Quick-Start Protocol

February 2025

dPCR CGT Assays

This protocol is optimized for the detection and quantification of cell and gene therapy (CGT) targets using the dPCR CGT Assay Kits (cat. no. 250230–250256) with the QIAcuity® Probe PCR Kit (cat. nos. 250101, 250102, 250103) in singleplex or multiplex reactions using the QIAcuity digital PCR (dPCR) instrument.

For detection and quantification of CGT targets using the dPCR CGT Assay Kits (cat. nos. 250300–250321), the QIAcuity MasterMix (cat. no. 1133251) is recommended.

The dPCR CGT Assays are provided in a ready-to-use 20x primer–probe mix, available in up to 3 fluorophore choices (FAM, HEX, and Cy5). These assays enable singleplexed as well as multiplexed CGT applications, including viral titer and vector copy number measurements.

The dPCR CGT Assays are shipped at ambient temperature and should upon receipt be stored protected from light at –30°C to –15°C in a constant-temperature freezer for long-term storage (e.g., 12 months). Under these conditions, the dPCR CGT Assay Kits are stable, without showing any reduction in performance and quality. After reconstitution, the assays are stable for at least 12 months. It is recommended to store the dPCR CGT assays in aliquots at –30°C to –15°C to avoid repeated freeze–thaw cycles.

Further information

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

Notes before starting

- Always start with the cycling conditions and primer concentrations specified in this protocol.
- A fluorescent reference dye is provided as a component of all QIAcuity MasterMixes for reliable detection of proper partition filling in the dPCR plates.
- Pipetting accuracy and precision affect the consistency of results. Make sure that no air bubbles are introduced into the wells of the dPCR plate during pipetting.

Procedure

Things to do before starting

- Resuspend the dPCR CGT Assays: Spin down the tube before opening it for the first time. Add 330 µL TE buffer to the tube to obtain a 20x stock and leave at room temperature (15–25°C) for 20 min. Vortex and spin down briefly.
- Thaw the QIAcuity Probe PCR Master Mix or QIAcuity MasterMix, dPCR CGT Assay, template DNA (e.g., adeno-associated virus [AAV] genome), and RNase-free water. Mix the individual solutions.

Reaction setup

1. Prepare a reaction mix according to Table 1. Due to the hot start, it is not necessary to keep samples on ice during reaction setup or while programming the QIAcuity.

   Note: It is recommended to prepare a 10% surplus to safely transfer the needed volume to the nanoplates.

2. Dispense appropriate volumes of the reaction mix, which contains all components except the template, into the wells of a standard PCR plate. Add template DNA into each well that contains the reaction mix.

   Note: The appropriate amounts of reaction mix and template DNA depend on various parameters. Refer to the *QIAcuity Application Guide* for details.

3. Transfer the contents of each well of the standard PCR plate to the wells of an 8.5k or 26k nanoplate.
4. Seal the nanoplate properly using the QIAcuity Nanoplate Seal provided in the QIAcuity Nanoplate kits. For sealing instructions, see the *QIAcuity User Manual*.

Table 1. Reaction setup

Component	Volume per reaction: Nanoplate 26k (24-well)	Volume per reaction: Nanoplate 8.5k (24-well and 96-well)	Final concentration
QIAcuity Probe PCR Master Mix or QIAcuity MasterMix	10 µL	3 µL	1x
20x dPCR CGT Assay-1	2 µL	0.6 µL	1x
Additional CGT Assays (2, 3, 4, 5) for up to 5-plex reaction (optional*)	2 µL	0.6 µL	1x
Restriction Enzyme (optional‡)	1–10 units	0.3–3 units	0.025–0.25 U/µL
RNase-free water	variable	variable	–
Template DNA§	variable	variable	–
Total reaction volume	**40 µL**	**12 µL**	–

* Add additional 20x dPCR CGT Assays or gene of interest assays for a multiplex reaction to detect multiple CGT targets at once (e.g., for vector copy number [VCN] quantification, a duplex with gene of interest and reference gene is recommended).

Important: Dye combinations must be different from those used in the 20x dPCR CGT Assay-1. For dye recommendations and the corresponding probe and channels available on the QIAcuity, see the *QIAcuity User Manual* or the *QIAcuity Application Guide*.

‡ For VCN analysis of gDNA, the recommended restriction enzyme is *Eco*RI. For AAV-derived DNA, the recommended restriction enzyme is *Hpa*II.

§ If target DNA is gDNA for VCN quantification, amounts ideally should lie within 30–50 ng/reaction.

Thermal cycling and imaging conditions

5. Set the cycling conditions under the dPCR parameters in the QIAcuity Software Suite or at the QIAcuity instrument according to Tables 2 and 3.

Table 2. Cycling conditions

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

Table 3. Imaging settings*

Channel	Exposure (ms)	Gain
Green (FAM)	500	6
Yellow (HEX)	500	6
Crimson (Cy5)	400	8
Orange	400	6
Red	300	4

* Imaging settings might need to be adjusted according to the assay. Always start with the recommended setting.

6. For multiple probe detection, activate the appropriate channel and deactivate the other channels in Imaging, under the dPCR parameters in the QIAcuity Software Suite or the QIAcuity instrument. Please always start with the recommended imaging settings in Table 3.
7. Place the nanoplate into the QIAcuity instrument and start the dPCR program.

Data analysis

1. To set up a plate layout according to the experimental design, open the QIAcuity Software Suite and define the reaction mixes, samples, and controls. Plate layout can be defined before or after the nanoplate run.

   Note: Refer to the *QIAcuity User Manual* for details about setting up the plate layout. After the nanoplate run, the raw data are automatically sent to the QIAcuity Software Suite.
2. For data analysis, open the QIAcuity Software Suite and select one nanoplate for analysis in Plate Overview of the software suite.

Note: Refer to the *QIAcuity Application Guide* and *QIAcuity User Manual* for details on how to analyze the data to get absolute quantification data.

Calculation of VCN

$$\mathrm{VCN} = 2 \times \frac{\mathrm{vector\ target\ copies}}{\mathrm{human\ reference\ target\ copies}}$$

Document Revision History

Date	Description
06/2022	Initial release
02/2025	New assays and reaction mix added. Information for VCN calculation added.