Product Profile

dPCR CNV Probe Assays

For locus-specific copy number variation (CNV) analysis using the QIAcuity® Digital PCR System

The dPCR CNV Probe Assays are hydrolysis probe-based assays for sensitive, precise and reproducible copy number variation analysis of an individual gene or region of interest (GOI) using digital PCR (dPCR). They consist of a ready-to-use 20x concentrated primer pair and a hydrolysis probe with a configurable dye (fluorophore) that allows multiplex detection of up to five targets in a single reaction. The assays are intended for use with the QIAcuity Probe PCR Kit.

- Choose from more than 200 wet-lab validated assays for the human genome, including references, centromeric references, single-copy and multi-copy targets
- Mix and match fluorophores for multiplex assays – the GOI target assays, available in three dyes, FAM™, ATTO™ 550 and Cy5®, can be combined with the reference and/or centromeric reference targets, available in two dyes, HEX™ and ROX™
- Simplify your assay design for complex sample types – our ready-to-use assays have been validated on a range of sample types including inhibited samples, formalin-fixed, paraffin-embedded (FFPE), cell lines, artificial templates and genomic DNA (gDNA)
- Follow a straightforward and rapid dPCR workflow on the QIAcuity Digital PCR System

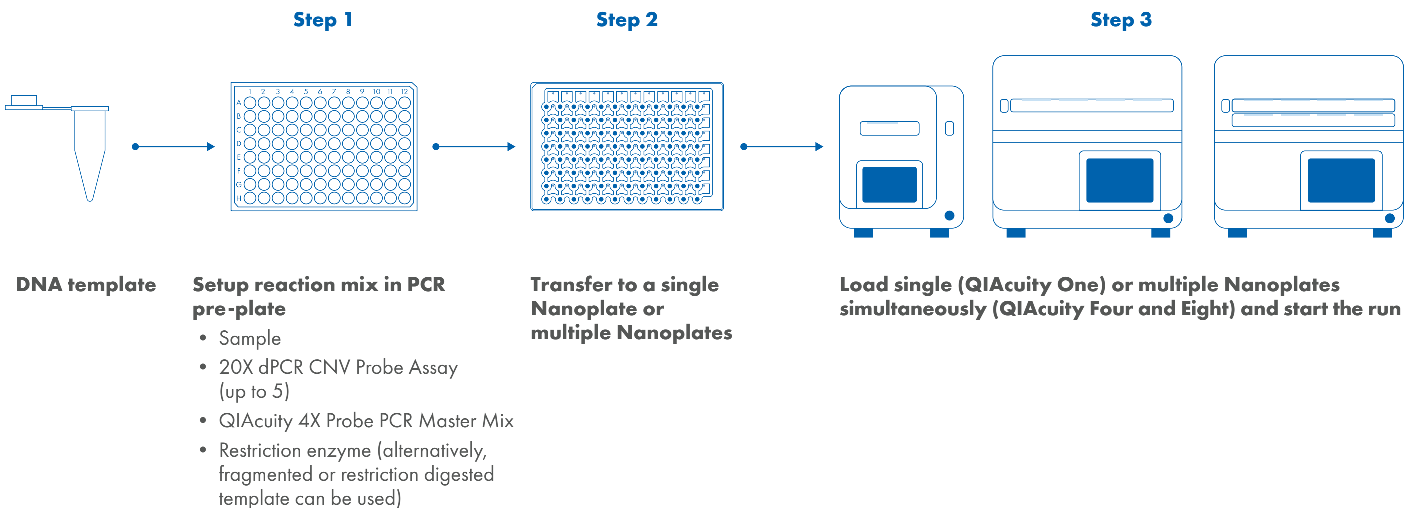


Figure 1. Complete the dPCR CNV analysis workflow in about 2 hours with minimal hands-on time.
For precise copy number determination, we recommend using either fragmented or restriction-digested templates. Restriction digestion using an enzyme that doesn't cut the amplicon of your gene or region of interest can be done either before transfer to the Nanoplate or directly within the Nanoplate. DNA templates are mixed with the ready-to-use QIAcuity Probe PCR Kit Master Mix and the dPCR CNV Probe Assay, and this mixture is aliquoted into each well of the dPCR pre-plate. The reaction mix is then mixed well via pipetting and is transferred into the Nanoplate wells. Multiple genes or targets of interest and reference assays can be analyzed in a single well in a multiplex reaction by combining the assays with different fluorophores. After loading and sealing the Nanoplate, the recommended cycling program is run on the QIAcuity instrument. The resulting copies per microliter for each target or reference assay are shown upon absolute quantification analysis using the QIAcuity Software Suite. For the calculation of copy number per genome, select Copy Number Variation Analysis in the QIAcuity Software Suite.

Explore the virtual workflow: **www.qiagen.com/applications/digital-pcr/workflow/qiacuity-demo**

Sensitive CNV detection with minimal sample input

The dPCR CNV Probe Assays can detect small copy number changes between samples – down to a single-copy difference – and with minimal template input. Reference assays and centromeric reference assays are available for the normalization of CNV in templates. The assays have been validated for multiple sample types.

Copy number alterations at individual loci can be detected using DNA from frozen, fresh and fixed sample types on the QIAcuity Digital PCR System in about 2 hours with minimal hands-on time (Figure 1). Table 1 gives an overview of targets covered by the dPCR CNV Probe Assays.

Table 1. Overview of targets

Gene of interest assays	Centromeric reference assays	Reference assays
205 assays	24 targets	4 assays

For a detailed target list, visit GeneGlobe®:
https://geneglobe.qiagen.com/product-groups/dpcr-cnv-probe-assays

Outstanding assay performance

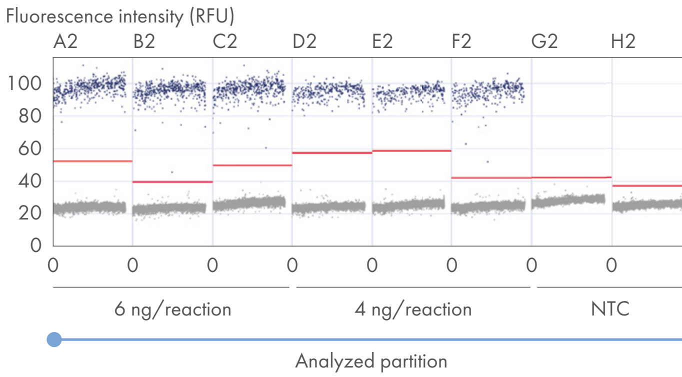
To demonstrate the excellent product amplification and separation between the negative and the positive partition clusters using the dPCR CNV Probe Assays, two different template input concentrations (6 ng/reaction and 4 ng/reaction) of the same gDNA sample were tested in triplicates, in two separate duplex reactions (reactions 1 and 2) using 8.5k 96-well Nanoplates.

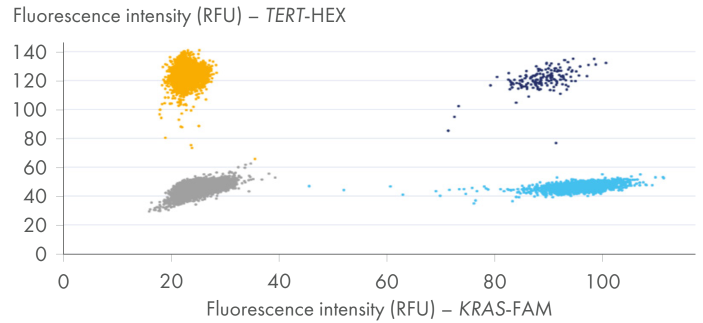
Furthermore, to compare the performance of different fluorophore combinations in multiplex, the target *KRAS* was labeled with FAM and the reference assay *TERT* with HEX in reaction 1, and *KRAS* with Cy5 and *TERT* with Atto 550 in reaction 2.

1D and 2D scatterplots of the two duplex reactions show that both dye combinations perform equally for the separation of positive and negative clusters as well as the signal intensity, independent of the template loading amounts of 6 ng/reaction and 4 ng/reaction (Figure 2). However, when required, a much lower template concentration can be used. Recommended template concentrations (minimum and maximum loading amounts) can be found in the **User Manual Extension Guide.**

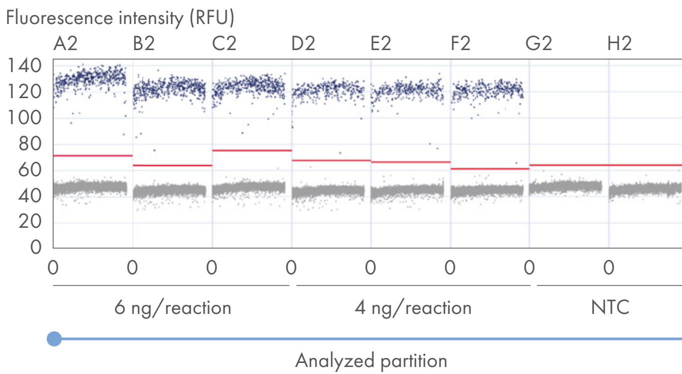
Reaction 1

***KRAS*-FAM**



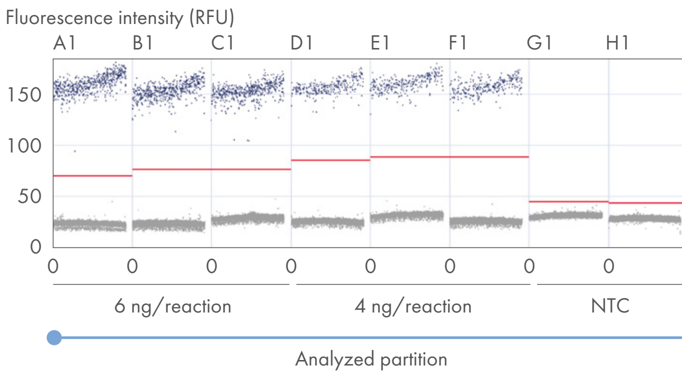


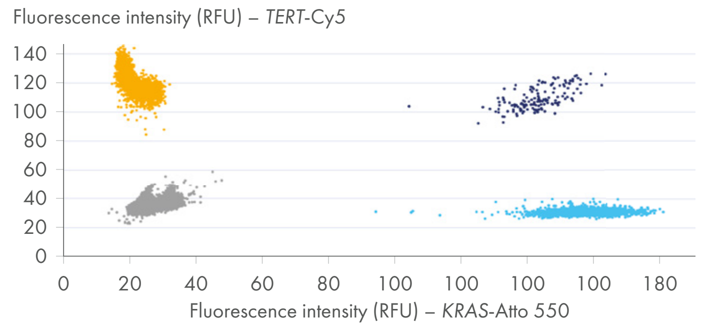
***TERT*-HEX**



Reaction 2

***KRAS*-Atto 550**





***TERT*-Cy5**

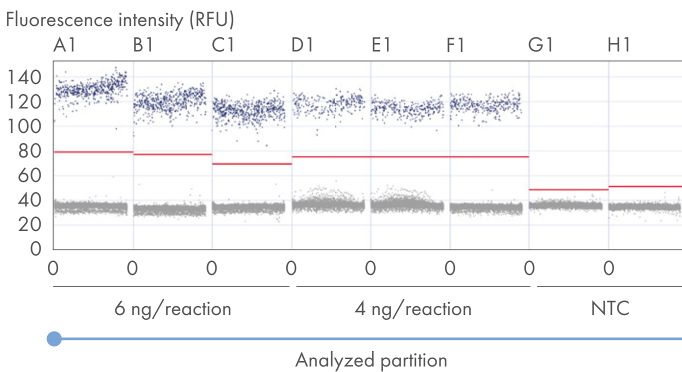


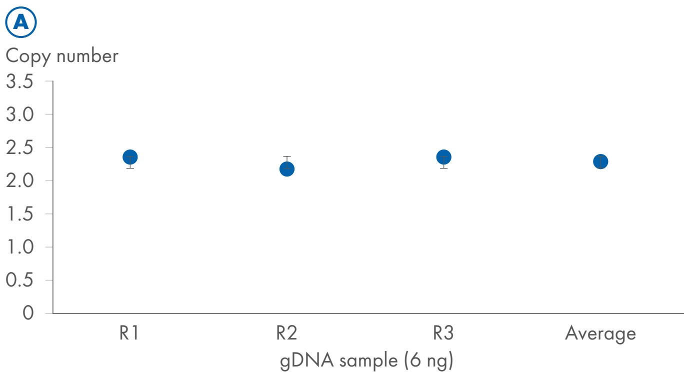
Figure 2. Evaluation of assay performance using two gDNA input amounts and two different dye combinations.
1D and 2D scatterplots of two duplex reactions for *KRAS* (target) and *TERT* (reference). dPCR, with three replicates per template concentration and two replicates of no template control (NTC), was performed using 8.5k 96-well Nanoplates and the QIAcuity Probe PCR Kit on the QIAcuity Digital PCR System.

Precise and reproducible copy number analysis

Copy number analysis using the QIAcuity system is highly reproducible, which eliminates the need for replicates. This allows for a cost-effective increase in sample throughput. In addition, the QIAcuity Software Suite, which comes with the instrument, automatically calculates the copy number, reducing the user's workload.

Figure 3 A demonstrates accurate and reproducible copy number analysis in a duplex reaction for the same gDNA template sample ran in three replicates on the QIAcuity instrument. The expected copy number of two copies per genome for the target *KRAS* was observed for all replicates. Figure 3 B shows the copy number per human genome for six different targets in the SK-BR3 cell line. Excellent distribution and cluster separation can be seen for both assays.

Figure 4. shows the linearity of detection using *KRAS* and *TERT* dPCR CNV Probe Assays using a dilution series of a gDNA template (5 to 3000 copies/µl).



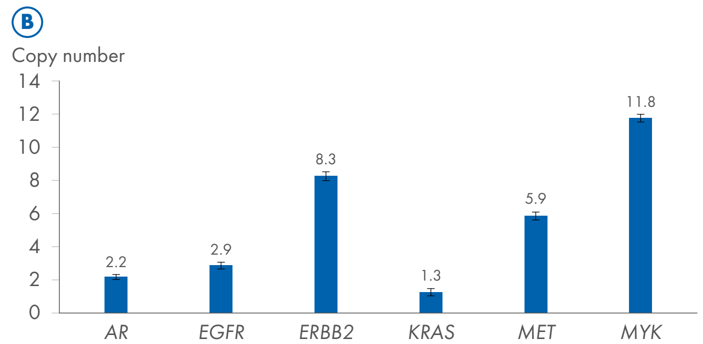
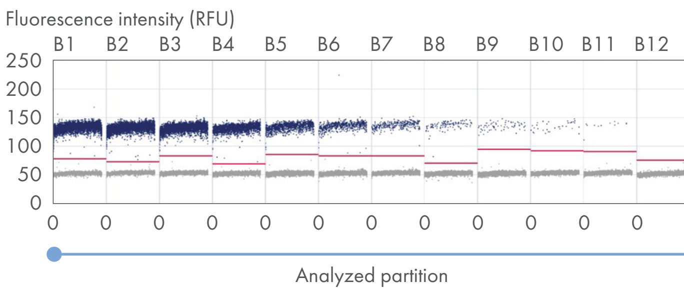
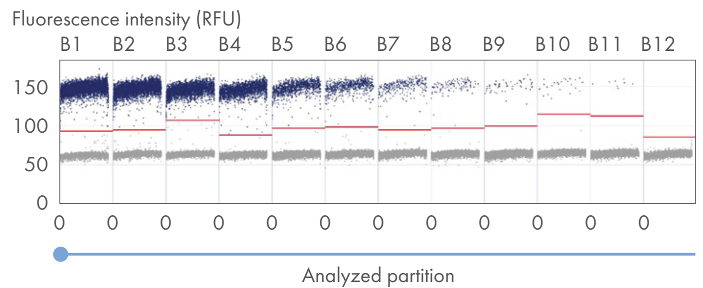


Figure 3. Evaluation of reproducibility across replicates and targets.
A Copy number determination of the target *KRAS* (and *TERT* as reference) in a gDNA sample ran as three replicates (R1, R2, R3) in a duplex reaction. **B** Copies per human genome observed for six different targets in the SK-BR3 cell line. For both A and B, dPCR was performed using 8.5k 96-well Nanoplates and the QIAcuity Probe PCR Kit on the QIAcuity Digital PCR System.





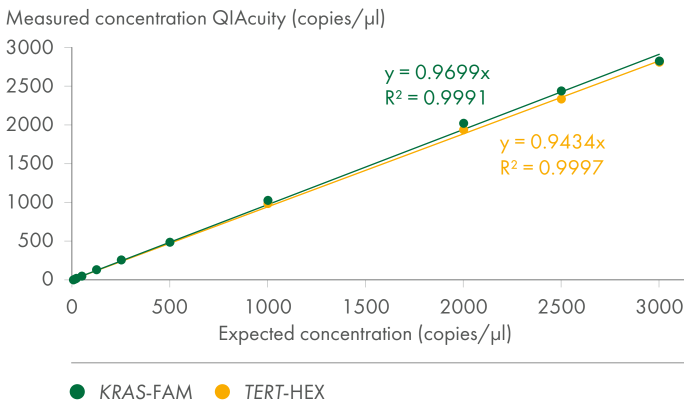


Figure 4. Linearity distribution and scatterplots of *KRAS* and *TERT* dPCR CNV Probe Assays.
A 5–3000 copies/µl dilution series of a gDNA template (B1-11) and NTC (B12) were loaded. The 1-D scatterplots for *KRAS* and *TERT* assays show excellent positive and negative partition cluster separations. dPCR was performed using 8.5k 96-well Nanoplates and the QIAcuity Probe PCR Kit on the QIAcuity Digital PCR System.

Multiplex CNV analysis of up to five targets per reaction

The flexible setup of the dPCR CNV Probe assays allows simultaneous screening of up to five targets in a single reaction. A 5-plex reaction setup on the QIAcuity Digital PCR System not only allows a high throughput but also is cost-efficient, as the cost per target is significantly reduced when compared to single-plex or duplex assays.

Figure 5 demonstrates reliable copy number determination of five targets in a multiplex reaction using a gDNA sample as input. Optimal primer and probe concentrations ensure accurate detection of CNVs in a 5-plex assay.

Furthermore, identical concentrations (in copies/µl) were observed across different multiplex setups – 2-plex, 3-plex, 4-plex and 5-plex.

The option to order a single assay with different dyes (FAM, HEX, ROX, Atto 550 and Cy5) enables flexible experimental design and analysis. Moreover, all dPCR CNV Probe Assays have been wet-lab validated to ensure robust performance, even when working with challenging samples such as FFPE.

Figure 5. The strong signal separation between channels in 5-plex.
1-D scatterplots show excellent positive and negative partition cluster separations for each of the 5 dPCR Probe CNV Assays multiplexed in a single reaction. In addition, identical concentrations were observed across different multiplex setups. dPCR, using 4 ng/reaction of gDNA input ran in two replicates and NTC, was performed using 8.5k 96-well Nanoplates and the QIAcuity Probe PCR Kit on the QIAcuity Digital PCR System.

Ordering Information

Product	Contents	Cat. no.
dPCR CNV Probe Assays	One tube of gene of interest/centromeric reference assay/reference dPCR Assay, 300 rxn, 1,000 rxn for 12 µl reaction in Nanoplate 8.5k	250210, 250212, 250213 (Respective Assay Cat. No. to be specified in GeneGlobe)
QIAcuity Probe PCR Kit (1ml, 5 ml)	1 ml or 5 ml Master Mix for the QIAcuity dPCR instrument; water	250101, 250102

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

For configuring and ordering the assays, please visit:
https://geneglobe.qiagen.com/product-groups/dpcr-cnv-probe-assays

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