

## Quick-Start Protocol

# 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. nos. 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.

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

dPCR CGT Assays are shipped at ambient temperature and should upon receipt be stored protected from light at  $-30^{\circ}\text{C}$  to  $-15^{\circ}\text{C}$  in a constant-temperature freezer for long-term storage (e.g., 12 months). Under these conditions 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  $-30^{\circ}\text{C}$  to  $-15^{\circ}\text{C}$  to avoid repeated freeze–thaw cycles.

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## Further information

- *QIAcuity Application Guide*: [www.qiagen.com/HB-2839](http://www.qiagen.com/HB-2839)
- *QIAcuity User Manual*: [www.qiagen.com/HB-2717](http://www.qiagen.com/HB-2717)
- Safety Data Sheets: [www.qiagen.com/safety](http://www.qiagen.com/safety)
- Technical assistance: [support.qiagen.com](http://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 the QIAcuity Probe PCR Master Mix 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 for 20 min. Vortex and spin down briefly.
- Thaw the QIAcuity Probe PCR Master Mix, 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 be able 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 depends on various parameters. Refer to the *QIAcuity Application Guide* for details.

**Table 1. Reaction setup**

Component	Volume per reaction		
	Nanoplate 26k (24-well)	Nanoplate 8.5k (24-well, 96-well)	Final concentration
4x QIAcuity Probe PCR Master Mix	10 µl	3 µl	1x
20x dPCR CGT Assay-1	2 µl	0.6 µl	1x
<b>Optional:</b> Additional CGT Assays (2, 3, 4, 5) for up to fiveplex reaction*	2 µl	0.6 µl	1x
RNase-free water	Variable	Variable	
Template DNA (added at step 4)	Variable	Variable	
<b>Total reaction volume</b>	<b>40 µl</b>	<b>12 µl</b>	

\* Add additional 20x dPCR CGT Assays or gene of interest assays for a multiplex reaction to detect multiple CGT targets at once.

**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 User Manual Extension: Application Guide*.

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

## Thermal cycling and imaging conditions

- 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
Initial heat activation	2 min	95°C
<b>2-step cycling (40 cycles)</b>		
Denaturation	15 s	95°C
Combined annealing and extension	30 s	60°C

**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.

- 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 at the QIAcuity instrument.

Please always start with the recommended imaging settings in Table 3.

- 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 about how to analyze the data to get absolute quantification data.

## Document Revision History

Date	Changes
06/2022	Initial release

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