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Quick-Start Protocol

QIAcuity® Probe PCR Kit

This protocol is optimized for the quantification of DNA or cDNA targets using the QIAcuity Probe PCR Kit (cat. nos. 250101, 250102, and 250103) with using TaqMan® probes in a singleplex or multiplex (up to 5 targets) reaction using QIAGEN's QIAcuity instruments for digital PCR (dPCR).

The QIAcuity Probe PCR Kit should be stored immediately upon receipt at –30 to –15°C in a constant-temperature freezer and protected from light. The QIAcuity Probe PCR master mix can also be stored protected from light at 2–8°C. Unless otherwise indicated on the label, the components are stable for 12 months without showing any reduction in performance under these conditions.

Dedicated protocols for the various types of QIAGEN's QIAcuity dPCR assays can be found in their respective quick-start protocols and the *QIAcuity User Manual Extension: Application Guide*.

Further information

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

Notes before starting

- A fluorescent reference dye is provided as a component of the QIAcuity Probe PCR Master Mix, for reliable detection of proper partition filling in the QIAcuity Nanoplates.
- For the highest efficiency in dPCR using TaqMan probes, amplicons should ideally be 60–150 bp in length. Similar to qPCR, longer amplicons can be used as well; however, assay performance might be impaired.

- Before performing multiplex analyses, choose suitable combinations of reporter dyes and quenchers that are compatible with multiplex analyses using the detection optics of the QIAcuity instruments. Only nonfluorescent quenchers should be used.
- Always start with the cycling conditions and primer concentrations specified in this protocol.
- The PCR must start with an initial incubation step of 2 min at 95°C to activate the QuantiNova® DNA Polymerase in the master mix.
- For ease of use, we recommend preparing a 10x or higher concentrated primer–probe mix containing target-specific primers and probe for each of your targets. A 10x primer–probe mix consists of 8 µM forward primer, 8 µM reverse primer, and 4 µM probe in TE buffer with low EDTA (0.1 mM).

Template DNA digestion

- DNA samples with ≥20 kb average length (e.g., genomic DNA 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, which in turn leads to accurate and precise quantification.
- Fragmentation of DNA via restriction digest is particularly important when copy number variation (CNV) analyses are performed, where multiple copies of a gene might be linked in tandem. 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. For QIAGEN's CNV and mutation detection assays, appropriate information can be found at **geneglobe.qiagen.com**.
- 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	*Cvi*QI	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 setup

1. Thaw the QIAcuity Probe PCR master mix, template DNA or cDNA, primers, probes, and RNase-free water. Vigorously mix the QIAcuity Probe PCR master mix and the individual solutions. Centrifuge briefly to collect liquids at the bottom of the tubes.
2. Prepare a reaction mix for the number of reactions needed according to Table 2. Due to the hot-start, it is not necessary to keep samples on ice during reaction setup or while programming the QIAcuity instrument.

Table 2. Reaction setup

	Volume/reaction		
Component	**Nanoplate 8.5k (24-well, 96-well)**	**Nanoplate 26k (24-well)**	**Final concentration**
4x Probe PCR Master Mix	3 µl	10 µl	1x
10x primer–probe mix 1*	1.2 µl†	4 µl†	0.8 µM forward primer 0.8 µM reverse primer 0.4 µM probe
10x primer–probe mix 2, 3, 4, 5* (for multiplex)	1.2 µl†	4 µl†	0.8 µM forward primer 0.8 µM reverse primer 0.4 µM probe
Restriction Enzyme (optional)	Up to 1 µl	Up to 1 µl	0.025–0.25 U/µl
RNase-free water	Variable	Variable	
Template DNA or cDNA (added at step 4)	Variable‡	Variable‡	
Total reaction volume	**12 µl**	**40 µl**	

* For respective dye recommendation for the probe and available channels on the QIAcuity, please see the *QIAcuity User Manual* or the *QIAcuity User Manual Extension: Application Guide*.

† Volume might vary, depending on concentration of the primer/probe mix used.

‡ Appropriate template amount depends on various parameters. Please see the *QIAcuity User Manual Extension: Application Guide* for details.

3. Vortex the reaction mix.
4. Dispense appropriate volumes of the reaction mix, which contains all components except the template, into the wells of a standard PCR plate. Then, add template DNA or cDNA into each well that contains the reaction mix.

   Note: The appropriate amount of reaction mix and template DNA depends on various parameters. Please refer to the *QIAcuity User Manual Extension: Application Guide* for details.

Note: For 2-step RT-PCR, the volume of the cDNA added (from the undiluted reverse-transcription reaction) should not exceed 15% of the final PCR volume.

5. Transfer the content of each well from the standard PCR plate to the wells of the nanoplate.
6. Seal the nanoplate properly using the QIAcuity Nanoplate Seal provided in the QIAcuity Nanoplate Kits.

   Note: For exact sealing procedure, please see the *QIAcuity System User Manual*.
7. If a restriction enzyme for DNA digestion has been included in the reaction, leave the plate for 10 min at room temperature.

Thermal cycling conditions

1. Program the cycler of the QIAcuity instrument according to Table 3.

Table 3. Cycling conditions

Step	Time	Temperature (°C)
PCR initial heat activation	2 min	95
2-step cycling (40 cycles)		
Denaturation	15 s	95
Combined annealing/extension	30 s	60*

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

2. Place the nanoplate into the QIAcuity instrument and start the dPCR program.

Document Revision History

Date	Changes
07/2020	Initial release
01/2021	Updated the URL for *QIAcuity User Manual Extension: Application Guide*.

Scan QR code for the *QIAcuity User Manual Extension: Application Guide*.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual.