QuantiNova® SYBR® Green PCR Kit

For unparalleled results using SYBR Green-based qPCR

Featuring enhanced specificity, sensitivity, speed, and process safety, the QuantiNova SYBR Green PCR Kit sets new standards in SYBR Green-based qPCR. A novel hot-start mechanism significantly increases the specificity of real-time PCR and optimized chemistry produces accurate quantitative results from cDNA or gDNA on any real-time PCR cycler with detection of even a single target copy. Finally, a built-in visual indicator ensures correct pipetting.

The QuantiNova SYBR Green Kit streamlines qPCR by delivering:

- Unmatched specificity due to a novel antibody-mediated hot-start mechanism
- Precise reaction setup with a built-in visual indicator to minimize pipetting errors
- Accurate results with robust detection of rare targets down to a single copy
- Magnified throughput and speed with ultrafast cycling
- Seamless performance when combined with QuantiTect® Primer Assays

A novel, highly stringent hot-start mechanism

The hot-start mechanism of the QuantiNova SYBR Green PCR Kit is a stringent barrier to non-specific amplification resulting in unmatched real-time PCR specificity. At low temperatures, the novel additive QuantiNova Guard stabilizes a binding complex between QuantiNova DNA Polymerase and the QuantiNova Antibody. QuantiNova DNA Polymerase remains inactive in this configuration. 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 PCR amplification (Figure 1). The heightened stringency of the hot-start prevents extension of nonspecifically annealed primers and the formation of primer-dimers. ▶

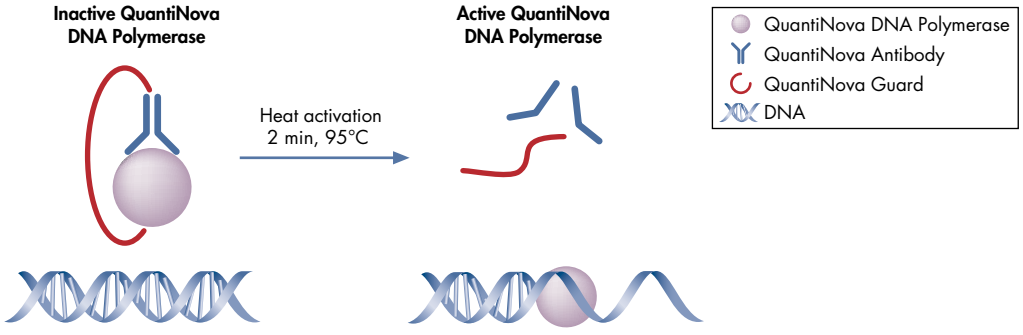


Figure 1. Mechanism of the highly stringent QuantiNova hot-start. Sequestered in a binding complex with the QuantiNova Antibody, QuantiNova DNA Polymerase is kept in an inactive state until the initial heat activation step denatures the complex-stabilizer QuantiNova Guard and the QuantiNova Antibody.

A built-in visual indicator of accurate reaction setup

The chemistry of the QuantiNova SYBR Green PCR Kit is enhanced with a built-in control that minimizes pipetting errors during reaction setup. The master mix contains an inert blue dye that does not interfere with the qPCR reaction, but improves the visibility of contents in the reaction vessel. The QuantiNova Yellow Template Dilution Buffer, used to dilute the template nucleic acid, contains an inert yellow dye. Upon adding the diluted template DNA to the master mix, the color of the solution changes from blue to green (Figure 2), providing a visual indication that each reaction was set up correctly.

Figure 2. Accurate reaction setup indicated by the built-in pipetting control. The QuantiNova SYBR Green PCR Master Mix contains an inert blue dye. Combined with QuantiNova Yellow Template Dilution Buffer, the resulting solution turns green, indicating that the reaction was set up correctly.

While the yellow dye of the QuantiNova Yellow Template Dilution Buffer is a fail-proof way of ensuring accurate reaction setup without impacting real-time PCR performance (Figure 3), the dilution buffer is not required to obtain superior results with the QuantiNova SYBR Green PCR Kit. Thus, the user is not restricted to a particular template buffer, just like the QuantiNova SYBR Green PCR Kit can be used with any real-time PCR cycler.

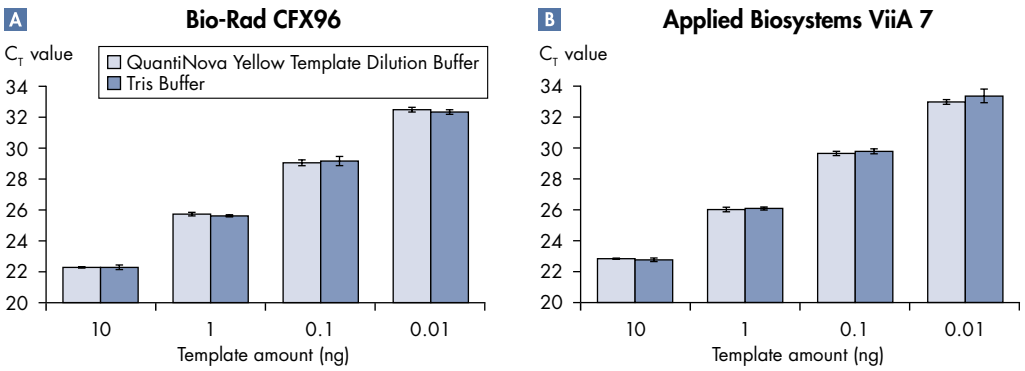


Figure 3. Maximum performance with and without QuantiNova Yellow Template Dilution Buffer. EGFR was amplified from HeLa cDNA using a QuantiTect Primer Assay and the QuantiNova SYBR Green PCR Kit. The template DNA was diluted from 10 ng to 0.01 ng using QuantiNova Yellow Template Dilution Buffer or Tris buffer, and reactions were performed on the A Bio-Rad® CFX96 and the B Applied Biosystems® ViiA® 7 instruments. Resulting C_T values were comparable for both buffers. All reactions were performed in triplicate.

Sensitive detection of single-copy targets

The unique formulation of the QuantiNova SYBR Green PCR Kit enables robust and precise detection of even single target copies (Figure 4). This extreme sensitivity is achieved even under ultrafast cycling conditions making the QuantiNova SYBR Green PCR Kit the optimal choice for challenging experiments with precious DNA samples that require highest sensitivity.

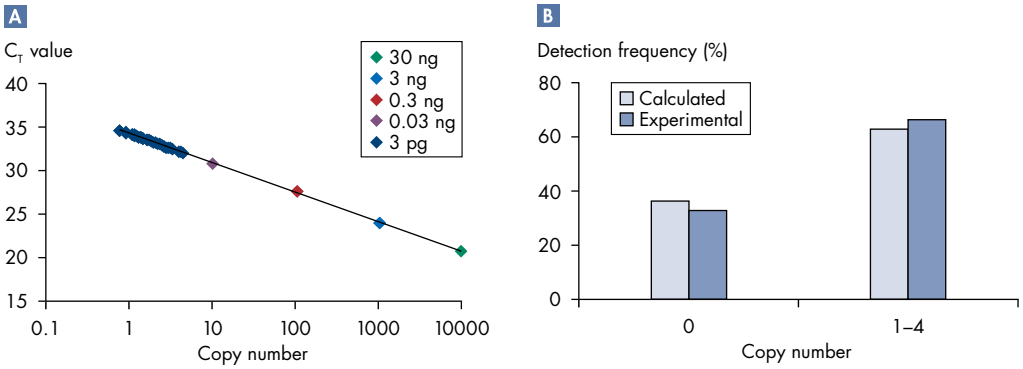


Figure 4. Robust and sensitive detection of single-copy targets. A The single-copy gene IL1R2 was detected in 30, 3, 0.3 and 0.03 ng of leukocyte genomic DNA using the QuantiNova SYBR Green PCR Kit to generate a calibration curve for C_T value versus number of target copies. The used volumes correspond to copy numbers ranging from 10 to 10,000 and the correlation to C_T value was highly linear. The calibration curve was subsequently used to determine the actual number of target copies in 60 reactions set up to theoretically contain a single copy. The reactions were aliquoted from a master mix and thus, statistical variation led to some reaction wells having more than one copy and others having no copies. B Theoretical number of copies expected in each reaction was calculated using Poisson's equation and compared to the actual number of copies determined with the calibration curve. The calculated and experimental frequencies of detection for low copy numbers were highly concordant, demonstrating the high sensitivity and robustness of the QuantiNova chemistry.

Top results with any template amount and cycler

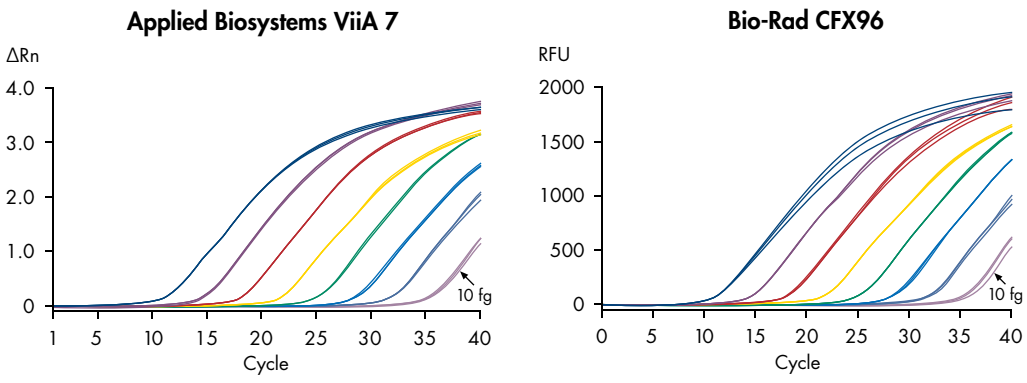
The QuantiNova SYBR Green PCR Kit accurately quantifies a wide range of template amounts. Detection is precise and consistent within a dynamic range of input material covering 8 orders of magnitude (Figure 5). The stringent specificity and exceptional sensitivity of the QuantiNova ▶

A

HeLa cDNA	ViiA 7	CFX96
100 ng	11.33	11.55
10 ng	14.67	14.94
1 ng	18.11	18.37
100 pg	21.74	22.12
10 pg	25.08	25.40
1 pg	28.57	28.74
100 fg	31.85	32.29
10 fg	35.39	36.02
No template control	–	–
PCR efficiency	95%	94%

Figure 5. Accurate quantification over a wide dynamic range. Ten-fold dilutions of HeLa cDNA ranging from 100 ng to 10 fg were quantified on the Applied Biosystems ViiA 7 instrument and the Bio-Rad CFX96 using an in-house assay for beta-actin. A The QuantiNova SYBR Green PCR Kit delivers accurate results over a wide dynamic range of 8 orders of magnitude. B This range is confirmed by the comparable amplification plots from both instruments.

B



chemistry produces optimal results on the QIAGEN® Rotor-Gene® Q, but can be used on any real-time PCR cycler, regardless of format, fast-cycling capacity, and need for a passive reference dye. ROX provided with the QuantiNova SYBR Green PCR Kit is simply added to the master mix if required. Amplification and quantification results using the Rotor-Gene Q, Agilent® Technologies Mx3005P, Applied Biosystems 7900 HT Fast, ViiA 7, StepOnePlus™, and 7500 Fast, Roche® LightCycler® 480, and Bio-Rad CFX96 are invariably robust and sensitive. Visit the QuantiNova SYBR Green PCR Kit catalog page at www.qiagen.com for details.

Setting new standards in SYBR Green-based real-time PCR

The QuantiNova SYBR Green PCR Kit combines the innovative QuantiNova hot-start with QIAGEN's proven buffer technology to deliver unparalleled specificity and sensitivity under a broad range of cycling conditions. As a result, the QuantiNova chemistry is easily incorporated into standing workflows with your instrument of choice. Along with features for easy reaction setup and robust performance even under ultrafast cycling, the QuantiNova SYBR Green PCR Kit ensures real-time PCR success at the first attempt.

Ordering Information

Product	Contents	Cat. no.
QuantiNova SYBR Green PCR Kit (100)	For 100 x 20 µl reactions: 1 ml 2x QuantiNova SYBR Green PCR Master Mix, 500 µl QuantiNova Yellow Template Dilution Buffer, 250 µl QN ROX Reference Dye, 1.9 ml RNase-Free Water	208052
QuantiNova SYBR Green PCR Kit (500)	For 500 x 20 µl reactions: 3 x 1.7 ml 2x QuantiNova SYBR Green PCR Master Mix, 500 µl QuantiNova Yellow Template Dilution Buffer, 1 ml QN ROX Reference Dye, 1.9 ml RNase-Free Water	208054
QuantiNova SYBR Green PCR Kit (2500)	For 2500 x 20 µl reactions: 15 x 1.7 ml 2x QuantiNova SYBR Green PCR Master Mix, 5 x 500 µl QuantiNova Yellow Template Dilution Buffer, 5 x 1 ml QN ROX Reference Dye, 5 x 1.9 ml RNase-Free Water	208056
QuantiTect Primer Assay (200)	For 200 x 50 µl reactions, 400 x 25 µl reactions, or 500 x 20 µl reactions: 10x QuantiTect Primer Assay (lyophilized)	Varies

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.

Get started with the QuantiNova SYBR Green PCR Kit at www.qiagen.com.

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