complete.



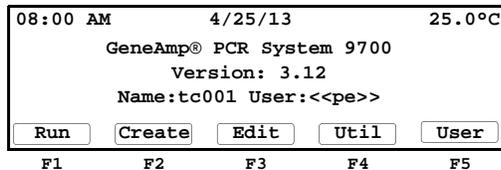
Note: CE clears an entry.

6. From the Configuration screen, set the instrument date:
- Use the circular key to select the **Date** field.
 - Press the **D/M/Y** or **Y/M/D** soft keys until the format you want for the current date displays in the Date field.
 - Use the numeric keys to enter the values into the **Day**, **Month**, and **Year** fields.

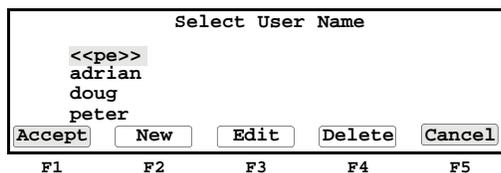
7. From the Main menu, create a user:

- a. From the Main menu, press **User** (F5).

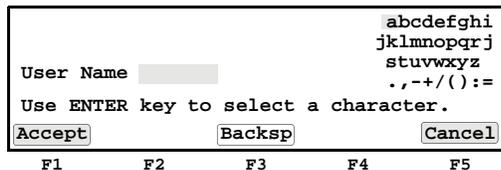
The Select User Name screen displays a list of names of all users who have been added to the instrument displays in a 4 x 5 matrix.



- b. Press **New** (F2) to add a new name to the list.

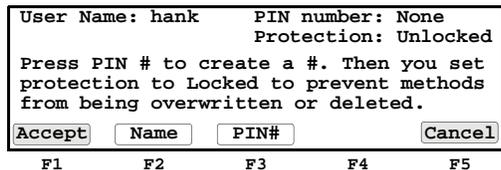


- c. In the User Name field, enter an alphanumeric name up to six characters in length, then press **Accept** (F1) to accept a name.



The name you add or the name you select from a list of existing user names becomes the current user name. All new methods that you create are stored by default under the current user name.

- d. Configure the Security Code screen according to your needs.



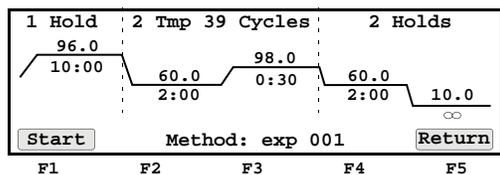
If you...	Then...
do not want to protect your methods	press Accept (F1) again.
want to protect your methods	see the <i>GeneAmp™ PCR System 9700 Base Module User Manual</i> (Pub. No. 4303481) for instructions on securing your methods.
want to return to the Main menu without adding the new user name	press Cancel (F5).

8. Program the GeneAmp™ PCR System 9700 with the method for thermal cycling chips.

Table 5 GeneAmp™ PCR System 9700 PCR Method

PCR protocol					Run speed	Reaction volume
Stage 1	Stage 2		Stage 3			
96.0°C	56.0°C	98.0°C	60.0°C	10.0°C	Standard	20 µL ^[1]
10 min	2 min	30 sec	2 min	∞		
1x (Hold)	39x (Cycles)		1x (Hold)			

^[1] The reaction volume on the instrument display does not refer to the reaction volume on the chip and should not be changed.



Note: For information on programming the GeneAmp™ PCR System 9700, see the *GeneAmp™ PCR System 9700 Base Module User Manual* (Pub. No. 4303481).

The installation of the GeneAmp™ PCR System 9700 is complete and the components are ready for use.



Specifications and Layout

- Component dimensions and weights 79
- QuantStudio™ 3D Digital PCR Instrument layout and connections 80
- Instrument clearances 80
- Electrical specifications 81
- Environmental requirements 81
- Network requirements 82

Component dimensions and weights

Ensure that the installation site bench space can accommodate the system component dimensions and weight.

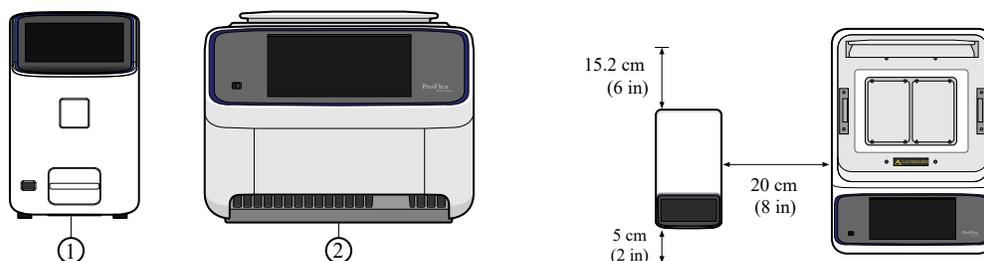
Table 6 Uncrated dimensions and weights

Component	Height	Length (depth)	Width	Weight
QuantStudio™ 3D Digital PCR Instrument	21 cm (8.3 in)	23.3 cm (9.2 in)	13.5 cm (5.3 in)	2.4 kg (5.3 lbs)
QuantStudio™ 3D Digital PCR Chip Loader	11 cm (4.3 in)	27 cm (10.6 in) ^[1]	14 cm (5.5 in)	3.0 kg (6.1 lbs)
ProFlex™ 2 × Flat PCR System (assembled)	27.2 cm (10.7 in)	56.5 cm (22 in)	33.0 cm (13 in)	19.6 kg (43.2 lbs)
Dual Flat Block GeneAmp™ PCR System 9700 (assembled)	26.0 cm (10.0 in)	41.0 cm (16.0 in)	28.0 cm (11.0 in)	11.8 kg (26.0 lbs)

^[1] Length increases to 38 cm (14.9 in) when open.

QuantStudio™ 3D Digital PCR Instrument layout and connections

The QuantStudio™ 3D Digital PCR Instrument and the ProFlex™ 2 × Flat PCR System (or Dual Flat Block GeneAmp™ PCR System 9700) can be placed on separate benches or collocated as shown in the following figure. If you place the units adjacent to one another, provide at least 20 cm (8 inches) clearance between them to allow for heat dissipation.



- ① QuantStudio™ 3D Digital PCR Instrument
- ② ProFlex™ 2 × Flat PCR System

Instrument clearances

The instruments require the following additional clearances:

IMPORTANT! For safety, the outlet used to power the instruments must be accessible at all times.

Component	Top	Front	Sides	Back
QuantStudio™ 3D Digital PCR Instrument	30.48 cm (12 in.)	30.48 cm (12 in.)	15.2 cm (6 in.)	15.2 cm (6 in.)
QuantStudio™ 3D Digital PCR Chip Loader	25.4 cm (10 in.)	15.2 cm (6 in.)	15.2 cm (6 in.)	25.4 cm (10 in.)
ProFlex™ 2 × Flat PCR System	15.5 cm (6.1 in.) ^[1]	—	—	23.0 cm (9 in.) ^[2]
Dual Flat Block GeneAmp™ PCR System 9700	20.38 cm (8 in.)	—	20.38 cm (8 in.)	20.38 cm (8 in.)

^[1] With the heated cover open.
^[2] Ensures adequate airflow and cooling.

Electrical specifications



WARNING! For safety, the power outlet used for powering the instrument must be accessible at all times. In case of emergency, you must be able to immediately disconnect the main power supply to all the equipment. Allow adequate space between the wall and the equipment so that the power cords can be disconnected in case of emergency.

IMPORTANT! Use only the approved power supply provided by Thermo Fisher Scientific to power the QuantStudio™ 3D Digital PCR Chip Loader.

- Electric receptacle is required
- Mains AC line voltage tolerances must be up to $\pm 10\%$ percent of nominal voltage

Note: Place the instrument power receptacle on an electrical circuit that is not shared with electrically noisy devices or devices that can cause power surges, such as refrigeration units.

The following table provides electrical specifications for the instrument and associated devices. For all indicated input voltages, a 15A circuit is required.

Device	Rated voltage	Rated frequency	Rated current	Rated power
QuantStudio™ 3D Digital PCR Instrument	100–240 VAC ^[1]	50/60 Hz	150 mA	10 VA
QuantStudio™ 3D Digital PCR Chip Loader	12 VDC	–	–	2.5 A max
ProFlex™ 2 × Flat PCR System	See the <i>ProFlex™ PCR System User Guide</i> (Pub. No. MAN0007697) for the ProFlex™ 2 × Flat PCR System electrical requirements.			
Dual Flat Block GeneAmp™ PCR System 9700	See the <i>GeneAmp™ PCR System 9700 Base Module User Manual</i> (Pub. No. 4303481) for the GeneAmp™ PCR System 9700 electrical requirements.			

^[1] If the supplied power fluctuates beyond the rated voltage, a power line regulator may be required. High or low voltages can adversely affect the electronic components of the instrument.

Environmental requirements

Ensure that the installation room is maintained under correct environmental conditions.

Condition	Acceptable Range
Installation site	Indoor use only
Humidity	Recommended range: 30% to 60% (noncondensing)
Temperature	Recommended range: 18°C to 22°C

(continued)

Condition	Acceptable Range
Thermal output	During operation the net thermal output, based on the actual current draw of the QuantStudio™ 3D Digital PCR Instrument, is expected to be approximately 12 W (41 BTU/hr).
Vibration	Ensure the instrument is not adjacent to strong vibration sources, such as a centrifuge, pump, or compressor. Excessive vibration will affect instrument performance.
Pollution	The instrument has a Pollution Degree rating of II. The instrument may only be installed in an environment that has nonconductive pollutants such as dust particles or wood chips. Typical environments with a Pollution Degree II rating are laboratories and sales and commercial areas. The noise output of the instrument is 0 dB at idle.
Other conditions	Ensure the room is away from any vents that could expel particulate material on the system components. Avoid placing the instrument, computer, and UPS unit adjacent to heaters, cooling ducts, or in direct sunlight.

Note: See the *ProFlex™ PCR System User Guide* (Pub. No. MAN0007697) for the ProFlex™ 2 × Flat PCR System environmental requirements. See the *GeneAmp™ PCR System 9700 Base Module User Manual* (Pub. No. 4303481) for the GeneAmp™ PCR System 9700 environmental requirements.

Network requirements

The QuantStudio™ 3D Digital PCR Instrument supports IPv4 TCP/IP communication and provides two methods for integrating the device into a local area network (LAN, also see “Materials required” on page 96):

- The fast Ethernet adapter (10/100 Mbps) with a RJ45-type connector on the rear panel of the instrument allows you to connect the device directly to a standard network port using a standard Category 6 Ethernet cable of the required length.
- An optional QuantStudio™ 3D Extended Arm WiFi Adapter (Cat. No. A28598) connected to the instrument enables a connection to compatible wireless hotspots.

The QuantStudio™ 3D Instrument requires the following if connected to a network:

- The room where the instrument will be deployed must contain at least one active network jack.
- If the instrument will be located more than 10 feet from the network jack, you must provide a shielded Category 6 Ethernet cable of sufficient length.
- If dynamic network configuration is not available, a static IP address must be reserved for the instrument.
- The configured DNS server must resolve internet domain names (such as "apps.lifetechnologies.com/" and "pool.ntp.org").

The names of any local file servers used as data destinations must also be available through DNS, or optionally WINS.

- If the instrument will transfer experiment (.eds) files directly to the QuantStudio™ 3D AnalysisSuite™ Software, then the instrument must have outbound Internet access through port 443/HTTPS to `apps.lifetechnologies.com/quantstudio3d/`.
- If the instrument will use Network Time Protocol (NTP) for date/time synchronization, the instrument must have outbound Internet access through port 123/UDP to the server pool at `pool.ntp.org`.
- If the instrument will transfer experiment (.eds) files to a Windows™ operating system-based or SAMBA file server, network credentials must be provided for authentication.

In addition to the requirements above, if the QuantStudio™ 3D Instrument will be connected to a wireless network, the access point must support:

- IEEE 802.11b/g/n wireless standards
- WEP, WEP-Enterprise, WPA- Personal, or WPA2-Personal security



Maintenance

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IMPORTANT! This chapter contains all user service procedures for the QuantStudio™ 3D Digital PCR Instrument. Procedures other than those described in this document must be performed by a qualified Service Engineer.

Cleaning the chip tray and sample block

If a chip leaks during thermal cycling or during a run, perform the following procedure to eliminate any residual contaminants from the chip tray or the thermal cycler.

Materials required

- Cotton or nylon swabs and lint-free cloths
- Deionized water
- Isopropanol
- Pipette (100- μ L) with pipette tips
- Powder-free gloves
- Safety glasses



Clean the chip tray or sample block



CAUTION! PHYSICAL INJURY HAZARD. Do not remove the covers to the QuantStudio™ 3D Digital PCR Instrument or thermal cycler. There are no components inside the instruments that you can safely service yourself. If you suspect a problem, contact technical support (see “Customer and technical support” on page 123).



CAUTION! PHYSICAL INJURY HAZARD. During instrument operation, the sample block can be heated to 100°C. Before performing the following procedure, be sure to wait until the sample block reaches room temperature.



CAUTION! Before using a recommended decontamination method other than that listed here, contact technical support to confirm that the proposed method will not damage the equipment.

IMPORTANT! Wear powder-free gloves when you perform this procedure.

1. Power off and unplug the QuantStudio™ 3D Instrument or thermal cycler.
2. Open the QuantStudio™ 3D Instrument chip tray or open the thermal cycler heated cover to expose the sample block.
3. If you are cleaning the thermal cycler, remove the Chip Adapters and Thermal Pads from the sample block.
4. If the contamination remains, clean the chip tray or sample block using isopropanol:
 - a. Pipet a small volume of 10% isopropanol solution onto surface that contacts the consumable, then use a cotton swab to scrub the surface.
 - b. Pipet a small volume of deionized water onto surface, then use a cotton swab to scrub the surface.
 - c. Use a lint-free cloth to absorb the excess deionized water.

IMPORTANT! Always use deionized water to rinse after cleaning with isopropanol solution.

5. Close the heated cover or chip tray, then plug in and power on the QuantStudio™ 3D Instrument or thermal cycler.



Configure the instrument settings

After you install the QuantStudio™ 3D Digital PCR Instrument, you can configure the instrument settings for your region as explained below.

(Optional) Set the instrument name

Note: The serial number of the QuantStudio™ 3D Digital PCR Instrument is the default instrument name. We recommend that you name the QuantStudio™ 3D Instrument uniquely. While not critical to network function, unique instrument names can aid with sample tracking and instrument troubleshooting. The QuantStudio™ 3D Instrument does not test the uniqueness of the instrument name when it is set.

1. From the Main Menu of the touchscreen, touch , then touch **Instrument Settings**.
 2. In the Instrument Settings menu, touch **Instrument Name**.
 3. In the Instrument Name screen, touch the Instrument Name field.
 4. In the Edit Instrument Name screen, enter up to a 16-character name for the instrument, then touch **Enter**.
-

IMPORTANT! The instrument name is an alphanumeric string used to identify the QuantStudio™ 3D Instrument on the network. The instrument name:

- Can be up to 16 characters long.
 - Must consist of alphabetic and numeric characters.
 - Can contain hyphens, but not as the first or last characters in the name.
 - Cannot include spaces or special characters (; : " < > * + = \ | ? ,).
-

5. Touch **OK** when done to save the setting, then touch  to return to the Main Menu.



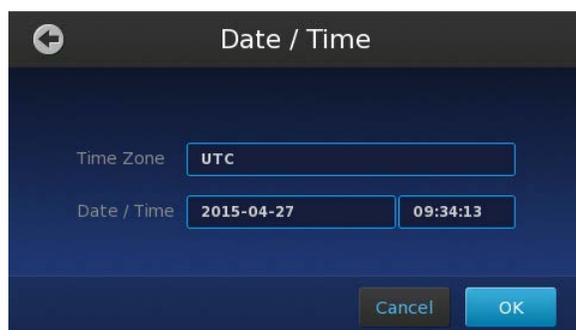


Set the date and time

Use this procedure to manually configure the time zone, date, and time settings for your instrument.

Note: If connected to a network, the QuantStudio™ 3D Digital PCR Instrument will automatically synchronize the date and time settings via Network Time Protocol (NTP). However, you will still need to manually set the instrument time zone.

1. From the Main Menu of the touchscreen, touch , then touch **Instrument Settings**.
2. In the Instrument Settings screen, touch **Date/Time**.
3. Set the time zone, date, and time:
 - a. Touch the **Time Zone** field, then select the correct continent and region from the lists.
 - b. Touch the **Date** field, enter the current date, then touch **Enter**.
 - c. Touch the **Time** field, enter the appropriate time units, then touch **Enter**.



4. Touch **OK** when done to save the setting.

Check the instrument firmware

Imaging and analysis of the QuantStudio™ 3D Digital PCR 20K Chip v2 requires firmware version 3.0 or later on the QuantStudio™ 3D Digital PCR Instrument. To check the firmware version:

1. On the instrument touchscreen Main Menu, touch .
2. In the Settings Menu, touch **Maintenance & Service**.



3. In the Maintenance & Service screen, touch **Software Update**. The firmware version is displayed on the screen after **Embedded SW Ver.**:



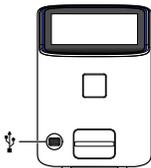
4. If the version is earlier than 3.0, update the firmware as follows.

Update the instrument firmware

You can download QuantStudio™ 3D Digital PCR Instrument firmware updates directly from thermofisher.com/support. You must log in to the website and include your instrument shipping and billing information, if they are not already associated with your www.thermofisher.com profile. After obtaining a firmware update, transfer the update to the instrument using a USB drive.

1. Download the firmware update:
 - a. Go to thermofisher.com/support.
 - b. Under **Product Support**, select **Instrument Support**.
 - c. Click **Instrument Management**, then click **Log in to the instrument management tool**. Find the link to **Software, Patches & Updates** on the left side of the **Instrument Management** dashboard.
 - d. In the **Software Downloads** page, select **QuantStudio™ 3D Digital PCR Software** from the **Digital PCR** menu.
 - e. In the product page for your instrument, click the **Download QuantStudio™ 3D Digital PCR Software** link to download the instrument firmware and save it on a USB drive.
 - f. On the same page, click on **User Instructions** to download and review the release notes for the update.
2. Confirm that the instrument touchscreen is displaying the Main Menu, then plug the drive into the USB port.

IMPORTANT! The instrument must display the Main Menu when you insert the USB drive. If not, the update may fail.





3. From the Main Menu of the touchscreen, touch , then touch **Maintenance & Service**.
4. In the Maintenance & Service screen, touch **Software Update** then wait while the instrument scans the USB drive for the firmware update.
5. In the Software Upgrade screen, touch **OK** to proceed with the update.



IMPORTANT! Do not remove the USB drive from the instrument until after the update is complete.

6. After the upgrade is complete and the instrument restarts, re-calibrate the touchscreen (if prompted), then re-check the firmware version to confirm the success of the update.

Returning your instrument for service

Before returning your QuantStudio™ 3D Digital PCR Instrument for service:

- Transfer any experiment (.eds) files from the instrument as explained in “Transfer the results” on page 48. This practice ensures that you will not lose data when the instrument is serviced.
- Confirm that the instrument is unloaded before you power off and pack the instrument. If the instrument is moved while loaded, the chip inside may become dislodged from the chip tray and obstruct the chip tray mechanism.

To return your instrument for service:

1. Contact your local customer care center or technical support group (see “Customer and technical support” on page 123) to obtain a copy of the Certificate of Instrument Decontamination, a service notification, a service call number, and packaging (if required).
2. Decontaminate the instrument.

IMPORTANT! If the chip tray becomes contaminated with radioactivity, use a commercially available decontaminant to remove the contamination. If the tray cannot be decontaminated, the instrument cannot be returned to Thermo Fisher Scientific for service.

3. Complete and sign a copy of the Certificate of Instrument Decontamination.
4. Fax the Certificate of Instrument Decontamination to the customer care center.



5. Pack the instrument in the provided packaging, without any accessories or power cords. Include a hard copy of the Certificate of Instrument Decontamination in the box.

Note: Repairs for instruments without the Certificate of Instrument Decontamination are delayed.

6. Affix the provided postage to the box, then ship the instrument to the designated facility.

The repair process requires 2 to 3 weeks.

Replace the instrument fuses

If the fuses of your QuantStudio™ 3D Digital PCR Instrument fail, perform the following procedure to replace them.

Materials required

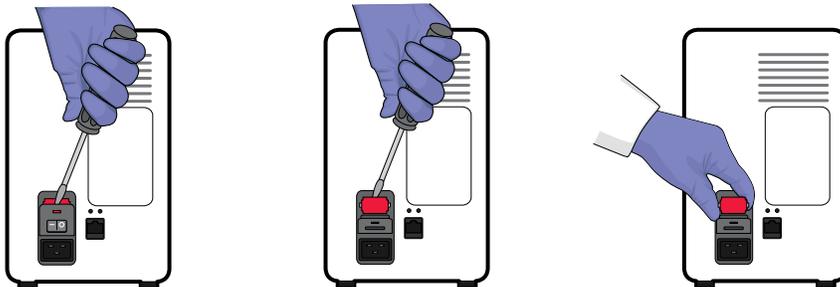
- Fuses, 1.6A, Time-Lag T, 250VAC, 5 × 20-mm (2)
- Safety glasses
- Powder-free gloves
- Screwdriver, flathead

Replace the fuses



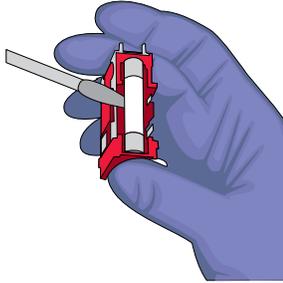
CAUTION! FIRE HAZARD. For continued protection against the risk of fire, replace fuses only with listed and certified fuses of the same type and rating as those currently in the QuantStudio™ 3D Digital PCR Instrument.

1. Power off, then unplug the QuantStudio™ 3D Digital PCR Instrument.
2. Using a flat-head screwdriver, pry open the fuse door, and remove the fuse holder.





3. Remove each fuse from its fuse holder and inspect it for damage. Carbon typically coats the inside of failed fuses.



4. Replace each failed fuse with a 1.6A, Time-Lag T, 250VAC, 5 × 20-mm Fuse.

Note: The voltage and amperage ratings are on the fuse holder.

5. Install the fuse holder.
6. Plug in, then power on the QuantStudio™ 3D Digital PCR Instrument. The installation is successful if the instrument powers on.

Note: Fuse failure can result from fluctuations in the supplied power to the QuantStudio™ 3D Digital PCR Instrument. To prevent further failures, consider installing an electrical protective device, such as a UPS or surge protector.

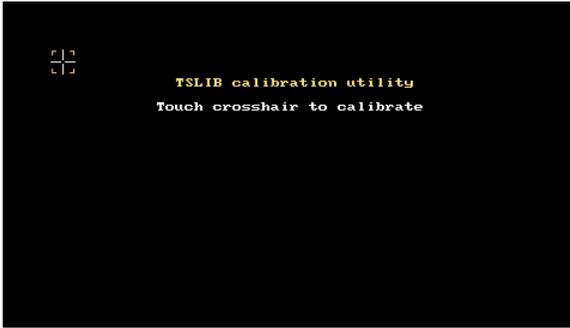
Calibrate the touchscreen

Calibrate the instrument touchscreen periodically as part of the routine maintenance of your instrument, if you observe a decrease in screen responsiveness over time, or if prompted after updating the instrument firmware.

1. From the Main Menu of the touchscreen, touch , then touch **Maintenance & Service**.
2. In the Maintenance & Service screen, touch **Screen Calibration**.



3. Touch each crosshair as shown in the sequence of calibration utility screens.



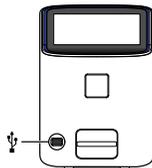
4. At the end of the calibration sequence, touch **OK** to save the new touchscreen calibration settings and restart the instrument.

View the instrument log

You can use the QuantStudio™ 3D Digital PCR Instrument touchscreen to view and export a log that summarizes the past 6 months of instrument activity. For each event, the log provides a description of the activity and the date/time when it occurred.

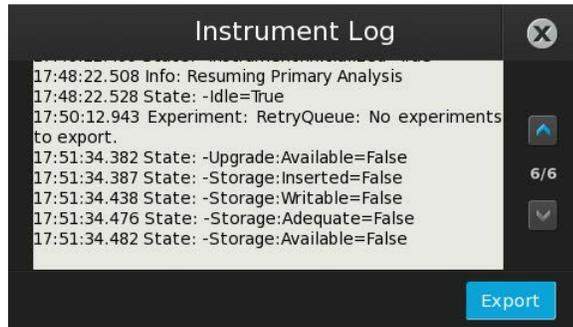
Note: The information conveyed in the instrument log is used primarily by technical support as a resource for troubleshooting instrument errors. If the QuantStudio™ 3D Digital PCR Instrument encounters an error during operation, the log is written to the experiment (.eds) file, which can be exported and sent to technical support (see “Customer and technical support” on page 123). If the instrument does not generate a file, then you can export the instrument log for distribution as explained below.

1. From the Main Menu of the touchscreen, touch , then touch **Maintenance & Service**.
2. In the Maintenance & Service screen, touch **View Log**.
3. In the Instrument Log screen, review the records of interest.
4. (Optional) Export the system log to a USB drive:
 - a. Plug the drive into the USB port on the front of the instrument.





- b. In the Instrument Log screen, touch **Export**, then wait for the instrument to export the log to a text file on the drive.



5. When you are finished, touch , then touch to return to the Main Menu.



■ Networking overview	94
■ Connect the instrument to the network	95

IMPORTANT! This document *does not* provide adequate detail to integrate the QuantStudio™ 3D Instrument into all possible network architectures. Because your laboratory network can contain advanced features (such as firewalls), we recommend that you consult a network administrator before connecting your instrument.

Networking overview

After installing the QuantStudio™ 3D Digital PCR Instrument and the QuantStudio™ 3D AnalysisSuite™ Software, you can connect the instrument to a local area network to enhance its functionality. When connected to a network, the instrument can upload data directly to the QuantStudio™ 3D AnalysisSuite™ Software or to a Windows™ operating system-based or SAMBA file server, for later import by the AnalysisSuite™ Software.

Note: The instrument supports a wired network connection without any additional hardware other than what is shipped with the instrument. The instrument can be configured for wireless networking through the installation of a wireless adapter, which is sold separately (see “Materials required” on page 96).

About the network port and wireless adaptor

The QuantStudio™ 3D Digital PCR Instrument is designed to integrate into a standard Local Area Network (LAN), both wired and wireless. The instrument features a Gigabit Ethernet port that provides direct connection to a network via a shielded cat 6 Ethernet cable, or wireless communication via the QuantStudio™ 3D Extended Arm WiFi Adapter.

The instrument supports the following networking technologies and standards:

- Communication via Transmission Control Protocol (TCP) over Internet Protocol (IPv4).
- Internet and local host name lookups via standard Domain Name Service (DNS).
- Local server lookups via Windows Name Service (WINS) (optional).
- Automatic configuration of IP, DNS, and WINS settings via Dynamic Host Configuration Protocol (DHCP), or manual configuration via the instrument touchscreen.
- Automatic data transfer to the QuantStudio™ 3D AnalysisSuite™ Software using the Hypertext Transfer Protocol over a Secure Socket Layer (HTTPS), or data export to a local Windows™ operating system-based or SAMBA file server over the Server Message Block (SMB) protocol.
- Date and time synchronization via Network Time Protocol (NTP).

The QuantStudio™ 3D Extended Arm WiFi Adapter sold for use with the instrument can be installed to an internal USB port behind the rear panel to enable wireless network connectivity. See the product documentation accompanying the adaptor for the network requirements, specifications, and product details.

Networking guidelines and best practices

- We recommend that you consult a network administrator before connecting the QuantStudio™ 3D Digital PCR Instrument to your laboratory network.
- We recommend that you name the QuantStudio™ 3D Digital PCR Instrument uniquely. While not critical to network function, unique instrument names can aid with sample tracking and instrument troubleshooting.

Note: The serial number of the QuantStudio™ 3D Digital PCR Instrument is the default instrument name. The QuantStudio™ 3D Digital PCR Instrument does not test the uniqueness of the instrument name when it is set.

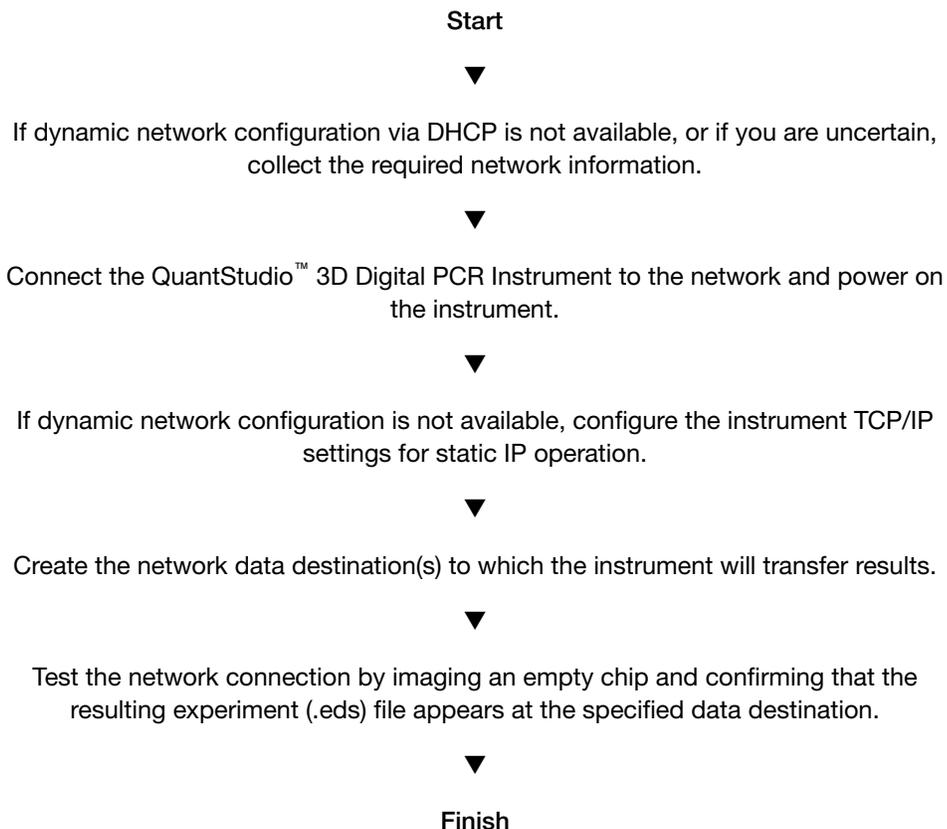
- Name QuantStudio™ 3D Digital PCR Instruments using numbers and letters. The instrument name can include hyphens, but not as the first or last character.

Connect the instrument to the network

IMPORTANT! The QuantStudio™ 3D Digital PCR Instrument is factory-configured for DHCP operation (automatic addressing); network configuration is necessary only if your network does not support dynamic configuration.

For server configuration information, see the *QuantStudio™ 3D AnalysisSuite™ Server System Quick Reference* (Pub. No. 4486248).

Network setup workflow



Materials required

Connection	Materials
Wired	CAT6 Ethernet cable of sufficient length with RJ45 connectors (for a 1000Mbit/s network connection or a CAT5 for a 100Mbit/s connection)
Wireless	QuantStudio™ 3D Extended Arm WiFi Adapter (Cat. No. A28598)

Required network information

Before beginning the installation, collect from a network administrator your network policy for obtaining IP addresses (DHCP or static IP).

If your network requires a static IP address assignment, also obtain the:

- Static IP address for the QuantStudio™ 3D Digital PCR Instrument
- Subnet mask (if applicable)
- Gateway address (if applicable)
- Internet domain name
- Domain Name Server address(es)



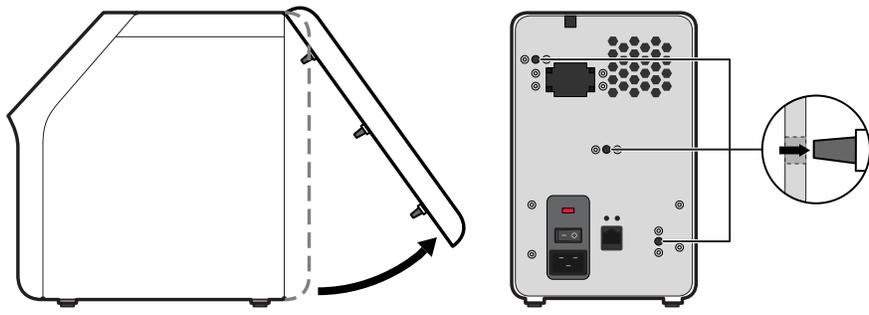
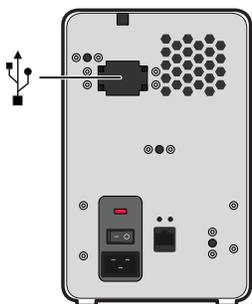
- Windows™ operating system domain (if applicable)
- WINS server address(es) (if applicable)

If connecting your instrument to a wireless network, obtain the following information for the local wireless hotspot:

- Wireless local area hotspot name
- Security policy requirement for the wireless hotspot (WPA, WEP, or WPA2)
- Network credentials for the wireless hotspot (password and user account if necessary)

Connect the QuantStudio™ 3D Digital PCR Instrument to a network

1. Prepare the physical connection to the network:

Connection	Action
Wired	<ol style="list-style-type: none"> 1. Power on the QuantStudio™ 3D Instrument. 2. Connect one end of a standard category-6 Ethernet cable to the RJ-45 port on the back panel of the instrument and the other end to an open network port.
Wireless	<ol style="list-style-type: none"> 1. If required for network setup, record the MAC address of the QuantStudio™ 3D Extended Arm WiFi Adapter, which is located on the underside of the adapter. 2. Power off and unplug the QuantStudio™ 3D Instrument. 3. Turn the QuantStudio™ 3D Instrument over, then use your fingers to carefully pry open the rear panel. <p>IMPORTANT! The rear panel is secured to the instrument by three pegs that require significant force to decouple. If necessary, insert a flathead screwdriver into the slot at the base of the panel to pry it from the instrument chassis.</p>  4. Turn the instrument over, then uncap the WiFi Adapter and insert it into the USB port <i>inside</i> the instrument. Firmly push on the adapter to ensure that it is plugged in completely. <p>The internal USB port is accessed through a small opening in the instrument chassis.</p>  5. Reattach the rear panel to the instrument chassis, then plug in and power on the QuantStudio™ 3D Instrument.

2. From the Main Menu of the touchscreen, touch , then touch **Instrument Settings**.

3. Choose the appropriate option:

If connecting your instrument to a...	Action
Wired network that supports DHCP	Network setup is complete. Note: The QuantStudio™ 3D Instrument is pre-configured for DHCP operation and automatically joins the network when connected.
<ul style="list-style-type: none"> Wireless network Wired network with specific requirements (such as static IP operation) 	Go to step 4.

4. In the Instrument Settings screen, touch **Network Setup**, then touch **Edit** in the network setup screen.



5. (Wireless connection only) If you are connecting your instrument to a wireless network:

- In the Network Setup screen, touch **Change Connection Type**.
- In the Choose Connection Type screen, touch **Wireless** .
- In the Available Wireless Network screen, touch the desired wireless hotspot.

Note: The signal strength of a wireless hotspot is indicated by the number of bars present in the wireless icon (). The presence of a lock icon () indicates that the hotspot is secure (WPA, WEP, or WPA2).

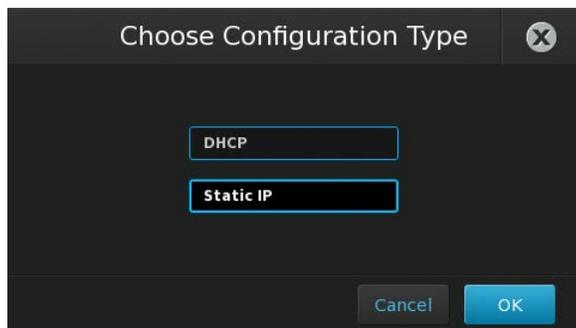


- d. If your selected wireless hotspot is secure, enter the required authentication information (user name/password), then touch **OK**.



If connecting your instrument to a network that supports DHCP, network setup is complete. Otherwise, go to the next step to configure the TCP/IP settings for the instrument.

6. Set the internet protocol (TCP/IP) properties for the QuantStudio™ 3D Instrument:
 - a. In the Network Setup screen, touch **Edit Settings**.
 - b. In the Network Setup settings, touch the **IP Configuration** field, touch the setting appropriate for your network (Static IP or DHCP), then touch **OK**.
 - **DHCP** – Select to allow your network DHCP server to automatically assign an IP address to the instrument.
 - **Static IP** – Select if you have a static IP address to assign to the instrument.



- c. If you elected to assign a static IP to the instrument, touch the following fields and enter the:
- **IP Address**—Enter the static IP address for the instrument.
 - **Netmask**—Enter a subnet mask for the network if applicable.
 - **Gateway**—Enter a default gateway for the network if applicable.

Note: For each setting, touch the associated field, then enter the setting using the keypad and touch **Enter** to accept the value.

The screenshot shows the 'Network Setup' dialog box. At the top, there is a back arrow and the title 'Network Setup'. Below that, 'IP Configuration' is set to 'Static IP'. There are three input fields: 'IP Address' with the value '10.43.32.97', 'Netmask' with '255.255.252.0', and 'Gateway' with '10.43.32.1'. To the right of the IP Address field is an upward arrow and the text '1/3'. To the right of the Netmask field is a downward arrow. At the bottom, there are 'Cancel' and 'OK' buttons.

- d. Touch , then configure the domain name service (DNS) settings:
- **DNS Domain** – Enter the domain name of the DNS server that you want the instrument to use.
 - **DNS Server** – Enter the IP addresses for the primary (top) and secondary (bottom) DNS servers.

IMPORTANT! DNS settings are required if you:

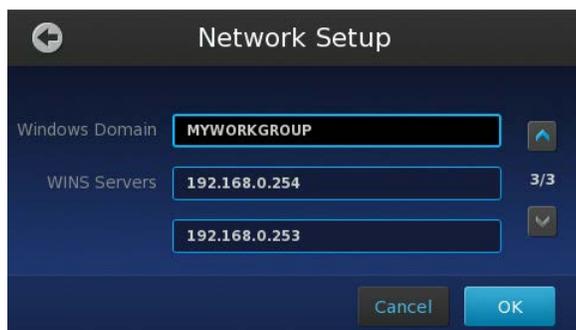
- Specify the Connect cloud-based platform as a data destination (for your QuantStudio™ 3D AnalysisSuite™ Software projects).
- Want to use Network Time Protocol (NTP) for instrument date/time synchronization.

The screenshot shows the 'Network Setup' dialog box. At the top, there is a back arrow and the title 'Network Setup'. Below that, 'DNS Domain' is set to 'mydomain'. There are two input fields for 'DNS Server': the top one contains '8.8.8.8' and the bottom one contains '8.8.4.4'. To the right of the top DNS Server field is an upward arrow and the text '2/3'. To the right of the bottom DNS Server field is a downward arrow. At the bottom, there are 'Cancel' and 'OK' buttons.

- e. Touch , then configure the Windows Internet Naming Service (WINS) settings:
- **Windows Domain** – Enter the domain name of the WINS server.
 - **WINS Server** – Enter the IP addresses for the primary (top) and secondary (bottom) WINS servers.

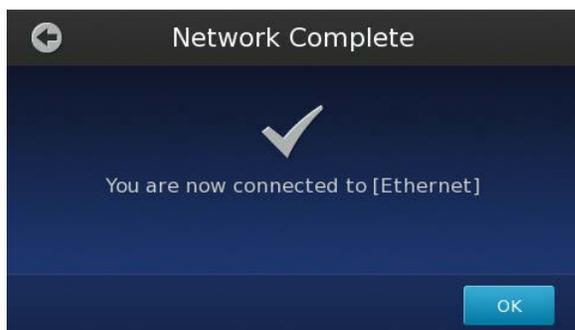
IMPORTANT! WINS may be required if you specify a file server as a data destination.

Note: During operation, the instrument attempts to resolve WINS names to IP addresses using the server specified in the top-most field. If unavailable, the instrument uses the server specified in the bottom-most field.



- f. When you are finished configuring your network settings, touch **OK**.

The touchscreen displays "Network Complete" when the instrument successfully connects to the network.



7. In the Network Complete screen, touch **OK**, then touch  to return to the Main Menu.
After the instrument has successfully connected to the network, the Network Setup screen displays the details of the network connection.

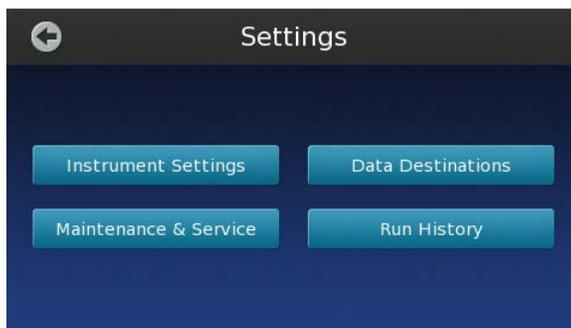


Note: If your wireless hotspot performs MAC filtering, you can obtain the Media Access Control (MAC) address of the QuantStudio™ 3D Instrument on the label of the WiFi Adapter that you installed in step 1.

Create a network destination to receive run data

The following procedure allows you to create network data destinations to receive experiment (.eds) files generated by the QuantStudio™ 3D Digital PCR Instrument. You can create data destinations for the QuantStudio™ 3D AnalysisSuite™ Software for direct upload, or for local Windows™ operating system-based or SAMBA file servers for later import. You can create an unlimited number of destinations and select from them prior to each run.

1. From the Main Menu of the touchscreen, touch .
2. In the Settings menu, touch **Instrument Settings**.



Note: To perform step 3 and step 4 firmware version 3.2 or later is required.

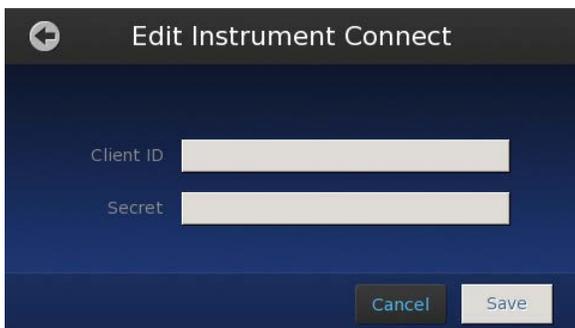
- In the Instrument Settings menu, touch **Instrument Connect** to enter your instrument-specific credentials to provide access to the Connect cloud-based platform.

Note: This information is provided by an external communication for each instrument at your site and only needs to be entered once.



- In the Edit Instrument Connect screen enter the following information for each instrument, then touch **Save**.

Field	Action
Client ID	Enter the instrument-specific Client ID.
Secret	Enter the instrument-specific secret.

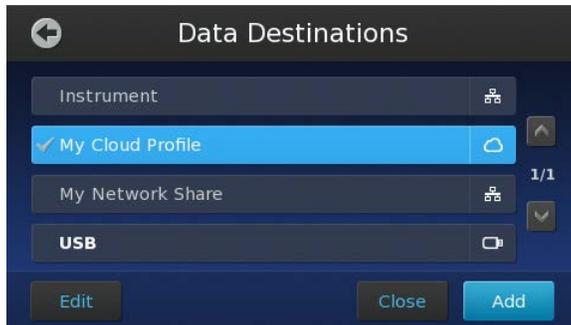


- In the Settings menu, touch **Data Destinations**.

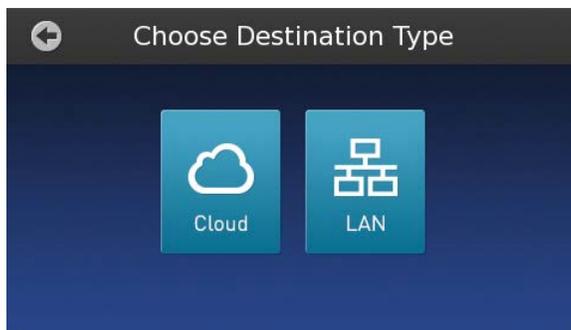


6. In the Data Destinations screen, touch **Add** to add a data destination.

Note: To edit an existing destination, touch the name of the destination.



7. In the Choose Destination Type screen, touch the desired network destination:
 -  **Cloud**—Touch to specify a Connect cloud-based platform data destination (for your QuantStudio™ 3D AnalysisSuite™ Software projects).
 -  **LAN**—Touch to specify a network file server data destination.



8. Touch each option, enter the appropriate data for the destination, and then touch **Save**.
 - **Cloud** data destination fields:

IMPORTANT! Make sure that the Client ID and Secret for your instrument has been added before entering your credentials for the Cloud data destination. See step 3 and step 4.

- **Name**—Enter a name to identify the Connect cloud-based platform data destination.

Note: The target name appears within the Destination Selection and Export screens.

- **Username**—Enter the user name that the instrument should use when accessing the Connect cloud-based platform.

- **LAN** data destination fields:

- **Target Name**—Enter a name to identify the LAN data destination.

Note: The target name appears within the Destination Selection and Export screens.

- **Server**—Enter the path of the file server using the following syntax, where `smb:` and `path` are optional:
 - `smb://servername/share/path`
 - `\\servername\share\path`
- **User Name**—Enter the user name that the instrument should use when authenticating to the file server.
- **Password**—Enter the password that the instrument should use when authenticating to the file server.

9. Repeat step 6 to step 8 to add additional data destinations as needed.

Upon saving the data destination, the QuantStudio™ 3D Instrument automatically attempts to connect to the specified location. The instrument saves the destination only if the connection is successful.

10. When you are finished adding data destinations, touch  to return to the Main Menu.



Load chips manually

For instructions on loading chips using the QuantStudio™ 3D Digital PCR Chip Loader, see “Load the chips using the QuantStudio™ 3D Digital PCR Chip Loader” on page 23.

The following procedure describes how to load the QuantStudio™ 3D Digital PCR 20K Chip v2. It can be used as an alternative method for loading chips if the chip loader is unavailable.

This procedure assumes that you have prepared your PCR reactions as explained in “Prepare the dPCR reaction mix for each sample” on page 22. Before proceeding, see “Guidelines for loading and sealing chips” on page 18.

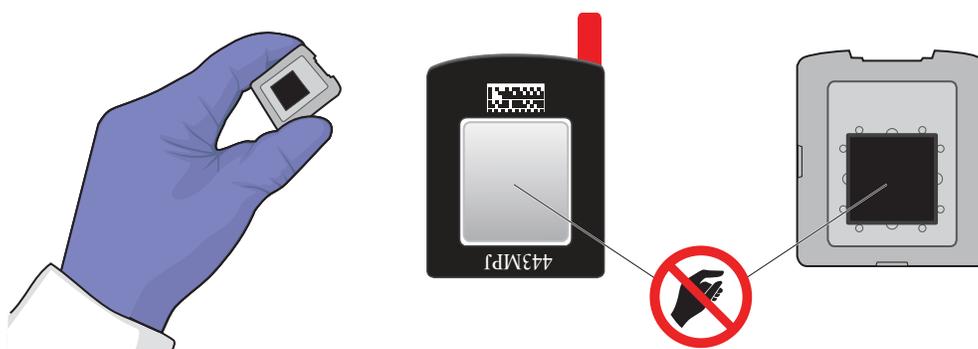
Guidelines for loading and sealing chips

To ensure proper processing and analysis of loaded chips, handle them according to the following guidelines:

- Always wear powder-free gloves when loading and sealing chips.

IMPORTANT! Never handle chips or chip lids without gloves. Oils from your hands can contaminate the components and interfere with thermal cycling and imaging.

- Hold the chip and lid gently by their sides.
 - If you accidentally touch the chip surface, discard the chip.
 - If you accidentally touch the chip lid window, clean it using a laboratory wipe that has been sprayed with isopropanol, then dry by wiping in one direction using a clean low-lint wipe.



- Use a permanent pen or marker to label the back of each loaded chip for sample tracking.
- Load each chip within 2 hours after opening it.
- Load chips in alphanumeric order (according to the chip ID printed on the lid) to avoid data entry errors.
- Apply the chip lid and add Immersion Fluid immediately after loading each chip to avoid evaporation.

- Load and seal chips in batches of up to 24 chips (the maximum number that can be loaded onto one thermal cycler).
- If you do not intend to load an opened chip immediately, cover the chip using the plastic plate included in the chip package to prevent contamination. If you plan to keep the opened chip for longer than a day, the chip should be stored with dessicant.
- Use all of the Immersion Fluid within 60 minutes of uncapping the syringe. After a syringe is opened, you cannot recap it for later use.
- Thermal cycle the chips within 2 hours after loading them.

Materials required

The following materials are required to load and seal chips for use on the QuantStudio™ 3D Digital PCR System.

- Prepared digital PCR reactions (from the previous section)
- QuantStudio™ 3D Digital PCR 20K Chip v2
- QuantStudio™ 3D Digital PCR Chip Lid v2
- QuantStudio™ 3D Digital PCR Sample Loading Bladess
- Immersion Fluid
- Immersion Fluid tip
- Isopropanol
- Gloves, powder-free, nitrile
- Heated block (capable of maintaining $40\pm 1^\circ\text{C}$)
- Scissors
- Low-lint polyester wipes (clean-room grade)
- Microcentrifuge
- Pipettes and tips, P10 to P1000
- Vortex mixer

Prepare the syringe containing the Immersion Fluid

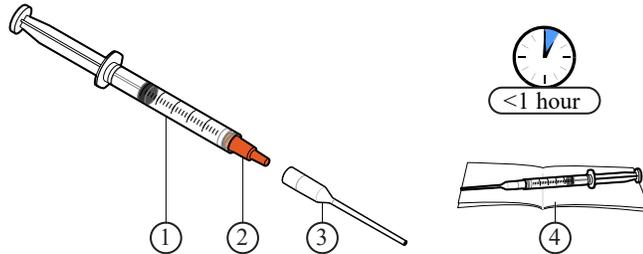
1. Remove the Immersion Fluid syringe, plunger, and tip from the packaging.
2. Before uncapping the syringe, gently pull back the plunger 1-2 mm and release it to break any resistance that may have formed during storage.
3. Remove the cap from the syringe, then attach the syringe tip by pushing it into place.

IMPORTANT! No twisting or screwing is required to attach the tip.

- Carefully depress the plunger until Immersion Fluid flows from the tip of the assembled syringe. Place it on a clean surface and proceed to the next steps.

IMPORTANT! Confirm that the tip is locked firmly in place before proceeding.

IMPORTANT! Open only one syringe at a time. Use all of the Immersion Fluid within 60 minutes of uncapping the syringe. After a syringe is opened, you cannot recap it for later use.



- Immersion Fluid syringe
- Cap (remove)
- Syringe tip (push to attach)
- Low-lint wipe

Load and seal the chips manually

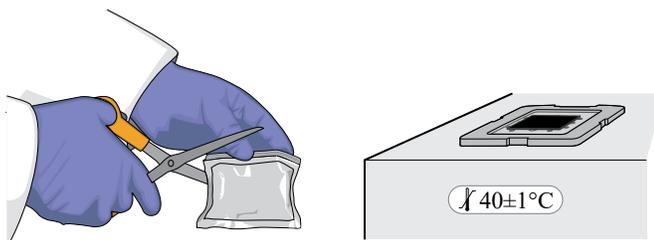
IMPORTANT! Wear powder-free gloves while preparing the chips.

- Preheat the heated block to 40°C, the optimal temperature for loading chips.

Note: The loading surface should be a flat, temperature-controlled surface that is large enough to accommodate the chip.

- Remove the following consumables from their packaging and place them on a clean, dry, lint-free surface:
 - QuantStudio™ 3D Digital PCR Chip Lid v2
 - QuantStudio™ 3D Digital PCR Sample Loading Blade
- Open the chip package, gently grasp the chip by its sides, then place it face-up on the heated block, preheated to 40°C.

IMPORTANT! Allow the chip to sit on the heated block for at least 5 seconds before loading it.

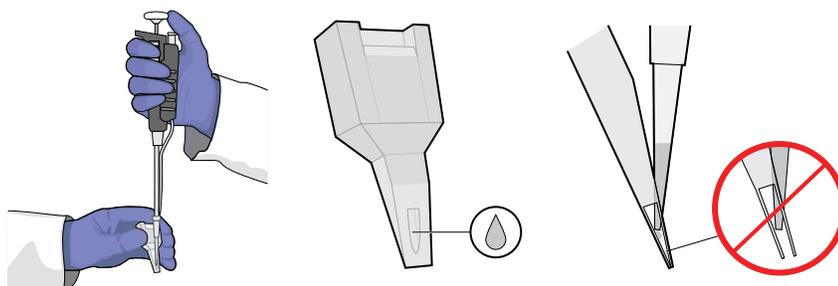


4. Load the PCR reaction onto the chip:

- a. Briefly vortex and centrifuge the prepared reaction (master mix, assay, and template), then aspirate 14.5 μ L of the solution into a P20 pipette. If the reactions were placed on ice, allow them to warm prior to loading.
- b. While holding the loading blade by its edges, carefully dispense the reaction into the sample-loading port. After loading, gently tap the top of the loading blade to spread the reaction across the full length of the blade.

While filling the loading blade:

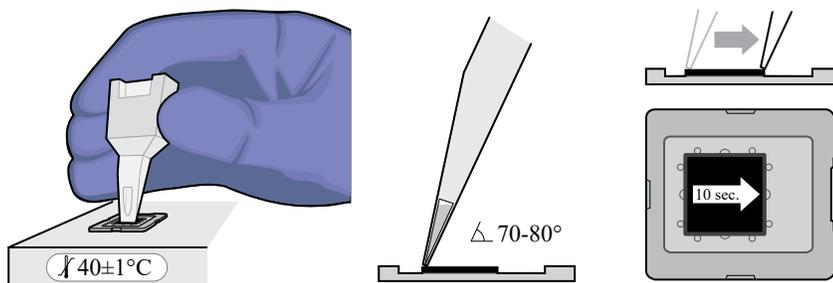
- Hold the pipette so that the tip meets the sample-loading port at a sharp angle and so it does not deflect either blade (forward or rear).
- Do *not* depress the pipette to the second stop. Doing so increases the chance of introducing an air bubble to the loading blade.
- Avoid creating air bubbles.



- c. While holding the loading blade at a 70–80° angle relative to the heated block so that the port faces up, place the edge of the blade at the end of the chip. Adjust the angle of the blade until you visually confirm that it is wetting the chip. Then, in one smooth motion, slowly drag the blade across the chip while applying a slight downward pressure to dispense the reaction.

IMPORTANT! Move the loading blade slowly. The time required to move the blade across the chip should be approximately 10–12 seconds.

IMPORTANT! Before you draw the blade across the chip, visually confirm that the loading blade is wetting the chip. If not, adjust the angle slightly.

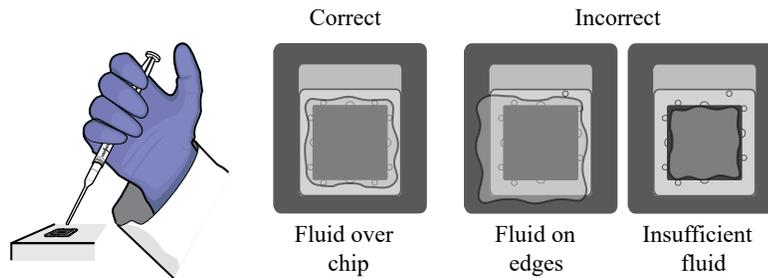


- d. Confirm that the loading blade contains no residual fluid, indicating that the reaction has been completely loaded onto the chip.

IMPORTANT! Do not apply the loading blade to the chip more than once.

- e. Allow the chip to sit on the heated block for 20 seconds while the PCR reaction stabilizes within the chip.
- f. Add several drops of Immersion Fluid directly to the chip so that the fluid covers the entire surface.

IMPORTANT! Do not allow the syringe tip to contact the chip.



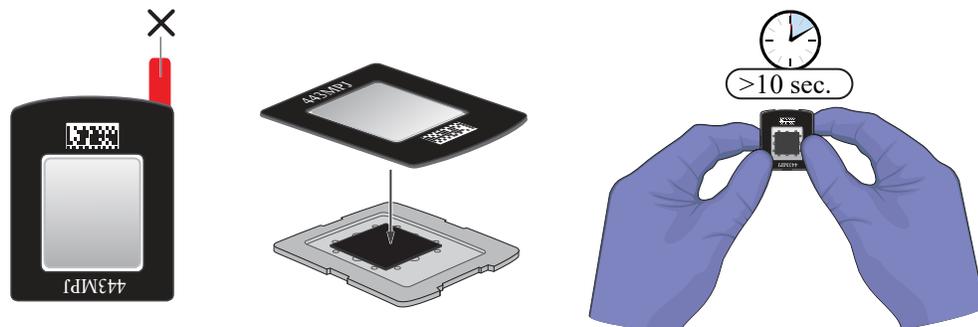
Note: A small amount of fluid running off the surface of the chip is acceptable.

5. Seal the chip:
- a. Remove the red protective film from the chip lid, then carefully place it onto the prepared chip so that the window in the lid aligns with the chip.

IMPORTANT! Before applying the chip lid, make certain that it is correctly oriented to the chip assembly.

- b. Pinch together all four sides and corners of the chip case for at least 10 seconds to seal it. While pressing, apply some lateral pressure to ensure a tight seal.

Note: You can remove the chip from the loading surface when you apply pressure to seal it.



Proceed to filling the chip case with Immersion Fluid.

Fill the chip case with Immersion Fluid

1. Hold the chip and lid assembly by its edges at a 45° angle so that air can escape from the fill port as you fill it.
2. Hold back the top half of the chip lid label to expose the fill port.

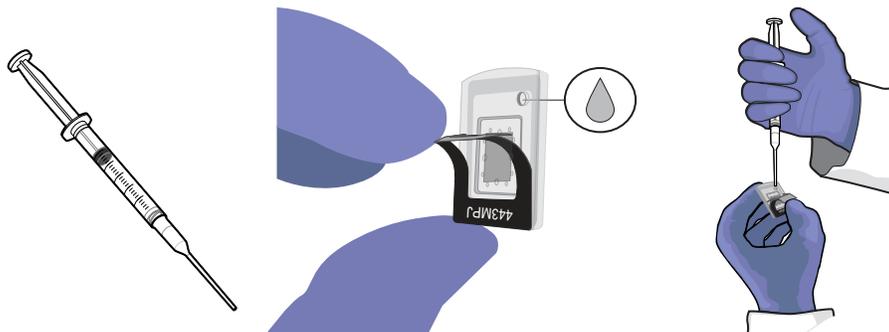


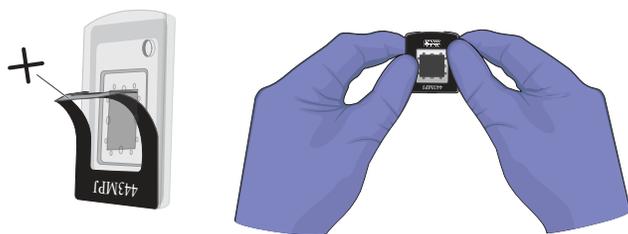
Figure 4 QuantStudio™ 3D Digital PCR Chip Lid v2

3. Carefully dispense Immersion Fluid into the port until the chip case contains an air bubble slightly larger than the fill port (<2–3 mm in diameter). Rotate the chip slightly to expose any hidden bubble. If a bubble larger than 2–3 mm is present, add additional fluid.
4. Using a low-lint wipe, remove any excess Immersion Fluid from the chip case to ensure optimal imaging.

Proceed to sealing the chip case.

Seal the chip case with the label

1. Gently pull back the top half of the label on the chip lid.
2. Remove the label backing and press the label firmly over the fill port for 5 seconds to ensure a tight seal.



IMPORTANT! Press only on the label region of the chip lid. Pressure placed on the window of the chip lid can expel the PCR reactions out of the chip wells.

3. Gently run your fingers over the entire label to seal the remainder of the label.

4. Inspect the sealed chip for potential problems:

- **Leaks** – Confirm that no fluid is leaking from the fill port or from the seal between the chip and lid.
- **Bubbles** – Check for large or multiple bubbles inside the chip case. One small air bubble is acceptable.
- **Correct lid orientation** – Confirm that the lid is correctly aligned on the chip.

If the sealed chip fails any of the criteria above, discard it and prepare another chip. The sealed chip cannot be opened and resealed.

5. Store the prepared chip in a clean, dry, dark location until you are ready to load it onto the thermal cycler.

Thermal cycle prepared chips within 2 hours after loading them.

Note: You can label the back of the chip with a permanent marker. This will not affect the imaging data.



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, see the “Documentation and Support” section in this document.

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.

Symbol	English	Français
	Caution, risk of danger Consult the manual for further safety information.	Attention, risque de danger Consulter le manuel pour d'autres renseignements de sécurité.
	Ultraviolet light	Rayonnement ultraviolet
	Protective conductor terminal (main ground)	Borne de conducteur de protection (mise à la terre principale)

(continued)

Symbol	English	Français
	<p>Do not dispose of this product in unsorted municipal waste</p> <p>⚠ 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.</p>	<p>Ne pas éliminer ce produit avec les déchets usuels non soumis au tri sélectif.</p> <p>⚠ MISE EN GARDE ! Pour minimiser les conséquences négatives sur l'environnement à la suite de l'élimination de déchets électroniques, ne pas éliminer ce déchet électronique avec les déchets usuels non soumis au tri sélectif. Se conformer aux ordonnances locales sur les déchets municipaux pour les dispositions d'élimination et communiquer avec le service à la clientèle pour des renseignements sur les options d'élimination responsable.</p>

Conformity symbols

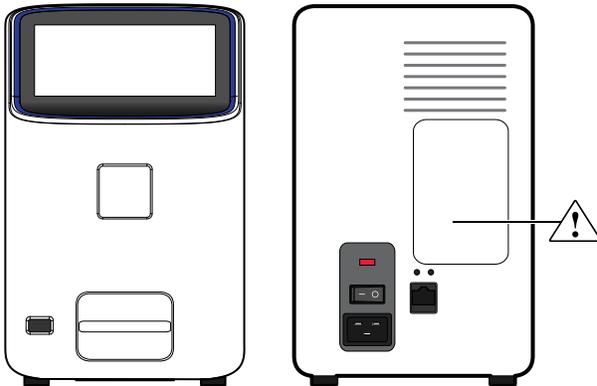
Conformity mark	Description
	Indicates conformity with safety requirements for Canada and U.S.A.
	Indicates conformity with European Union requirements for safety and electromagnetic compatibility.
	Indicates conformity with Australian standards for electromagnetic compatibility.

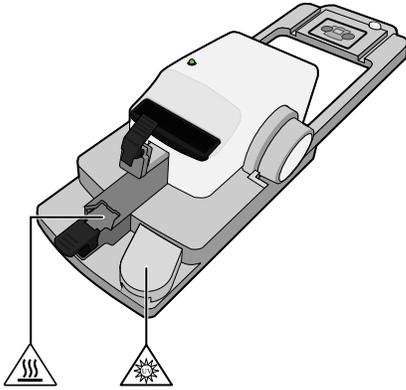
Safety alerts on this instrument

Additional text may be used with one of the symbols described above when more specific information is needed to avoid exposure to a hazard. See the following table for safety alerts found on the instrument.

English	French translation	Location on Instrument
<p> CAUTION! Hazardous chemicals. Read the Safety Data Sheets (SDSs) before handling.</p>	<p> CAUTION! Produits chimiques dangereux. Lire les fiches signalétiques (FS) avant de manipuler les produits.</p>	Rear instrument panel
<p> CAUTION! Hazardous waste. Refer to SDS(s) and local regulations for handling and disposal.</p>	<p> CAUTION! Déchets dangereux. Lire les fiches signalétiques (FS) et la réglementation locale associées à la manipulation et à l'élimination des déchets.</p>	

Location of safety alerts on the instrument





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.

Electrical safety



WARNING! Fuse Installation. Before installing the instrument, verify that the fuses are properly installed and the fuse voltage matches the supply voltage. Replace fuses only with the type and rating specified for the unit. Improper fuses can damage the instrument wiring system and cause a fire.



AVERTISSEMENT ! Installation des fusibles. Avant d'installer l'instrument, vérifier que les fusibles sont correctement insérés et que leur tension correspond à celle fournie par le circuit d'alimentation. Ne remplacer les fusibles que par des modèles du type et de la puissance spécifiés pour l'appareil. L'utilisation de fusibles inadaptés peut endommager le circuit électrique de l'instrument et provoquer un incendie.



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 specified in the manufacturer's user documentation. It is the responsibility of the operator (or other responsible person) to ensure the following requirements are met:

- No decontamination or cleaning agents are used that could cause a HAZARD as a result of a reaction with parts of the equipment or with material contained in the equipment.
- The instrument is properly decontaminated a) if hazardous material is spilled onto or into the equipment, and/or b) prior to having the instrument serviced at your facility or sending the instrument for repair, maintenance, trade-in, disposal, or termination of a loan (decontamination forms may be requested from customer service).
- Before using any cleaning or decontamination methods (except those recommended by the manufacturer), users should 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.

Safety and electromagnetic compatibility (EMC) standards

The instrument design and manufacture complies with the following standards and requirements for safety and electromagnetic compatibility.



Safety

Reference	Description
EU Directive 2006/95/EC	European Union “Low Voltage Directive”
CSA C22.2 No. 61010-1 IEC 61010-1 UL 61010-1 IEC/CSA/UL 61010-1	<i>Safety requirements for electrical equipment for measurement, control, and laboratory use – Part 1: General requirements</i>
IEC 61010-2-010 ^[1]	<i>Safety requirements for electrical equipment for measurement, control, and laboratory use – Part 2-010: Particular requirements for laboratory equipment for the heating of materials</i>

^[1] Applies only to the QuantStudio™ 3D Digital PCR Chip Loader.

EMC

Reference	Description
Directive 2004/108/EC	European Union “EMC Directive”
EN 61326-1	<i>Electrical Equipment for Measurement, Control and Laboratory Use – EMC Requirements – Part 1: General Requirements</i>
FCC Part 18 (47 CFR)	U.S. Standard “Industrial, Scientific, and Medical Equipment”
AS/NZS 2064	<i>Limits and Methods of Measurement of Electromagnetic Disturbance Characteristics of Industrial, Scientific, and Medical (ISM) Radiofrequency Equipment</i>
ICES-001, Issue 3	<i>Industrial, Scientific and Medical (ISM) Radio Frequency Generators</i>

Environmental design

Reference	Description
Directive 2002/96/EC	European Union “WEEE Directive” – Waste electrical and electronic equipment

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 adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's 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 necessary) 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.



WARNING! HAZARDOUS WASTE (from instruments). Waste produced by the instrument is potentially hazardous. Follow the guidelines noted in the preceding General Chemical Handling warning.

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.



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)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
<https://www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2009-P.pdf>
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

Documentation and support

Related documentation

The following related documents are available:

Document	Publication number
<i>QuantStudio™ 3D Digital PCR System Quick Reference</i>	MAN0008159
<i>QuantStudio™ 3D AnalysisSuite™ Software User Guide</i>	MAN0008161
<i>QuantStudio™ 3D AnalysisSuite™ Server System Quick Reference</i>	4486248
<i>QuantStudio™ 3D AnalysisSuite™ Software Help: Relative Quantification (server)</i>	MAN0009636
<i>QuantStudio™ 3D AnalysisSuite™ Software Help: Absolute Quantification (server)</i>	MAN0009635
<i>QuantStudio™ 3D AnalysisSuite™ Software Help: Base module (server)</i>	MAN0009634

Note: For additional documentation, see “Customer and technical support” on page 123.

Customer and technical support

Visit thermofisher.com/support for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
 - Product FAQs
 - Software, patches, and updates
 - Training for many applications and instruments
- Order and web support
- Product documentation
 - User guides, manuals, and protocols
 - Certificates of Analysis
 - Safety Data Sheets (SDSs; also known as MSDSs)

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at www.thermofisher.com/us/en/home/global/terms-and-conditions.html. If you have any questions, please contact Life Technologies at www.thermofisher.com/support.

