Quick-Start Protocol April 2022

dPCR CNV Probe Assays

This protocol is optimized for the quantification of gene-specific regions in human DNA using the dPCR CNV Probe Assays (cat. nos. 250210, 250212, 250213) with the QIAcuity® Probe PCR Kit (cat. nos. 250101, 250102, 250103) in a duplex or multiplex reaction using the QIAcuity digital PCR (dPCR) instrument, to detect copy number variation (CNV) in the human genome. The dPCR CNV Probe Assays are available in 5 different dyes: FAM, HEX, Atto550, ROX, and Cy5. For further information, please refer to the dPCR CNV Probe Assays product page (**www.qiagen.com/dpcr-cnv-probe-assays**).

The dPCR CNV Probe Assays are shipped on dry ice and should upon receipt be immediately stored protected from light in a constant-temperature freezer at –30 to –15°C for long-term storage (12 months) or at 2–8°C for short-term storage (6 months). Under these conditions, the components are stable, without showing any reduction in performance and quality.

Further information

- *QIAcuity User Manual Extension: 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**

Notes before starting

- A reference target assay with a known copy number per genome can be used to identify CNVs for target(s) of interest in test samples. For consistency of CNV analysis, same reference target assay(s) and target-of-interest assay(s) should be used across test samples.
- We recommend using multiple reference target assays depending on experimental conditions. Please refer to the *QIAcuity User Manual Extension: QIAcuity Application Guide* for additional information on reference target assay selection.
- We recommend that the quality and source of any additional positive and negative control sample(s) be comparable to that of test samples, if possible.
- dPCR CNV Probe Assays come as a 20x primer/probe mix in a single tube.

- Always start with the recommended 20x assay concentration and dPCR cycling conditions specified in this protocol.
- Pipetting accuracy and precision affect the consistency of results. Make sure that no bubbles are introduced into the wells of the QIAcuity Nanoplate during pipetting.

Template DNA digestion

- Before partitioning, use restriction digestion to fragment DNA samples with an average length of ≥20 kb, to ensure even distribution of template throughout the QIAcuity Nanoplate, for accurate and precise quantification.
- DNA fragmentation via restriction digestion is particularly important when 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.
- To perform restriction digestion directly in reaction mix, add selected restriction enzyme during reaction setup. Do not use restriction enzymes that cut within target amplicon regions.
- We recommend using EcoRI-HF, PvuII, XbaI (6-cutters), AluI, CviQI, HaeIII (4-cutters), which are validated to digest template DNA in 10 min at RT in QIAcuity Probe PCR Master Mix without impairing the subsequent PCR amplification (Table 1). For additional assay-specific restriction enzyme recommendations, please go to **geneglobe.qiagen.com** or refer to the product data sheet.

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, dPCR CNV Probe Assays, and RNase-free water. Mix the individual solutions.
2. Prepare a reaction mix 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.

3. Vortex the reaction mix.

Table 2. Reaction setup

Component	Volume/reaction		
	Recommended: Nanoplate 8.5k (24-well, 96-well)	**Optional: Nanoplate 26k (24-well)**	**Final concentration**
4x Probe PCR Master Mix	3 µl	10 µl	1x
20x dPCR CNV Probe Assay 1*	0,6 µl	2 µl	1x
20x dPCR CNV Probe Assay 2,3,4,5*	0,6 µl	2 µl	1x
Restriction Enzyme† (highly recommended‡)	0.3–3 units	1–10 units	0.025–0.25 U/µl
RNase-free water	Variable	Variable	
Template gDNA (added at step 4)	Variable§	Variable§	
Total reaction volume	**12 µl**	**40 µl**	

* For respective dye recommendations for the CNV Probe Assays and available channels on QIAcuity, as well as for multiplexing, please see the *QIAcuity User Manual* or the *QIAcuity User Manual Extension: QIAcuity Application Guide*.

† For selection of restriction enzymes, please refer to assay specifications and the *QIAcuity User Manual Extension: QIAcuity Application Guide*.

‡ For long gDNA.

§ Template gDNA amounts ideally should lie within 30–100 ng/reaction and should not exceed 450 ng/reaction when detecting CNV targets present in 2 copies/diploid genome.

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 into each well that contains the reaction mix.

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

5. Transfer the contents of each well of the standard PCR plate to the wells of a 24-well 26k or 96-well 8.5k nanoplate.
6. Seal the nanoplate properly using the QIAcuity Nanoplate Seal provided in the QIAcuity Nanoplate Kits. For exact sealing procedure, see the *QIAcuity 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 (15–25°C).

Thermal cycling and imaging conditions

1. Set the cycling conditions under the dPCR parameters in the QIAcuity Software Suite or at the QIAcuity instrument according to Table 3.

2. For Probe-based detection, activate the required channels in **Imaging**, under the dPCR parameters in the QIAcuity Software Suite or at the QIAcuity instrument.

Table 3. Cycling conditions

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

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. The plate layout can be defined before or after the nanoplate run.

   Note: Refer to the *QIAcuity User Manual* for details on setting up the plate layout.

2. After the nanoplate run, the raw data is automatically sent to the QIAcuity Software suite.
3. For data analysis, open the QIAcuity Software Suite and select the individual nanoplate for the analysis in **Plate Overview** of the software suite.

   Note: Refer to the *QIAcuity User Manual Extension: QIAcuity Application Guide* and the *QIAcuity User Manual* for details on how to analyze the data and calculate copy numbers per human genome based on the reference(s) you have added to the nanoplate.

Document Revision History

Date	Changes
04/2022	Initial release

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