Product Profile

QuantiNova Probe RT-PCR Kit

For probe-based, one-step RT-qPCR with in-process safety measures

Reliable gene expression analysis requires sensitive and reproducible reverse transcription and RNA amplification. Target RNA quantitation can be significantly impacted by the variability introduced by RNA extraction, the RT-PCR reaction or set-up. These variables can lead to under- or overestimation of expression profiles due to inhibitor presence, premature enzyme activity, gDNA carry-over adding to the specific transcript signal and more. The QuantiNova® Probe RT-PCR Kit, with its built-in controls, detects and helps you avoid these sources of misquantitation. Additionally, our unique, two-phase hot-start mechanism enables true, room temperature reaction set-up and at least 2 hours of room temperature stability preventing premature enzyme activity and creation of artifacts. Ensure precise and reproducible real-time RT-PCR results with the QuantiNova Probe RT-PCR Kit.

The QuantiNova Probe RT-PCR Kit provides:

- Internal control for in-process verification of successful RT-PCR
- Unique, two-phase hot-start procedure for room-temperature set-up
- Visual pipetting control, preventing pipetting errors
- Duplex capability, enabling inclusion of internal control or reference gene
- gDNA reduction for improved quantitation accuracy
- Accurate quantitation over several logs of template

Complete confidence in your target quantitation

Easily confirm successful experiments with internal control RNA

IC RNA is transcribed into cDNA and amplified alongside your target RNA – Reporting instrument or chemistry failures, errors in assay set-up and the presence of inhibitors (Figure 1). Use of the included IC is optional. The assay for the probe-based detection of IC is available separately with the choice of two different dyes. The duplex capacity of the QuantiNova Probe RT-PCR Kit allows simultaneous detection of IC and target RNA in one reaction.

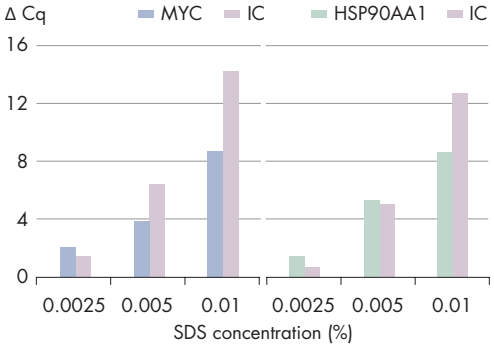

Figure 1. Reliable monitoring of RT-PCR inhibition. Reactions containing total RNA (1ng/rxn) from HeLa cells and internal control (IC) RNA were spiked with increasing amounts of SDS, a potent RT-PCR inhibitor. In a duplex reaction, MYC and HSP90AA1 were co-amplified with the IC RNA (using the QuantiNova IC Probe Assay). Resulting Cq shifts were comparable for the internal control and the endogenous target transcripts, demonstrating that the IC RNA can be used to detect the presence of inhibitors.

True, room temperature set-up and reaction stability with our unique two-phase hot-start mechanism

Our unique, two-phase hot-start mechanism enables true, room temperature reaction set-up and at least 2 hours of stability at room temperature, preventing premature enzyme activity and creation of artifacts (Figure 2). Say goodbye to inconvenient pipetting on ice with the QuantNova Probe RT-PCR Kit – Perfectly designed for automated reaction set-up and greater accuracy and workflow efficiency.

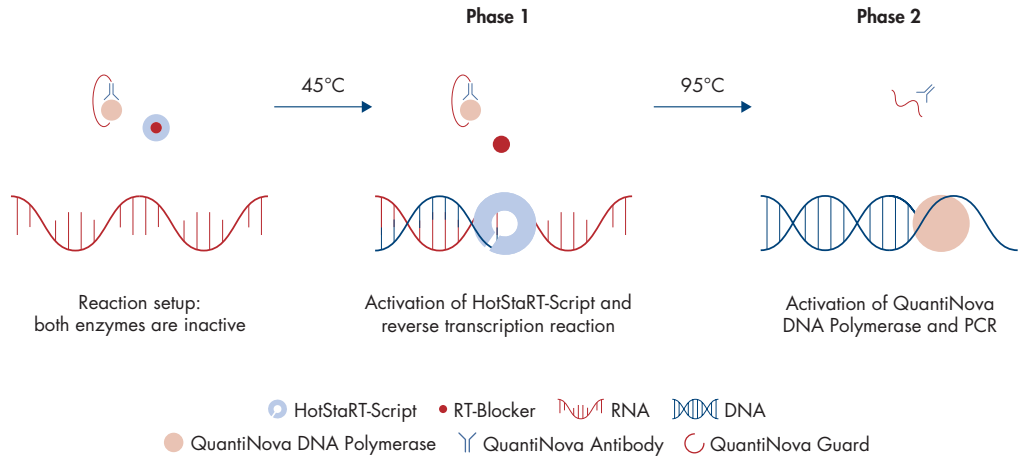

Figure 2. Principle of the novel QuantiNova two-phase hot-start mechanism. At ambient temperature, the HotStaRTScript is inhibited by the RT-blocker and the QuantiNova DNA polymerase is kept inactive by QuantiNova Antibody and QuantiNova Guard. At 45°C, the RT is activated while the QuantiNova DNA polymerase remains inactive. At 95°C, the RT enzyme is denatured and the DNA polymerase is activated.

Prevent pipetting errors with our built-in, visual pipetting control

With the built-in, visual pipetting control, you can easily verify master mix dispensing and template addition – preventing pipetting errors and stress during reaction set-up. This is particularly useful when working with 96 or 384 wells.

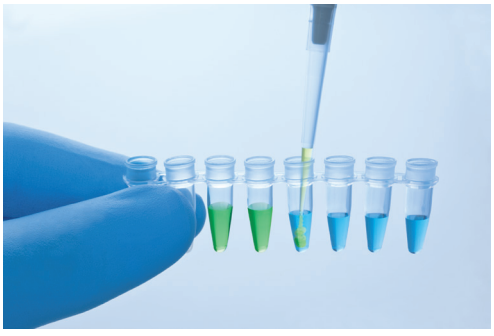

Figure 3. Accurate reaction set-up indicated by the built-in pipetting control. The master mix contains an inert blue dye, which when combined with the QuantiNova yellow template dilution buffer, turns the resulting solution green. This visual confirmation indicates that the reaction was set-up correctly.

Minimize RT-qPCR variations with integrated genomic DNA reduction

Minimizing effects of genomic DNA is crucial for accurate gene expression results, particularly when design of RNA-specific primers or probes is not possible. gDNA presence can add to the specific transcript signal, leading to over-estimation of the expression profile. With integrated gDNA reduction, work and costs associated with separate DNase digestion are reduced.

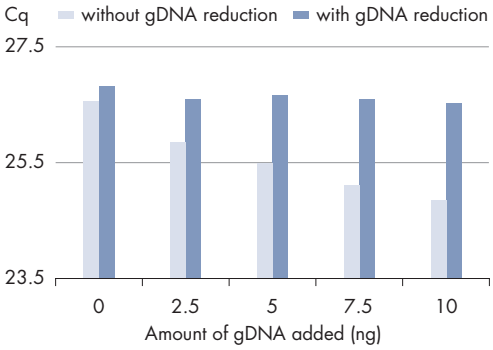

Figure 4. Increased reliability of gene expression results using gDNA reduction. Total HeLa RNA (10 ng/rxn) samples were spiked with increasing amounts of human genomic DNA. Expression of MGAT1 was analyzed by RT-qPCR. MGAT1 is a single-exon gene that does not allow the discrimination of RNA and gDNA by assay design. Without the gDNA reduction step, Cq values decreased linearly, misleadingly indicating an increase in expression rate. In contrast, the expression levels remained similar when using the gDNA reduction step.

Precise quantitation over a wide dynamic range

Accurately quantitate low-copy RNA targets over a wide linear range on any common cycler with the HotStaRT-Script Reverse Transcriptase, QuantiNova DNA Polymerase and the unique composition of the RT-PCR buffer included in the QuantiNova Probe RT-PCR Kit.

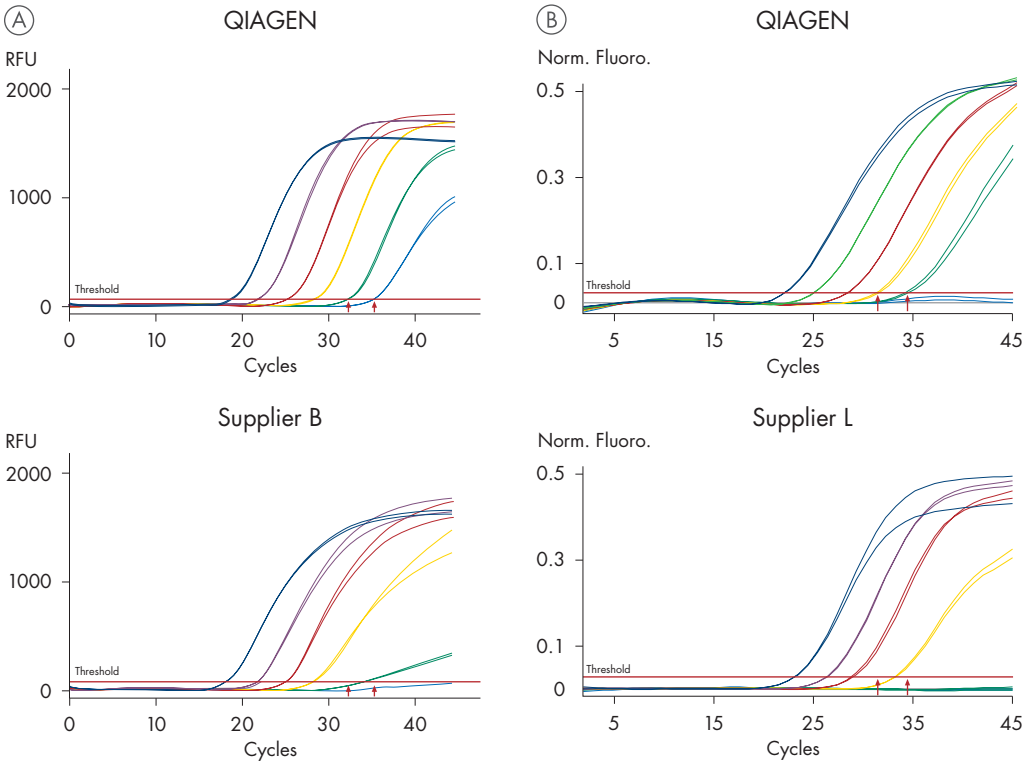

Figure 5. Superior sensitivity of the QuantiNova Probe RT-PCR Kit. QuantiNova Probe RT-PCR Kit performance was compared to probe RT-PCR kits from supplier B and L. Reactions were run in duplicate using 10-fold dilutions of HeLa total RNA (100 ng – 10 pg) and TaqMan® assays for MYC **A** or CDKNB1 **B**. Results show a high correlation between kits for higher template amounts; however, sensitivity was higher with the QuantiNova Probe RT-PCR Kit at 100 and 10 pg of total RNA (red arrows).

Base your results on facts, not artifacts with state-of-the-art performance and in-process controls

Combining an extraordinarily fast protocol with superior performance and integrated controls, the QuantiNova Probe RT-PCR Kit ensures data interpretation is not compromised by artifacts or variables, but based on the true expression profile. An efficient, ultra-fast workflow and optimal results are achieved in combination with the QuantiNova IC Assay.

Ordering Information

Product	Contents	Cat. no.
QuantiNova Probe RT-PCR Kit (100)	For 100 x 20 µl reactions: 1 ml QuantiNova Probe RT-PCR Master Mix, 20 µl QuantiNova Probe RT Mix, 20 µl Internal Control RNA, 500 µl Yellow Template Dilution Buffer, 250 µl ROX Reference Dye, 1.9 µl RNase-Free Water	208352
QuantiNova Probe RT-PCR Kit (500)	For 500 x 20 µl reactions: 3 x 1.7 ml QuantiNova Probe RT-PCR Master Mix, 100 µl QuantiNova Probe RT Mix, 100 µl Internal Control RNA, 500 µl Yellow Template Dilution Buffer, 1 ml ROX Reference Dye, 2 x 1.9 µl RNase-Free Water	208354
QuantiNova Probe RT-PCR Kit (2500)	For 2500 x 20 µl reactions: 15 x 1.7 ml QuantiNova Probe RT-PCR Master Mix, 5 x 100 µl QuantiNova Probe RT Mix, 5 x 100 µl Internal Control RNA , 5 x 500 µl Yellow Template Dilution buffer, 5 x 1 ml ROX Reference Dye, 10 x 1.9 µl RNase-Free Water	208356
QuantiNova IC Probe Assay (200)	For 200 reactions: 400 µl primer / probe mix (10x). Detects internal control RNA; use with QuantiNova Probe PCR Kit or QuantiNova Probe RT-PCR Kit	205813
QuantiNova IC Probe Assay Red 650 (500)	For 500 reactions: 1000 µl IC Probe Assay Red (Cy5 analog label, available as an accessory only)	205824

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