

## dPCR CNV Probe Assays

This protocol is optimized for the quantification of gene-specific regions in human, mouse, or rat DNA using the dPCR CNV Probe Assays (cat. nos. 250210, 250212, 250213, 250214) with the QIAcuity® Probe PCR Kit (cat. nos. 250101, 250102, 250103) in a singleplex or multiplex reaction using the QIAcuity digital PCR (dPCR) instrument, to detect copy number variations (CNV). 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 at [www.qiagen.com](http://www.qiagen.com)

### Wet-lab Validated dPCR CNV Probe Assays

The wet-lab validated dPCR CNV Probe Assays (cat. no. 250210, 250212, and 250213) are shipped ready-to-use on dry ice. Upon receipt, the assays should be stored protected from light in a constant-temperature freezer at  $-30^{\circ}\text{C}$  to  $-15^{\circ}\text{C}$  for long-term storage (12 months) or at  $2-8^{\circ}\text{C}$  for short-term storage (6 months). Repeated freeze-thaw cycles should be avoided. If possible, store the assay in aliquots. Under these conditions, the components are stable, without showing any reduction in performance and quality.

### Custom dPCR CNV Probe Assays

The custom dPCR CNV Probe Assays (cat. no. 250214) are shipped lyophilized at ambient temperature. Upon receipt, the lyophilized custom dPCR CNV Probe Assay should be stored protected from light between at  $2-8^{\circ}\text{C}$  for short term storage and between  $-30^{\circ}\text{C}$  to  $-15^{\circ}\text{C}$  in a constant-temperature freezer for long term storage. After reconstitution, it is recommended to store the dPCR CNV Probe Assay at  $-30^{\circ}\text{C}$  to  $-15^{\circ}\text{C}$ . Repeated freeze-thaw cycles should be avoided. If possible, store the assay in aliquots. Under these conditions, the dPCR CNV Probe Assay is stable until the expiry date listed on the vial.

## Further information

- *dPCR CNV Probe Assay Handbook*: [www.qiagen.com/HB-3542](http://www.qiagen.com/HB-3542)
- *QIAcuity User Manual*: [www.qiagen.com/HB-2717](http://www.qiagen.com/HB-2717)
- *QIAcuity User Manual Extension*: [www.qiagen.com/HB-2839](http://www.qiagen.com/HB-2839)
- Safety Data Sheets: [www.qiagen.com/safety](http://www.qiagen.com/safety)
- Technical assistance: [support.qiagen.com](http://support.qiagen.com)

## Important points 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, the 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. Refer to the *QIAcuity User Manual Extension: QIAcuity Application Guide* for more information on reference target assay selection.
- dPCR CNV Probe Assays are used as a 20x primer/probe mix. Always start with the 20x assay concentration and dPCR cycling conditions in this protocol.
- Pipetting accuracy and precision affect the consistency of results. Ensure that no bubbles are introduced Nanoplate wells while pipetting.
- Pipetting accuracy and precision affects the consistency of results. Be sure that all pipets and instruments have been checked and calibrated according to the manufacturer's recommendations. Also make sure that no bubbles are introduced into the wells of the QIAcuity Nanoplate during pipetting.

## Things to do before starting

- Reconstitute custom dPCR CNV Probe Assays to a 20x concentration with nuclease-free water according to their product datasheets, which can be retrieved from each user's GeneGlobe account.
- Thaw genomic DNA and QIAcuity Probe Mastermix on ice (4°C). After thawing, mix gently by repeated pipetting or quick vortex, then quick spin.

## Template DNA Digestion

- Before partitioning, DNA samples with an average length of  $\geq 20$  kb should be digested. This ensures 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).
- The restriction enzymes in Table 1 are validated to digest template DNA in 10 min at RT in QIAcuity Probe PCR Master Mix without impairing the subsequent PCR amplification. For assay-specific restriction enzyme compatibility, please go to [geneglobe.qiagen.com](https://geneglobe.qiagen.com) or refer to the product data sheet that is provided with each assay.

**Table 1. List of recommended enzymes**

6-Cutter restriction enzymes		4-Cutter restriction enzymes	
EcoRI	0.25 U/ $\mu$ L EcoRI-HF <sup>®</sup> , NEB* 0.025 U/ $\mu$ L Anza™ 11 EcoRI, TFS <sup>†</sup>	AluI	0.025 U/ $\mu$ L AluI, NEB 0.025 U/ $\mu$ L Anza 44 AluI, TFS
PvuII	0.025 U/ $\mu$ L PvuII, NEB 0.025 U/ $\mu$ L Anza 52 PvuII, TFS	CviQI	0.025 U/ $\mu$ L CviQI, NEB 0.025 U/ $\mu$ L Csp6I (CviQI), TFS
XbaI	0.025 U/ $\mu$ L Anza 12 XbaI, TFS	Haell	0.025 U/ $\mu$ L BsuRI (HaellI), TFS

\* NEB, New England Biolabs.

† TFS, Thermo Fisher Scientific.

## 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 (8-well, 24-well)	Final concentration
4x Probe PCR Master Mix	3 $\mu$ L	10 $\mu$ L	1x
20x dPCR CNV Probe Assay 1*	0.6 $\mu$ L	2 $\mu$ L	1x
20x dPCR CNV Probe Assay 2,3,4,5*	0.6 $\mu$ L	2 $\mu$ L	1x
Restriction Enzyme <sup>†</sup> (highly recommended <sup>†</sup> )	0.3–3 units	1–10 units	0.025–0.25 U/ $\mu$ L
RNase-free water	Variable	Variable	
Template gDNA (added at step 4)	Variable <sup>§</sup>	Variable <sup>§</sup>	
<b>Total reaction volume</b>	<b>12 <math>\mu</math>L</b>	<b>40 <math>\mu</math>L</b>	

\* 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*.

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

<sup>‡</sup> For gDNA with an average length  $\geq$ 20 kb.

<sup>§</sup> 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. 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
<b>2-step cycling (40 cycles)</b>		
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

4. 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.

5. After the nanoplate run, the raw data is automatically sent to the QIAcuity Software suite.
6. 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.

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## Document Revision History

Date	Changes
03/2024	Initial release.



Scan QR code for the *QIAcuity User Manual*.

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