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Quick-Start Protocol

QIAcuity® EG PCR Kit

This protocol is optimized for the quantification of DNA or cDNA targets using the QIAcuity EG PCR Kit (cat. nos. 250111, 250112, and 250113) with the dsDNA-binding dye EvaGreen® in a singleplex reaction using QIAGEN's QIAcuity instruments for digital PCR (dPCR).

The QIAcuity EG PCR Kit should be stored immediately upon receipt at –30 to –15°C in a constant-temperature freezer and protected from light. The QIAcuity EG 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 6 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 EG PCR master mix for reliable detection of proper partition filling in the dPCR Nanoplates.
- For the highest efficiency in dPCR, 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.

- 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 mix. A 10x primer mix consists of 4 µM forward primer and 4 µM reverse primer in TE buffer with low EDTA (0.1 mM).
- For 2-step RT-PCR, use the QuantiTect® Reverse Transcription Kit for the first step to synthesize the cDNA.

  Important: The QuantiNova Reverse Transcription Kit is not recommended.

  The volume of the cDNA added (from the undiluted reverse-transcription reaction) should not exceed 10% of the final PCR volume if using the QuantiTect Reverse Transcription Kit. If using another RT kit, it should not exceed 5%.

Template DNA digestion

- DNA samples with an average length ≥20 kb (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	*Alu*I	0.025 U/µl AluI, NEB 0.025 U/µl Anza 44 AluI, Thermo Fisher Scientific
*Pvu*II	0.025 U/µl PvuII, NEB 0.025 U/µl Anza 52 PvuII, Thermo Fisher Scientific	*Cvi*QI	0.025 U/µl CviQI, NEB 0.025 U/µl Csp6I (CviQI), Thermo Fisher Scientific
*Xba*I	0.025 U/µl Anza 12 XbaI, Thermo Fisher Scientific	*Hae*III	0.025 U/µl BsuRI (HaeIII), Thermo Fisher Scientific

Procedure

Reaction setup

1. Thaw the QIAcuity EG PCR master mix, template DNA or cDNA, primers, and RNase-free water. Mix the individual solutions.
2. Prepare a reaction mix according to Table 2. Due to the hot-start polymerase, 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**
3x EvaGreen PCR Master Mix (FAM channel)	4 µl	13.3 µl	1x
10x primer mix	1.2 µl*	4 µl*	0.4 µM forward primer 0.4 µM reverse primer
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**	

* Volume might vary, depending on concentration of the primer 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.

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 at room temperature for 10 min.

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
3-step cycling (40 cycles)		
Denaturation	15 s	95
Annealing	15 s	55–62*
Extension	15 s	72
Cooling down	5 min	40

* 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 of *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.