applied biosystems

QuantStudio™ 3D Digital PCR System

Catalog Number A29154 or A29157

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Note: For safety and biohazard guidelines, refer to the "Safety" appendix in the *QuantStudio™ 3D Digital PCR System User Guide* (Pub. no. MAN0007720). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

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.

Start

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Set up the dPCR reaction by mixing sample, master mix, and assay(s).

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Load the dPCR reaction onto a QuantStudio™ 3D Digital PCR Chip v2 or QuantStudio™ 3D Digital PCR Chip, apply the lid, load the assembly with Immersion Fluid, then seal the loading port.

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Perform the PCR using the ProFlex™ 2x Flat PCR System or Dual Flat Block GeneAmp™ PCR System 9700.

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Read the chip using the QuantStudio™ 3D Digital PCR Instrument.

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Review the results on the instrument touchscreen.

▼

Store or discard the chip.

▼

Analyze the data using the QuantStudio™ 3D AnalysisSuite™ Software.

▼

Finish

Prepare the dPCR reaction mix

Prepare the PCR reactions for loading on the QuantStudio™ 3D Digital PCR Chip v2 or QuantStudio™ 3D Digital PCR Chip. The example volumes below assume that you are running two chips per human gDNA sample at 10 ng/µL.

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 or QuantStudio™ 3D Digital PCR Master Mix (depending on your chip type)
   - TaqMan® Assay(s)
2. Dilute your DNA samples as necessary so that the concentration of target sequence in the final reaction is between 200 and 2,000 copies/µL.
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 reaction mix at room temperature. Scale the volumes appropriately, depending on the number of samples.

Material	Volume (µL)		
	Per chip	1 sample/ 2 chips [1]	10 samples/ 20 chips[1]
Master Mix (v2 or v1, depending on chip type)	7.25	17.4	174.0
TaqMan® Assay(s), 20X (primer/probe mix)	0.725	1.74	17.4
Water	1.525	3.66	36.6
Total volume	9.5	22.8	228.0

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

5. Using a permanent marker, label a 0.5- or 1.5-mL reaction tube for each sample.
6. Vortex then briefly centrifuge the DNA sample(s).
7. Transfer 22.8 µL of PCR reaction mix to each labeled reaction tube.

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8. Transfer 12 µL of each DNA sample, diluted as appropriate, to the corresponding reaction tube. Mix well by gently pipetting up and down after each transfer (or gently vortex on low-medium speed).

9. Cap the reaction tubes, then briefly centrifuge them and 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 prior to loading.

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

For instructions on loading chips manually, see the *QuantStudio™ 3D Digital PCR System User Guide* (Pub. no. MAN0007720).

Prepare the chip loading workspace

WARNING! ULTRAVIOLET LIGHT HAZARD. Looking directly at a UV light source can cause serious eye damage. Never look directly at a UV light source and always prevent others from UV exposure. Wear appropriate protective eyewear and clothing.

1. Plug in and power on the chip loader, then wait until the status light illuminates solid green, indicating that the chip nest has reached operating temperature (≤20 minutes depending on room temperature).

2. Remove the following consumables from their packaging and place them on a clean, dry, lint-free surface:
   - Chip lid (appropriate for your chip type)
   - QuantStudio™ 3D Digital PCR Sample Loading Blade

Prepare the Chip Sealant (v1 chip lids only)

Note: The QuantStudio™ 3D Digital PCR Chip Lid v2 does not require the use of sealant. If you are using this lid, skip these steps.

1. Remove the Chip Sealant syringe, plunger, and tip from the protective packaging.

2. Remove the protective caps from both ends of the syringe, twist and push the tip to lock it into place, then insert the plunger into the opposite end of the syringe.

3. Place the assembled syringe within its protective package and store it in a dark location until ready for use, and then proceed to the next steps.

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. Unscrew the cap from the syringe, then attach the syringe tip by pushing it into place.

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.

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 Chip v2 or QuantStudio™ 3D Digital PCR Chip. 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.

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

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.

6. Briefly vortex and centrifuge the prepared dPCR reaction (from "Prepare the dPCR reaction mix" on page 1), 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.

7. Press the black loading button to load the chip.

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

8. 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.

9. 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).

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

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. QuantStudio™ 3D Digital PCR Chip Lid v2 only: Hold back the top half of the chip lid label to expose the fill port.
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.

Seal the chip case with the label (v2 chip lids only)

Note: The following procedure only applies to the QuantStudio™ 3D Digital PCR Chip Lid v2. If you are not using the v2 chip lid, skip this section.

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.
3. Gently run your fingers over the entire label to seal the remainder of the label.
4. Inspect the sealed chip for leaks, bubbles, and correct lid orientation.
5. Store the prepared chip in a clean, dry, dark location until you are ready to load it onto the thermal cycler.

Seal the chip case with Chip Sealant (v1 chip lids only)

Note: The QuantStudio™ 3D Digital PCR Chip Lid v2 does not require the use of sealant. If you are using this chip lid, skip these steps.

The following steps use the Chip Sealant syringe.

1. Hold the Chip Sealant syringe tip just above (or in slight contact with the inside wall) of the fill port of the sealed chip case. Carefully fill the port with sealant, ensuring that the fluid touches the walls of the port. To complete the seal, create a dome of sealant over the top of the port.
2. Insert the chip assembly fill port-first into the UV-Curing Station on the Chip Loader. Push the chip into the station until the ultraviolet light illuminates.
3. When the light shuts off (approximately ≥15 seconds), remove the chip and place it on a clean, dry, lint-free surface.
4. Visually inspect the sealed chip for leaks, bubbles, and correct lid orientation.
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.

Thermal cycle the chips

Perform the PCR

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).
3. 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.

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4. Lay the QuantStudio™ Thermal Pads over the chips.
5. Close and engage the heated cover of the thermal cycler.
6. 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.

Table 1 ProFlex™ 2x Flat PCR System PCR Method

PCR Protocol					Cover Temp.	Rxn. Vol.
Stage 1	Stage 2		Stage 3			
96.0°C	60.0°C	98.0°C	60.0°C	10.0°C[1]	70.0°C	1 nL
0:10:00	0:02:00	0:00:30	0:02:00	∞		
1x	39x		1x			

[1] Optional step.

Table 2 GeneAmp™ PCR System 9700 PCR Method

PCR Protocol					Run Speed	Rxn. Vol.
Stage 1	Stage 2		Stage 3			
96.0°C	60.0°C	98.0°C	60.0°C	10.0°C[1]	Std.	20 µL
10:00	2:00	0:30	2:00	99:59		
1x	39x		1x			

[1] Optional step.

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 ≤25°C. Alternatively, the chips can remain on the block for up to 24 hours at 10°C.

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.
   b. Allow the thermal cycler to sit for at least 10 minutes with the heated covered 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.
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.
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.

Chip imaging

Specify the chip well volume

The QuantStudio™ 3D Digital PCR Chip v2 and QuantStudio™ 3D Digital PCR Chip have different well volumes (755 pL and 809 pL respectively), so you need to specify the appropriate chip type/well volume before imaging.

1. From the Main Menu of the touchscreen, touch to open the Settings menu, then touch **Instrument Settings** followed by **Well Volume**.
2. In the Well Volume screen, select the chip type you are imaging or touch **User-defined** to enter a custom volume.
3. Click on **OK** to save your settings, then to return to the Settings menu.

Image the chips

IMPORTANT! Before imaging, confirm that the latest firmware is installed on the QuantStudio™ 3D Digital PCR Instrument. Imaging and analysis of the QuantStudio™ 3D Digital PCR Chip v2 requires firmware version 3.0 or later.

Note:

1. If you selected **USB** in the Data Destinations screen (as described above), insert a USB key into the USB port on the front of the instrument.
2. 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.
3. 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.
4. *(Optional)* During the run, touch the Add Prefix field to enter a prefix for each experiment (.eds) file name.
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.

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

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