November 2024

Quick-Start Protocol

QIAcuity® High Multiplex Probe PCR Kit

This protocol is optimized for the quantification of DNA targets using the QIAcuity High Multiplex Probe PCR Kit with hydrolysis probes in a multiplex reaction using QIAGEN's QIAcuity instruments for digital PCR (dPCR).

The QIAcuity High Multiplex Probe PCR Kit should be stored immediately upon receipt at –30°C to –15°C in a constant-temperature freezer and protected from light. Under these conditions, the components are stable for 12 months without showing any reduction in performance and quality, unless otherwise indicated on the label.

Further information

- *QIAcuity User Manual*: **www.qiagen.com/HB-2717**
- *QIAcuity User Manual Extension*: **www.qiagen.com/HB-2839**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- The QIAcuity High Multiplex Probe PCR Kit requires the QIAcuity Software Suite version 3.0 or newer.

- Refer to the *QIAcuity User Manual* and *QIAcuity User Manual Extension* for guidance on assay design and experimental setup for the QIAcuity platform.
- The QuantiNova® (QN) Internal Control DNA dPCR supplied with the kit can be used optionally as an amplification control. To do so, users must separately purchase the QuantiNova IC Probe Assay (200) (cat. no. 205813), which can be detected in the yellow channel on QIAcuity instruments.
- **Important**: DO NOT purchase the QuantiNova IC Probe Assay Red 650 (500) (cat. no. 205824), as it is not optimized to work with QIAcuity.
- QN Internal Control DNA dPCR is supplied at a concentration of ~2.5 x 10^5 copies/µL. Each lot of DNA has been prequantified with QIAcuity dPCR. The exact concentration is printed on the tube label.

Template DNA digestion

- DNA samples with ≥20 kb average length (e.g., gDNA purified via spin column with silica membrane, or salting out method) should be fragmented by restriction digestion before partitioning. Enzymatic fragmentation of larger DNA ensures even distribution of template throughout the QIAcuity Nanoplate for accurate and precise quantification.
- Restriction digestion is not required for highly fragmented DNA (e.g., FFPE DNA or circulating DNA) or cDNA.
- Care should be taken to use enzymes that will not cut within the amplified sequence. Visit **geneglobe.qiagen.com** for information on QIAGEN's dPCR CNV Probe, dPCR Microbial DNA Detection, and dPCR LNA Mutation assays.
- The following validated enzymes will digest DNA in 10 min at room temperature (15–25°C) when added directly to the QIAcuity reaction mix at the indicated concentrations:

Table 1. Validated restriction enzymes

6-cutter restriction enzymes		4-cutter restriction enzymes	
*Eco*RI	0.25 U/µL EcoRI-HF®, NEB® 0.025 U/µL Anza™ 11 EcoRI, Thermo Fisher Scientific (TFS)	*Alu*I	0.025 U/µL AluI, NEB 0.025 U/µL Anza 44 AluI, TFS
*Pvu*II	0.025 U/µL PvuII, NEB 0.025 U/µL Anza 52 PvuII, TFS	*CviQ*I	0.025 U/µL CviQI, NEB 0.025 U/µL Csp6I (CviQI), TFS
*Xba*I	0.025 U/µL Anza 12 XbaI, TFS	*Hae*III	0.025 U/µL BsuRI (HaeIII), TFS

Procedure

Reaction mix setup

1. Thaw the 4x QIAcuity High Multiplex Probe PCR Master Mix, template, primers, probes, and RNase-Free Water. Vigorously mix the QIAcuity High Multiplex Probe PCR Master Mix and the individual solutions. Centrifuge the tubes briefly to settle the liquids.
2. Prepare a master mix according to Table 2 or Table 3 for the desired Nanoplate format.
3. Vortex thoroughly the reaction mix. Dispense appropriate volumes of the reaction mix into the wells of a standard 96-well PCR pre-plate.

   Note: The pre-plate may be assembled at room temperature.
4. Add template DNA to wells containing the reaction mix. Thoroughly mix the template DNA with the reaction mix by pipetting up and down.

Table 2. Preparing reaction mix for detecting up to 8 targets per reaction (one target per channel in up to 8 channels)

Component	Volume per reaction		Final concentration
	Nanoplate 8.5k (24-well and 96-well)	Nanoplate 26k (8-well and 24-well)	
4x QIAcuity High Multiplex Probe PCR Master Mix	3 µL	10 µL	1x
20x Primer–probe mix 1–8*	0.6 µL (each)	2 µL	variable†
Restriction Enzyme (optional)	up to 1 µL	up to 1 µL	0.025–0.25 U/µL
RNase-Free Water	variable	variable	–
Template DNA (added at step 4)‡	variable	variable	–
Total reaction volume	**12 µL**	**40 µL**	–

* For dye recommendations, see the *QIAcuity User Manual* or the *QIAcuity User Manual Extension*.

† For multiplex reactions detecting a single target in any of the six standard QIAcuity channels (Green, Yellow, Orange, Red, Crimson, and Far Red), final assay concentrations of 0.8 µM forward primer, 0.8 µM reverse primer, and 0.4 µM probe are recommended. When using long Stokes-shift dyes, variable assay concentrations are required. Refer to the *QIAcuity High Multiplex Probe PCR Kit Handbook* for more details.

‡ Appropriate template amount depends on various parameters.

Table 3. Preparing reaction mix for detecting up to 12 targets per reaction with amplitude-based multiplexing

Component	Volume per reaction		Final concentration
	Nanoplate 8.5k (24-well and 96-well)	Nanoplate 26k (8-well and 24-well)	
4x QIAcuity High Multiplex Probe PCR Master Mix	3 µL	10 µL	1x
20x Primer–probe mix 1–12* (for multiplex)	variable	variable	variable†
Restriction Enzyme (optional)	up to 1 µL	up to 1 µL	0.025–0.25 U/µL
RNase-Free Water	variable	variable	–
Template DNA (added at step 4)‡	variable	variable	–
Total reaction volume	**12 µL**	**40 µL**	–

* For dye recommendations, see the *QIAcuity User Manual* or the *QIAcuity User Manual Extension*.

† For multiplex reactions that employ amplitude-based multiplexing, variable assay concentrations are required. Refer to the *QIAcuity High Multiplex Probe PCR Kit Handbook* for more details.

‡ Appropriate template amount depends on various parameters.

dPCR protocol for all QIAcuity instruments

1. Transfer the contents of each well in the pre-plate to the wells of a Nanoplate.
2. Seal the Nanoplate properly using the QIAcuity Nanoplate Seal provided in the QIAcuity Nanoplate Kits.
3. Place the Nanoplate into the QIAcuity instrument and start the dPCR program.
4. Image the plate using default imaging settings.

Table 4. QIAcuity dPCR cycling program

Step	Time	Temperature
Initial denaturation	2 min	95°C
2-step cycling (40 cycles)	–	–
Denaturation	15 s	95°C
Combined annealing/extension	30 s	60°C*

* Temperature during annealing/extension and number of cycles might vary depending on assay type.

Document Revision History

Date	Description
11/2024	Initial release