Quick-Start Protocol September 2024

dPCR Microbial DNA Detection Assays and Custom dPCR Microbial Assays

This protocol is optimized for the quantification of microbial DNA targets using dPCR Microbial DNA Detection Assays (cat. no. 250207) or Custom dPCR Microbial Assays (cat. no. 250208) together with the QIAcuity® Probe PCR Kit (cat. nos. 250101, 250102, 250103), the QIAcuity UCP Probe PCR Kit (cat. nos. 250121, 250122) or, when analysing RNA targets in a 1-step RT-dPCR, the QIAcuity OneStep Advanced Probe Kit (cat. nos. 250131, 250132). Assays can be used in single-plex or multiplex reactions with 26K and 8.5K nanoplates (cat. nos. 250001, 250011, 250021) using QIAGEN's QIAcuity instruments for digital PCR (dPCR).

dPCR Microbial DNA Detection Assays and Custom dPCR Microbial Assays consist of 2 primers and one hydrolysis probe that are lyophilized in a single tube. The lyophilized primer-probe mix is shipped at ambient temperature and should upon receipt be stored protected from light at –30 to –15°C in a constant-temperature freezer. Under these conditions, the Assay is stable until the expiry date listed on the vial.

Further information

- *Microbial DNA dPCR Handbook*: **www.qiagen.com/HB-3059**
- *QIAcuity 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**
- Custom dPCR Microbial Assays online design tool: **www.geneglobe.qiagen.com/customize/dpcr/custom-dpcr-microbial-assay**

Notes before starting

- The assays come as a primer-probe mix lyophilized in a single tube and have to be resuspended prior usage. For short term storage, the dissolved dPCR Assay mix can be stored at 2–8°C. Repeated freeze-thaw cycles should be avoided. If possible, store the assay in aliquots.
- The dPCR Microbial DNA Detection Assays contain oligo material to conduct 200 reactions on a 26K nanoplate.
- The Custom dPCR Microbial Assays come in 2 sizes containing oligo material for 150 or 400 reactions on the 26K nanoplate.
- Set up all reaction mixtures in an area separate from that used for DNA isolation and PCR product analysis (post-PCR).
- To compensate for any potential environmental contamination, it is required to run at least one No Template Control (NTC) sample for the identification assays.
- Use disposable tips containing hydrophobic filters to minimize cross-contamination.
- Start with the cycling conditions specified in the protocol. The cycling conditions have been optimized for this type of assay.
- Dilutions of DNA templates in QuantiTect® Nucleic Acid Dilution Buffer can be stored at 4°C for at least 1 week.

Procedure

Assay resuspension

For ease of use, we recommend preparing a 20x concentrated primer-probe mix. To achieve a 20x resuspension, add the amounts specified in Table 1 to the tube, using sterile, nuclease-free 10 mM TE buffer (pH 8.0) with low EDTA (0.1 mM). Mix thoroughly and let the solution stand for 20 minutes at ambient temperature to ensure the primer-probe mix fully dissolves. We do not recommend resuspension in water.

Table 1. Assay resuspension to achieve a 20x primer-probe mix

Assay Product (cat. no.)	Amount of 10 mM TE buffer (pH 8.0) with low EDTA (0.1 mM) to be added (µL)
dPCR Microbial DNA Detection Assay 200 rxns (250207)	400
Custom dPCR Microbial Assay 150 rxns (250208)	330
Custom dPCR Microbial Assay 400 rxns (250208)	880

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. This ensures even distribution of templates throughout the partitions of the QIAcuity Nanoplate well, which in turn leads to accurate and precise quantification. For individual samples, like fragmented DNA, this step might not be required. The recommended restriction enzyme for each assay is listed on respective product pages in **GeneGlobe.qiagen.com** or refer to the product data sheet that is provided with each assay.

Reaction Setup

1. Thaw the selected master mix, template DNA, microbial assay(s), and RNase/DNase-free water. Vigorously mix the 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 Tables 2 or 3 and according to the master mix selected. Due to the hot-start capability, it is not necessary to keep samples on ice during reaction setup or while programming the QIAcuity instrument.

Table 2. Reaction setup for QIAcuity Probe PCR MM and QIAcuity UCP Probe MM

Component	Volume/reaction		
	Nanoplate 8.5k (96-well)	Nanoplate 26k (24-well)	Final concentration
4x QIAcuity Probe Mastermix or 4x QIAcuity UCP Probe Mastermix	3 µL	10 µL	1x
20x primer-probe mix 1	0.6 µL	2 µL	1x
Each additional primer-probe mix	0.6 µL	2 µL	1x
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**	

* Appropriate template amount depends on various parameters. Please see the *QIAcuity Application Guide* for details.

Table 3. Reaction setup for QIAcuity OneStep Advanced Probe MM

	Volume/reaction		
Component	**Nanoplate 8.5k (96-well)**	**Nanoplate 26k (24-well)**	**Final concentration**
4x OneStep Advanced Probe MM	3 µL	10 µL	1x
100x OneStep Advanced RT Mix (Reverse Transcription)	0.12 µL	0.4 µL	1x
20x primer-probe mix 1	0.6 µL	2 µL	1x
Each additional primer-probe mix	0.6 µL	2 µL	1x
Enhancer GC* (optional)	1.5 µL	5 µL	
RNAse-free water	Variable	Variable	
Template DNA (added at step 4)	Variable†	Variable†	
Total reaction volume	**12 µL**	**40 µL**	

* Enhancer GC is recommended for use with all Applied Biosystems TaqMan assays, amplicons >150 bp in length, GC-rich amplicons, and RNA targets containing challenging secondary structures.

† Appropriate template amount depends on various parameters. Please see the *QIAcuity Application Guide* for details.

Note: Prepare a volume of master mix 10% greater than that required for the total number of PCR reactions to be performed. This should include positive and negative control reactions.

3. Mix the reaction mix thoroughly.
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 (with Probe and UCP Probe MM) or RNA (with OneStep Advanced MM) into each well that contains the reaction mix and mix thoroughly.
5. Transfer the content of each well from the standard PCR plate into the wells of the nanoplate.
6. Seal the nanoplate properly using the QIAcuity Nanoplate Seal provided in the QIAcuity Nanoplate Kits.
7. If a restriction enzyme for DNA digestion has been included in the reaction, leave the plate for 10 min at ambient temperature.

Thermal Cycling

1. Program the cycler of the QIAcuity instrument according to Tables 4 or 5.
2. Place the nanoplate into the QIAcuity instrument and start the dPCR program.

Table 4. Thermal cycling conditions for QIAcuity Probe MM and QIAcuity UCP Probe MM

Step	Time	Temperature (°C)
PCR initial heat activation	2 min	95
2-step cycling (35 cycles*)		
Denaturation	15 s	95
Combined annealing/extension	60 s	58

* Number of cycles might vary depending on sample type. Additional 5 cycles might increase signal to noise separation.

Table 5. Thermal cycling conditions for QIAcuity OneStep Advanced Probe MM

Step	Time	Temperature (°C)
Reverse Transcription	40 min	50
RT Enzyme Inactivation	2 min	95
2-step cycling (35 cycles*)	-	-
Denaturation	5 s	95
Combined annealing/extension	60 s	58

* Number of cycles might vary depending on sample type. Additional 5 cycles might increase signal to noise separation.

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Document Revision History

Date	Changes
09/24	Initial release.

Scan QR code for *QIAcuity Application Guide*. For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual.