Product Profile

QuantiNova® Reverse Transcription Kit

For fast cDNA synthesis with in-process safety measures for real-time two-step RT-PCR

Reliable gene expression analysis relies on quantitative and reproducible reverse transcription of various RNA molecules present in sample materials. Variations in extraction and purification of target RNA, or in real-time RT-PCR setup and performance can significantly impact on the quantification of target RNA. These variables can potentially lead to reduced expression profiles (e.g., caused by inhibitors) or to an overestimation of expression profiles (e.g., caused by genomic DNA carryover adding to the specific transcript signal). The QuantiNova Reverse Transcription Kit detects and minimizes these sources of misquantification, ensuring precise and reproducible real-time RT-PCR results.

The QuantiNova Reverse Transcription Kit provides:

- Fast and controlled cDNA synthesis, including Internal Control RNA (IC RNA)
- Genomic DNA removal for precise RNA quantification
- Wide input range (10 pg – 5 µg, upscalable)
- Simple and very fast 20 min procedure

Get results faster and benefit from integrated safety measures

The QuantiNova Reverse Transcription Kit provides a very fast, convenient procedure for cDNA synthesis with integrated genomic DNA removal and an additional internal control. Genomic DNA contamination in RNA samples is effectively eliminated by the gDNA Removal Mix, and the internal control RNA can be used to test successful reverse transcription and amplification. Genomic DNA removal and cDNA synthesis take only 20 minutes with the QuantiNova Reverse Transcription Kit.

QuantiNova IC RNA confirms successful experiments

Detecting variations in cDNA synthesis or qPCR allows the reproducibility of your results to be monitored. The newly developed QN IC RNA is a defined transcript that can be optionally added to samples and transcribed into cDNA. It is intended to report instrument or chemistry failures, errors in assay setup and the presence of inhibitors (Figure 1). ▷

While the IC RNA is provided in the kit, the respective assays for either SYBR® Green- or probe-based detection of IC RNA from cDNA are available separately. To completely monitor the real-time two-step RT-PCR procedure, we recommend the QuantiNova SYBR Green PCR Kit or the QuantiNova Probe PCR Kit for cDNA quantification. Both kits provide a visual pipetting control, a novel stringent hot-start and ultrafast qPCR protocols. The duplex capacity of the QuantiNova Probe PCR Kit allows simultaneous detection of IC RNA and target RNA in one reaction.

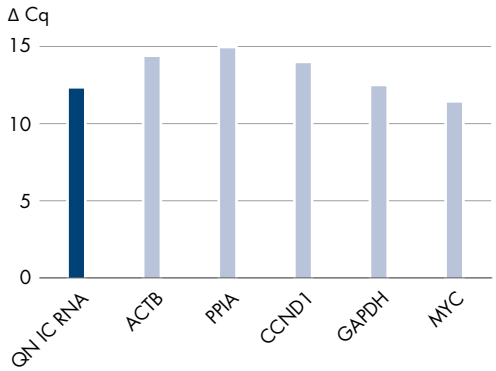

Figure 1. Internal control reliably detects the presence of inhibitors. Real-time two-step RT-PCR analysis of various target genes and the QuantiNova Internal Control RNA (QN IC RNA). One microgram of total RNA was purified from human whole blood, and the QN IC RNA was added to the RT reaction. RNA samples with inhibitor (0.003% sodium dodecyl sulfate) and without were tested in parallel. Real-time PCR was performed in triplicate on a Bio-Rad® CFX384 using a 1:100 cDNA dilution and the QuantiNova SYBR Green PCR Kit. Resulting Cq shifts for the internal control and endogenous target transcripts were comparable, demonstrating that the IC RNA can be used to detect the presence of inhibitors.

Eliminate RT-qPCR variations by eliminating genomic DNA

Varying amounts of gDNA can add to the specific transcript signal, leading to an overestimation of the expression profile. Elimination of genomic DNA is crucial for accurate gene expression results and when it's not possible to design RNA-specific primers or probes, for example, when analyzing single-exon genes. With our gDNA Removal Buffer, time is saved and costs are reduced, since a separate DNase digestion during or after purification of RNA samples is not required (Figure 2).

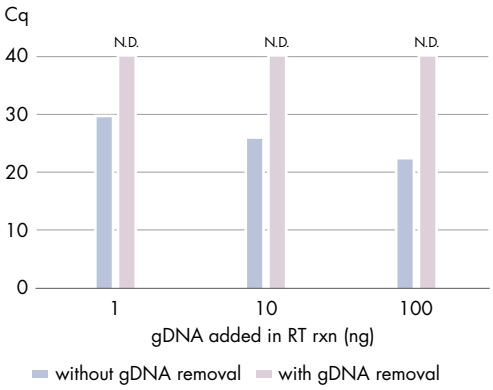

Figure 2. Efficient removal of contaminating gDNA ensures precise quantification of transcripts. Real-time two-step RT-PCR analysis of ACTB with and without gDNA removal step. Genomic DNA purified from human whole blood was used. Different amounts of gDNA (100 ng, 10 ng, 1 ng) were spiked into the cDNA synthesis reactions prepared using the QuantiNova Reverse Transcription Kit. Real-time PCR was performed in triplicate on an Applied Biosystems® 7500 Fast Real-Time PCR System using a 1:10 cDNA dilution and the QuantiNova Probe PCR Kit. Without gDNA removal, genomic DNA contamination resulted in a strong signal. This signal was successfully eliminated by the integrated gDNA removal step (N.D. = not detected).

Precise quantification over a wide dynamic range

The high RNA affinity of the QuantiNova Reverse Transcriptase enables high yields of cDNA from any RNA template. Difficult templates, such as those with high GC-content or complex secondary structure, are also successfully reverse transcribed. The Reverse Transcription Mix contains a specially optimized mix of oligo-dT and random primers that enable cDNA synthesis from all regions of RNA transcripts, including 5' regions. The kit provides high yields of cDNA template for real-time PCR analysis, regardless of where the amplified target region is located on the transcript, and provides greater sensitivity in the detection of low-abundance genes. The QuantiNova Reverse Transcription Kit offers accurate results over a wide range of input amounts, including up to 5 µg RNA in the standard protocol (Figure 3), which can also be doubled to accommodate exceptionally large amounts of RNA.

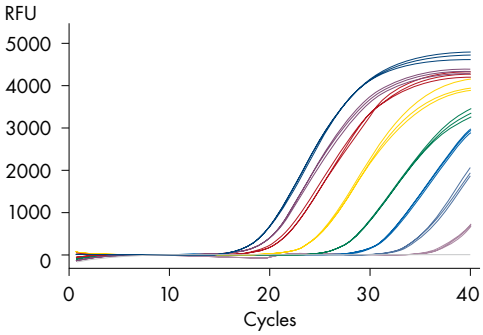

Figure 3. Precise quantification from 5µg – 10 pg RNA. Real-time two-step RT-PCR analysis of RPS27A. Total RNA was purified from human whole blood. cDNA was then synthesized from a serial dilution of RNA using the QuantiNova Reverse Transcription Kit (5 µg – 10 pg RNA). Real-time PCR was performed in triplicate on a Bio-Rad CFX384 using a 1:100 cDNA dilution and the QuantiNova Probe PCR Kit. The amplification plot shows a high dynamic range of the cDNA synthesis for the complete tested range (5 µg – 10 pg total RNA).

High-quality performance and in-process safety measures for relevant data interpretation

By combining an extremely fast protocol with superior performance and integrated safety measures, the QuantiNova Reverse Transcription Kit ensures your data is not compromised by artifacts or variables, but based on the true expression profile. Achieve an efficient, ultra-fast workflow with optimal results by combining this kit with the QuantiNova IC Assays and the QuantiNova SYBR Green PCR Kit or the QuantiNova Probe PCR Kit for cDNA quantification.

Ordering Information

Product	Contents	Cat. no.
QuantiNova Reverse Transcription Kit (10)	20 µl 8x gDNA Removal Mix, 10 µl Reverse Transcription Enzyme, 40 µl Reverse Transcription Mix (containing RT primers), 20 µl IC RNA, 1.9 ml RNase-Free Water	205410
QuantiNova Reverse Transcription Kit (50)	100 µl 8x gDNA Removal Mix, 50 µl Reverse Transcription Enzyme, 200 µl Reverse Transcription Mix (containing RT primers), 100 µl IC RNA, 1.9 ml RNase-Free Water	205411
QuantiNova Reverse Transcription Kit (200)	4 x 100 µl 8x gDNA Removal Mix, 4 x 50 µl Reverse Transcription Enzyme, 4 x 200 µl Reverse Transcription Mix (containing RT primers), 4 x 100 µl IC RNA, 4 x 1.9 ml RNase-Free Water	205413
QuantiNova IC Probe Assay (200)	400 µl primer/probe mix (10x) for 200 reactions, detects IC RNA; use with QN Probe PCR Kit or QN Probe RT-PCR Kit	205813
QuantiNova IC SYBR Green Assay (500)	QuantiTect Primer Assay for SYBR-based detection of QN IC RNA; available via GeneGlobe® (sufficient for approx. 500 x 20 µl rxn). For use with QN SYBR Green PCR Kit or QN SYBR Green RT-PCR Kit	
qPCR Kits		
QuantiNova Probe PCR Kit (100)	1 ml 2x QN Probe PCR Master Mix, 250 µl QN ROX Reference Dye, 500 µl QN Yellow Template Dilution Buffer, 1.9 ml Water	208252
QuantiNova SYBR Green PCR Kit (100)	1 ml 2x QN SYBR Green PCR Master Mix, 500 µl QN Yellow Template Dilution Buffer, 250 µl QN ROX Reference Dye, 1.9 ml Water	208052
QuantiTect Primer Assay (200)	10x QuantiTect Primer Assay (lyophilized) supplied in single tube	249900
Instruments		
Rotor-Gene Q 5plex System	Real-time PCR cycler with 5 channels (g, laptop computer, software	9001640
QIAgility System HEPA/UV (incl. PC)	Robotic workstation for automated PCR setup (with UV light and HEPA filter), notebook computer, and QIAgility Software	9001532

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.

Ensure your results are based on facts – not artifacts. Visit **www.qiagen.com/QNRTKit**.