QuantiNova® Probe PCR Kit

For highly sensitive, specific and ultrafast probe-based, real-time PCR

The QuantiNova Probe PCR Kit enhances the specificity and sensitivity of probe-based real-time PCR. The kit has been optimized to provide accurate, singleplex or duplex, cDNA or gDNA analysis on various real-time PCR cyclers. It features a built-in control for visual identification of correct pipetting. Reactions are stable for up to 100 hours at 30°C, facilitating convenient room temperature handling of multiple samples and automated workflows.

Benefits of the QuantiNova Probe PCR Kit:

- Accurate detection of rare targets down to one copy
- Duplex capabilities for more results per reaction
- Reaction stability of 100 hours at 30°C making it ideal for flexible workflows
- Visual indication of correct pipetting resulting in fewer pipetting errors
- High specificity resulting in better results
- Increased throughput due to ultrafast cycling

Novel, highly stringent, hot-start mechanism

The QuantiNova Probe PCR Kit provides the highest specificity in real-time PCR, because of a novel, antibody-mediated, hot-start mechanism (Figure 1). At low temperatures, the QuantiNova DNA Polymerase is kept in an inactive state by the QuantiNova Antibody and a novel additive, QuantiNova Guard, which stabilizes the complex. This improves the stringency of the hot-start and prevents extension of nonspecifically annealed primers and formation of primer–dimers. Within 2 minutes of raising the temperature to 95°C, the QuantiNova Antibody and QuantiNova Guard are denatured and the QuantiNova DNA Polymerase is activated, enabling the PCR amplification.

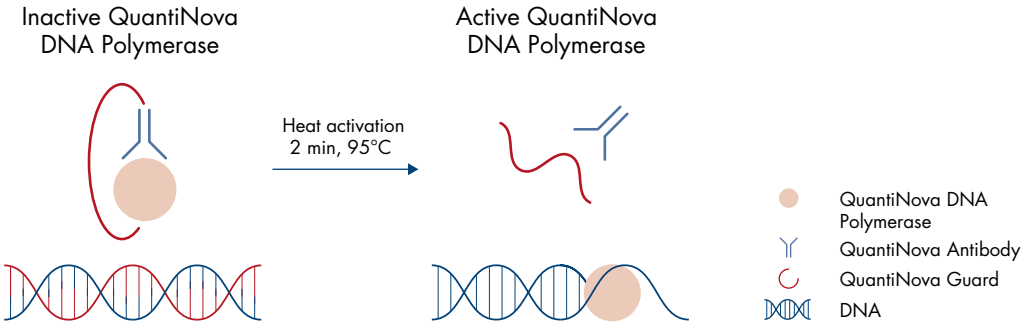

Figure 1. Principle of the novel QuantiNova hot-start mechanism. The QuantiNova DNA Polymerase is kept in an inactive state by the QuantiNova Antibody and QuantiNova Guard until the initial heat activation step.

Reaction stability of up to 100 hours

The real-time PCR mix can be stored at 30°C for up 100 hours without impairing the performance of the subsequent reactions (Table 1). The outstanding stability, even after extended storage at room temperatures without the use of any cooling agent, makes the QuantiNova Probe PCR Kit deal for high-throughput reaction setup and plate-stack handling.

Table 1. Effect of storage on reaction stability

	Mean C_T values			
	QuantiNova Probe PCR Kit		Probe PCR kit from supplier L	
ng	0 hrs	100 hrs	0 hrs	100 hrs
10	24.37	24.49	24.35	27.49
1	27.78	27.90	27.77	30.97
0.1	31.16	30.98	31.37	34.48
No template	Not detected	Not detected	Not detected	Not detected

Built-in visual control for correct pipetting

The master mix supplied with the QuantiNova Probe PCR Kit contains an inert blue dye that does not interfere with the real-time PCR, but increases visibility in the tube or well. The sample dilution buffer contains an inert yellow dye. When the template nucleic acid, diluted with the sample dilution buffer, is added to the master mix, the color of the solution changes from blue to green (Figure 2), providing a visual indication of correct pipetting and reaction setup.

Figure 2. Built-in pipetting control. The QuantiNova Probe PCR master mix contains an inert blue dye that turns green on addition of the template nucleic acid diluted in the yellow nucleic acid dilution buffer, providing a visual indication of correct pipetting.

Highly consistent results on various real-time cyclers

The QuantiNova Probe PCR Kit can be used on any real-time cycler. ROX™ is provided in a separate tube and can be added if using a cycler that uses ROX as a passive reference dye. The QuantiNova Probe PCR Kit delivers highly consistent results among different cyclers in terms of sensitivity, reproducibility, and efficiency. The consistency of the results is maintained, despite varying fast-cycling capacities of the different cyclers that result in different cycling protocols.

Accurate and robust detection of a single DNA molecule

The high sensitivity of the QuantiNova Probe PCR Kit results in accurate and robust detection of even a single target copy (Figure 3).

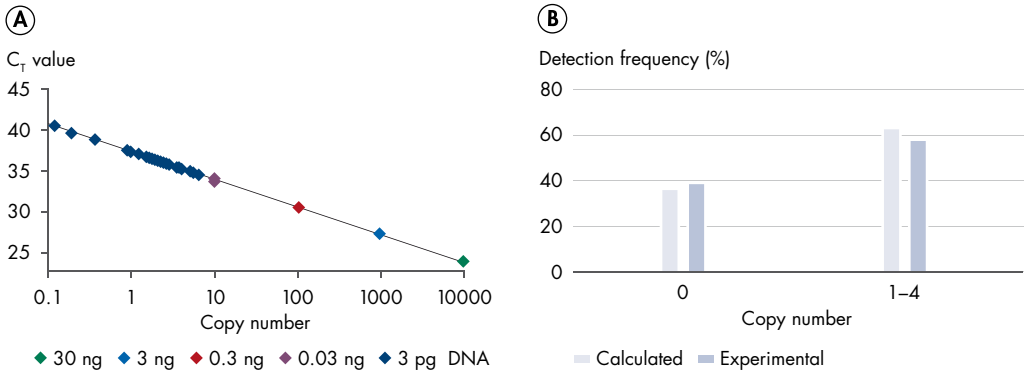

Figure 3. Sensitive and robust detection of a single target copy. A The QuantiNova Probe PCR Kit was used to detect the single-copy gene, CFTR, in leukocyte genomic DNA using 30, 3, 0.3, and 0.03 ng gDNA, corresponding to 10^4 to 10 copies, and the results were plotted to create a calibration curve. The plot of copy number versus C_T value demonstrates high linearity. A master mix sufficient for 60 reactions was set up and 180 pg template gDNA, corresponding theoretically to 1 target copy per reaction was added to the master mix. The master mix was pipetted into 60 wells. However, due to statistical variations, some wells had more than 1 target copy while others had none. The calibration curve was used to determine the actual number of copies within each of the 60 reactions. **B** The expected number of copies per reaction was calculated theoretically using Poisson's equation and compared to the actual number of copies determined using the calibration curve. There was a high concordance between the single-copy number results obtained using the calibration curve and Poisson's equation, demonstrating the high sensitivity and robustness of the QuantiNova Probe PCR Kit.

Detection of two targets in one reaction

The QuantiNova Probe PCR Kit enables accurate quantification of two targets having widely differing abundance in a single tube. This saves time, money, and reduces the amount of sample material needed (Figure 4).

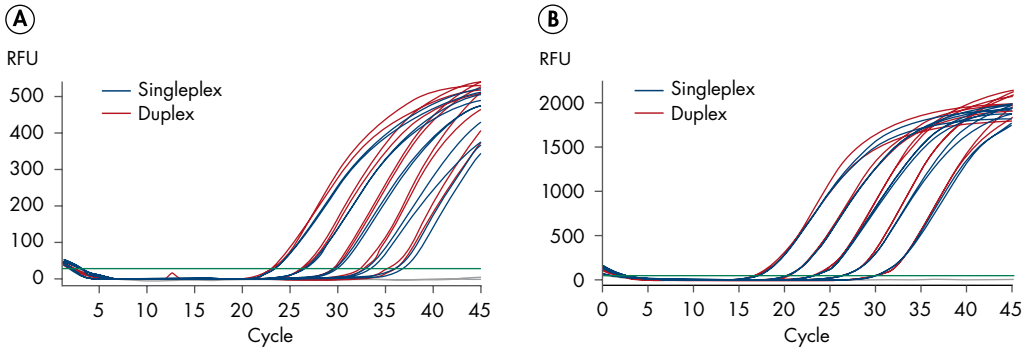

Figure 4. Comparable results in duplex and singleplex PCR. Duplex and singleplex PCR was performed on a Bio-Rad CFX96™ cycler using TaqMan® Assays for GAPD and TNF with the QuantiNova Probe PCR Kit. Ten-fold dilutions of leukocyte DNA (from 100 ng to 10 pg) were used as templates and reactions were run in triplicate. Overlay of the TNF amplification curve for the singleplex and duplex reactions and overlay of the GAPD amplification curve for the singleplex and duplex reactions. The plots demonstrate the comparability and reliability of the results for singleplex and duplex amplification using the QuantiNova Probe PCR Kit.

Superior performance for difficult targets

The unique combination of QIAGEN's proprietary and well-proven buffer technology along with the new QuantiNova DNA Polymerase, QuantiNova Antibody, and QuantiNova Guard, ensures real-time PCR success at the first attempt, without the need for costly and time-consuming optimization, even with challenging real-time PCR assays (Figure 5).

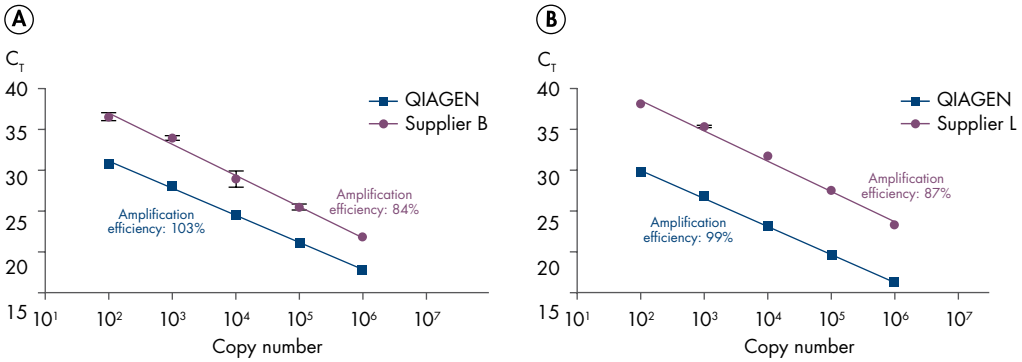

Figure 5. Superior results with challenging assays. The performance of the QuantiNova Probe PCR Kit was compared to a probe PCR kit from supplier B on a Bio-Rad CFX Connect cycler **A** and a probe PCR kit from supplier L **B** on a ViiA7™ cycler. Reactions were run in triplicate using 10-fold dilutions of plasmid DNA (10^6–10^2 copies per reaction) and a TaqMan assay detecting a 500 bp amplicon and using a minor-groove binding probe. The QuantiNova Probe PCR Kit provides significantly lower C_T values, higher reproducibility, and higher reaction efficiency, compared to the probe PCR kits from suppliers B and L.

Ordering Information

Product	Contents	Cat. no.
QuantiNova Probe PCR Kit (100)	1 ml 2x QuantiNova Probe PCR Master Mix, 250 µl QN ROX Reference Dye, 500 µl QuantiNova Yellow Template Dilution Buffer, and 1.9 ml Water for 100 x 20 µl reactions	208252
QuantiNova Probe PCR Kit (500)	3 x 1.7 ml 2x QuantiNova Probe PCR Master Mix, 1 ml QN ROX Reference Dye, 500 µl QuantiNova Yellow Template Dilution Buffer, and 1.9 ml Water for 500 x 20 µl reactions	208254
QuantiNova Probe PCR Kit (2500)	15 x 1.7 ml 2x QuantiNova Probe PCR Master Mix, 5 x 1 ml QN ROX Reference Dye, 5 x 500 µl QuantiNova Yellow Template Dilution Buffer, and 5 x 1.9 ml Water for 2500 x 20 µl reactions	208256

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.

Learn more about the QuantiNova Probe PCR Kit at **www.qiagen.com/QuantiNova-Probe-PCR-Kit**!