Product Profile

Validated assays for the QIAcuity™ Digital PCR System

For highly precise mutation detection, copy number variation analysis and gene expression analysis

dPCR LNA® Mutation Assays

- Locked nucleic acid (LNA) technology increases assay specificity and sensitivity
- Duplex assay design detects mutated and wild-type sequences
- Two dye combinations allow detection of two targets in the same reaction

dPCR Copy Number Assays

- Predesigned assays for all genes in the human genome deliver reliable results
- Three design locations per gene – 5', middle, 3'– to amplify your region of interest
- Simple and straightforward EvaGreen®-based dPCR format enhances usability

QuantiNova® LNA PCR Assays

- Over 1.3 million assays detect any human, mouse or rat mRNA or lncRNA
- Short LNA-enhanced primers provide exceptional sensitivity and specificity
- EvaGreen-based dPCR allow accurate and convenient transcript analysis

dPCR LNA Mutation Assays for reliable DNA sequence mutation detection

dPCR LNA Mutation Assays are LNA-enhanced, duplex, hydrolysis probe-based assays for highly precise and sensitive mutation detection, intended for use with QIAcuity Probe PCR Kits. These dPCR wet-lab validated assays can reliably detect individual sequence mutations, selected from comprehensive curated databases such as COSMIC, with a sensitivity down to 0.1% in a single nanoplate well. Even higher sensitivity can be achieved by splitting the reaction into multiple wells and combining the analysis. The choice of two different fluorescent dye combinations allows the detection of mutant and wild-type sequences as well as multiplexing analysis of two target mutations in one well.
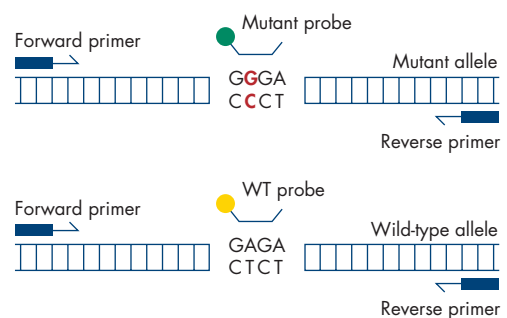

Figure 1. dPCR LNA Mutation Assay setup. The assay, provided in a single-tube format, contains a primer pair and two probes – a mutant probe and a wild-type (WT) probe – for detecting both mutant and wild-type alleles in the same reaction.
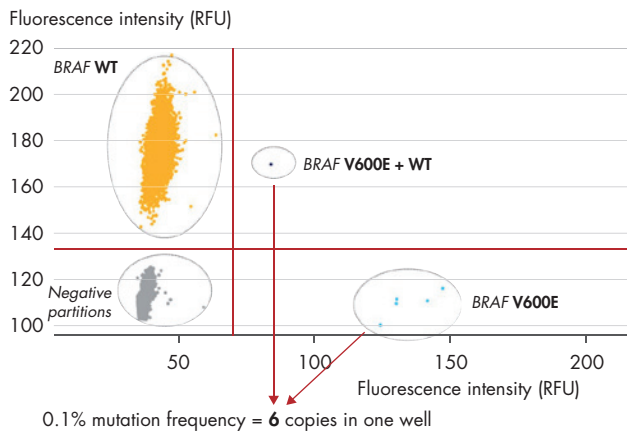
Figure 2. *BRAF* V600E detection at 0.1% in FFPE samples. Test sample with 0.1% mutation frequency was created by spiking 0.3 ng Horizon™ FFPE samples into 30 ng healthy WT gDNA. The measured mutation frequency was 0.13% with 0.24 copies/µl. The figure shows the 2D scatter plot of a single well with 6 positive copies detected in the green channel (FAM).

LNA-enhanced for highest specificity

dPCR LNA Mutation Assays are duplex reactions with competing probes, as shown in Figure 1. Detecting mutant and wild-type alleles in the same reaction warrants the highest specificity of the hydrolysis probes. LNA provides this enhanced specificity and also increases sensitivity. The assay product is available in two different dye combinations – FAM™/HEX™ and Atto™ 550/ROX™ (mutant/WT) – enabling the detection of two targets in the same 4plex reaction.

Highly sensitive detection of *BRAF* V600E in FFPE samples

Detecting mutations in heterogeneous samples, with only a few cells carrying the mutation, calls for a highly sensitive and specific assay. The dPCR LNA Mutation Assays on the QIAcuity Nanoplate 26K (with 26,000 partitions) deliver the possible lowest detection limits. In combination with the 4x concentrated QIAcuity Probe Master Mix, you can load up to 27 µl of sample for detecting the rarest mutations. Figure 2 shows the successful detection of *BRAF* V600E mutation at 0.1% sensitivity using FFPE samples.

dPCR Copy Number Assays for locus-specific copy number variation analysis

dPCR Copy Number Assays enable specific, accurate, reproducible and easy-to-interpret copy number change analysis for an individual gene or region of interest. The assay comes in a ready-to-use 25x-concentrated format, intended for use with the QIAcuity EG PCR Kit. Assays for more than 200 targets have been dPCR wet-lab validated. All other in-silico designs have been bench-verified and are ready to use in NGS follow-up studies, specific target screening and other related studies.

The detection is intercalating dye-based, which is EvaGreen on the QIAcuity. The dye is provided in the master mix of the QIAcuity EG PCR Kit and detected in the green channel of the QIAcuity system. Reference assays, available as single and multi-copy, are detected in a separate well on the same nanoplate. To achieve highly precise copy number determination using dPCR, perform a restriction enzyme digestion of the sample DNA. Information on choosing the correct restriction enzyme, one that doesn't cut the amplicon of a particular gene or region of interest, is provided with the assay.

Accurate copy number call of *MYC* with 8.5K partitions in MCF-7 cell line

In the QIAcuity dPCR System, you can flexibly choose between two nanoplate types – 26K and 8.5K. For copy number variation analysis, dPCR runs with ≤8000 valid partitions can provide the best performance. Using less reagents and a 96-well nanoplate processed in less than two hours, the QIAcuity System brings copy number determination using dPCR to a new level. Figures 3 and 4 show accurate *MYC* copy number determination in MCF-7 cell line using QIAcuity Nanoplate 8.5K 96-well.
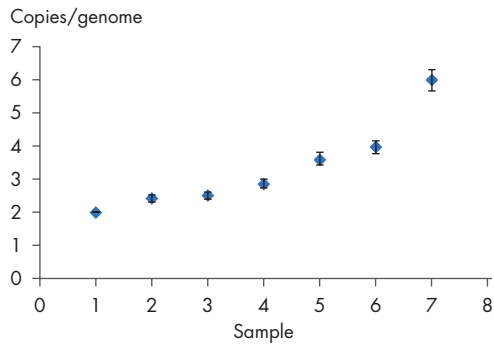
Figure 3. _MYC_ copy number determination in MCF-7 cell line. Copy number plot of _MYC_ normalized with _TERT_ as reference. The samples S1–S7 are WT/MCF-7 mixtures containing increasing amounts of MCF-7 DNA: S1=0%, S2=11%, S3=20%, S4=33%, S5=43%, S6=50% and S7=100%. _MYC_ copy number determined using the QIAcuity System matched the expected numbers: S1=2, S2=2.4, S3=2.8, S4=3.3, S5=3.7, S6=4 and S7=6. The WT, MCF-7 and mixture samples were analyzed with 4 ng/reaction.
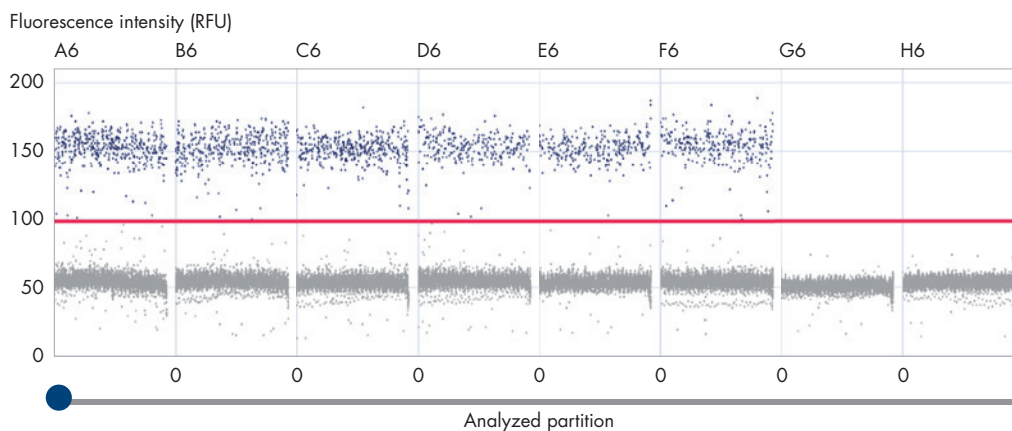

Figure 4. dPCR Copy Number Assay validation. 1D scatter plot showing single-well assay validation data of dPCR Copy Number Assay _MYC_ with different human gDNA input amounts (**A6–C6**: 6 ng/reaction, **D6–F6**: 4 ng/reaction, **G6**: NTCs; Green channel for EvaGreen detection).

QuantiNova LNA PCR Assays for dPCR-based gene expression analysis

QuantiNova LNA PCR Assays provide highly sensitive and accurate LNA-enhanced digital PCR quantification assays for mRNA and lncRNA. The assays are designed for use with universal reverse transcription (RT), followed by either real-time PCR or digital PCR amplification using EvaGreen for detection. In dPCR use on the QIAcuity instrument, the assays are run with the QIAcuity EG PCR Kit, and the QuantiTect® Reverse Transcription Kit is recommended for the RT reaction.

Detect small expression changes with the highest precision

The forward and reverse PCR amplification primers are LNA-enhanced, with the LNAs placed intelligently in the primers to fully optimize the primer performance. The result is exceptional sensitivity and specificity with extremely low background, enabling accurate quantification of very low levels of mRNA/lncRNA, as shown in Figure 5.
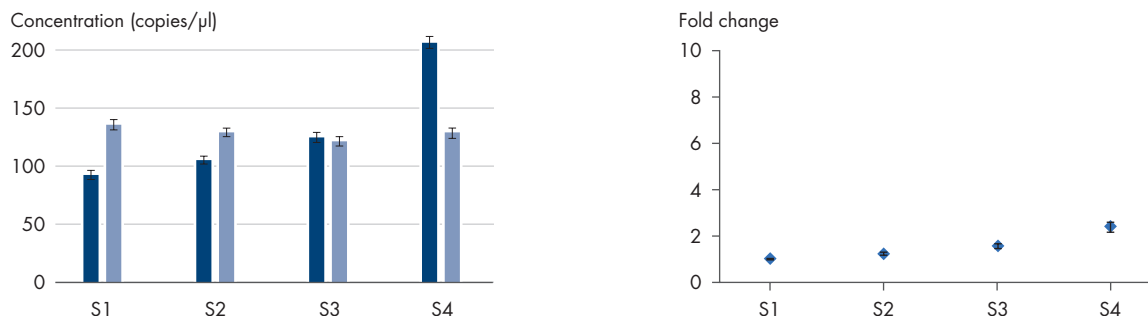
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Figure 5. IL-4 gene expression analysis – detecting small expression changes with the highest precision. Synthetic IL4 RNA was spiked into non-IL4 expressing Universal Human Reference RNA (Thermo Fisher Scientific). IL4 fold-expression changes in samples S2, S3 and S4 were calculated using S1 as reference sample and HPRT as reference target. Figure 5A shows mean concentration in copies/µl (from 3 technical replicates/sample) for the target IL4 (dark blue) and reference HPRT (light blue). Figure 5B shows the mean fold change (from 3 technical replicates/sample) in IL4 expression: S1 = 0 (reference), S2 = 1.3, S3 = 1.5 and S4 = 2.3.

Ordering Information

Product	Contents	Cat. no.
dPCR LNA Mutation Assays	Single tube containing 30x-concentrated assay with choice of FAM + HEX or Atto 550 + ROX detection dyes; 200 or 1000 dPCR reactions of 40 µl each	250200, 250201
dPCR Copy Number Assays	Single tube containing 25x-concentrated assay; 200 or 1000 dPCR reactions of 12 µl each	250205, 250206
QuantiNova LNA PCR Assays	mRNA/lncRNA-specific primer mixture in a single tube; for 400 or 1500 dPCR reactions	249990, 249992
QIAcuity Probe PCR Kit	1 ml, 5 ml or 25 ml 4x concentrated QIAcuity Probe Mastermix and Water	250101, 250102, 250103
QIAcuity EG PCR Kit	1 ml, 5 ml or 25 ml 3x concentrated QIAcuity EvaGreen Mastermix and Water	250111, 250112, 250113
QuantiTect Reverse Transcription Kit	For 50/200/400 x 20 µl reactions: gDNA Wipeout Buffer, Quantiscript® Reverse Transcriptase, 5x Quantiscript RT Buffer, RT Primer Mix, Water	205311, 205313, 250314

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.

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