Quick start guide: QuantStudio Absolute Q DNA Digital PCR Master Mix

This quick start guide provides concise instructions for preparing a digital PCR (dPCR) reaction for the Applied Biosystems™ QuantStudio™ Absolute Q™ Digital PCR System. For detailed instructions on preparing and performing a dPCR experiment, refer to the QuantStudio™ Absolute Q™ Digital PCR Installation, Use, and Maintenance Guide (Pub. No. MAN0025621).

Getting started

- QuantStudio Absolute Q MAP16 Digital PCR Plate
- 5 gaskets (per plate)
- Absolute Q DNA Digital PCR Master Mix (5X)
- QuantStudio Absolute Q Isolation Buffer
- Nuclease-free water
- Low-retention pipette tips
- Tabletop centrifuge
- Digital PCR assay

Reagent	Final concentration	Volume per reaction	Volume per reaction with 10% overage*
Water	–	Fill to 9 µL	Fill to 10 µL
Absolute Q DNA Digital PCR Master Mix (5X)	1X	1.8 µL	2 µL
Digital PCR assay (40X or 20X)	1X	0.23 µL (40X) or 0.45 µL (20X)	0.25 µL (40X) or 0.50 µL (20X)
DNA sample	1–11,000 copies/µL**	Variable	Variable
Total	–	9 µL	10 µL

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

** DNA copy number and dilution calculator can be found at thermofisher.com/dna-calculator

Prepare digital PCR reactions

1 Vortex the master mix well and combine the reagents shown in the table above in the order listed.

2 Mix dPCR reagents well.

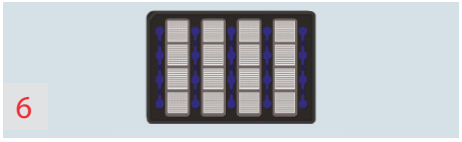
3 Centrifuge at 10,000 x g for 1 minute (rotor centrifuge).

Load the QuantStudio Absolute Q MAP16 plate

Handle the MAP plate by its frame and work on a level, dust-free surface. Proceed to step 4, using a new pipette tip for each well.

At a 45° angle, load 9 µL of the dPCR mixture to the bottom of the well. Pipette dPCR mixture only to the first stop.

At a 45° angle, load 15 µL of Absolute Q Isolation Buffer. Pipette only to the first stop.



Apply 5 gaskets to the MAP plate. Orient the gaskets such that the "A" tail is on top of the plate.

Setting up the run

7 Select the columns of the MAP plate that have been loaded.

8 Define PCR thermal parameters. Set preheat step to 96°C for 10 minutes. No post-cycling steps required or recommended.

9 Select channel dye from the available options.

10 Select "Start" to begin dPCR run

Find out more at thermofisher.com/absoluteq

ThermoFisher SCIENTIFIC

COL25388 0821