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 nanoliter 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 singletube 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.
**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**

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 with identifiable threshold between the four populations.

**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.
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 is exceptional sensitivity and specificity with extremely low background, enabling accurate quantification of very low levels of mRNA/lncRNA, as shown in Figure 5.
### Ordering Information

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<th>Product</th>
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<tr>
<td>dPCR LNA Mutation Assays</td>
<td>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</td>
<td>250200, 250201</td>
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<tr>
<td>dPCR Copy Number Assays</td>
<td>Single tube containing 25x-concentrated assay; 200 or 1000 dPCR reactions of 12 μl each</td>
<td>250205, 250206</td>
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<tr>
<td>QuantiNova LNA PCR Assays</td>
<td>mRNA/IncRNA-specific primer mixture in a single tube; for 400 or 1500 dPCR reactions</td>
<td>249990, 249992</td>
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<tr>
<td>QIAcuity Probe PCR Kit</td>
<td>1 ml, 5 ml or 25 ml 4x concentrated QIAcuity Probe Mastermix and Water</td>
<td>250101, 250102, 250103</td>
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<tr>
<td>QIAcuity EG PCR Kit</td>
<td>1 ml, 5 ml or 25 ml 3x concentrated QIAcuity EvaGreen Mastermix and Water</td>
<td>250111, 250112, 250113</td>
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<td>QuantiTect Reverse Transcription Kit</td>
<td>For 50/200/400 x 20 μl reactions: gDNA Wipeout Buffer, Quantiscript® Reverse Transcriptase, 5x Quantiscript RT Buffer, RT Primer Mix, Water</td>
<td>205311, 205313, 250314</td>
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Find more dPCR assays at: [www.qiagen.com/qiacuity-dpcr-assays](http://www.qiagen.com/qiacuity-dpcr-assays)