December 2021

QuantiNova® LNA® PCR Handbook

For highly sensitive, real-time RT-PCR detection of mRNA and lncRNA using SYBR® Green

Sample to Insight

QIAGEN

Contents

Kit Contents

Assay (1 single tube containing dried primermix)	Catalog no. (200 reactions)	Catalog no. (750 reactions)
QuantiNova LNA PCR Assay	249990	249992
QuantiNova LNA PCR Custom Assay	249910	249911
QuantiNova LNA PCR Reference Assay	249920	249921

Panel (one 96- or 384-well plate containing dried primer-mix for 1 reaction in each well)	Catalog no.
QuantiNova LNA PCR Focus Panels	249950
QuantiNova LNA PCR lncRNA Focus Panels	249951
QuantiNova LNA PCR Flexible Panels	249960
QuantiNova LNA PCR Custom Panels	249970

Product	Catalog no.
QuantiNova Reverse Transcription Kit (for 10, 50, or 200 reactions)	205410, 205411, or 205413
QuantiNova SYBR Green PCR Kit (for 100, 500, or 2500 reactions)	208052, 208054, or 208056
QuantiNova SYBR Green RT-PCR Kit (for 100, 500, or 2500 reactions)	208152, 208154, or 208156
QuantiNova IC SYBR Green Assay (500)	QT02589307*

* Available from **geneglobe.qiagen.com**

Shipping and Storage

The QuantiNova LNA PCR Assays and Panels are shipped at room temperature (15–25°C). Upon receipt, store QuantiNova LNA PCR Assays and Panels at 2–8°C for short-term storage or at –30 to –15°C in a constant-temperature freezer for long-term storage. After resuspension of the PCR Assays, it is recommended to store these in aliquots at –30 to –15°C to avoid repeated freeze-thaw cycles. Under these conditions, all components are stable for at least 12 months if not otherwise indicated on the label.

Intended Use

The QuantiNova LNA PCR Assays and Panels are intended for molecular biology applications. These products are not intended for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of the product. We recommend all users of QIAGEN® products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at **www.qiagen.com/safety** where you can find, view, and print the SDS for each QIAGEN kit and kit component.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of QuantiNova LNA PCR Assays and Panels are tested against predetermined specifications to ensure consistent product quality.

Introduction

QuantiNova LNA PCR Assays and Panels provide highly sensitive and accurate locked nucleic acid (LNA)-enhanced real-time PCR quantification assays for mRNA and lncRNA targets in an easy-to-handle format. They are designed for use with universal reverse transcription (RT), followed by real-time PCR amplification using SYBR Green for detection.

The QuantiNova LNA PCR Assays and Panels are developed and optimized for use with QIAGEN QuantiNova reagents. To obtain optimal results, the use of QuantiNova LNA PCR products in combination with the following QIAGEN QuantiNova products is recommended:

- For 2-step RT-PCR procedures: QuantiNova Reverse Transcription Kit (cat. no. 205410, 205411, or 205413) and QuantiNova SYBR Green PCR Kit (cat. no. 208052, 208054, or 208056).
- For 1-step RT-PCR procedures: QuantiNova SYBR Green RT-PCR Kit (cat. no. 208152, 208154, or 208156).

The QuantiNova Reverse Transcription Kit provides a fast, convenient procedure for efficient reverse transcription and effective genomic DNA removal. The kit is designed for use as part of real-time 2-step RT-PCR and provides high cDNA yields for sensitive quantification of even low-abundance transcripts. This kit also includes QuantiNova Internal Control (QN IC) RNA template to test successful reverse transcription and amplification.

The QuantiNova SYBR Green PCR Kit provides the highest specificity in real-time PCR because of a novel antibody-mediated hot-start mechanism. At low temperatures, the QuantiNova DNA Polymerase is kept in an inactive state by the QuantiNova Antibody and QuantiNova Guard, a novel additive that stabilizes the complex. This improves the stringency of the hot-start mechanism and prevents extension of nonspecifically annealed primers and formation of primer–dimers.

The QuantiNova SYBR Green RT-PCR Kit combines a unique 2-phase hot-start and PCR buffer system in the ready-to-use Master Mix to enable 1-step qRT-PCR with QuantiNova LNA PCR

Assays and Panels. This QuantiNova SYBR Green RT-PCR Kit also includes QuantiNova Internal Control (QN IC) RNA template to test successful reverse transcription and amplification.

Table 1. Descriptions of QuantiNova LNA system components

Component	Description
QuantiNova LNA PCR Assays	Single primer-mix designed for highly sensitive and rapid real-time quantification of single mRNA and lncRNA targets
QuantiNova LNA PCR Panels	Panels of primer-mixes designed for highly sensitive and rapid real-time quantification of mRNA and lncRNA targets in 96- or 384-well plates
QuantiNova Reverse Transcription Enzyme	An optimized enzyme developed for use in real-time 2-step PCR
QuantiNova gDNA Removal Mix	Buffer and enzyme for effective removal of genomic DNA contamination from starting RNA samples, including an RNase inhibitor
QuantiNova Reverse Transcription Mix	Buffer optimized for reverse transcription with QuantiNova Reverse Transcription Enzyme; contains an optimized combination of oligo dT and random primers and includes Mg^{2+} and dNTPs. It allows high cDNA yields from all regions of RNA transcripts, even from 5′ regions.
QuantiNova Internal Control (QN IC) RNA	Synthetic transcript for monitoring successful reverse transcription
QuantiNova DNA Polymerase	A modified form of a recombinant 94 kDa DNA polymerase originally isolated from *Thermus aquaticus*. QuantiNova DNA Polymerase is provided in an inactive state and has no enzymatic activity at ambient temperature. The enzyme is activated by a 2 min 95°C incubation step.
QuantiNova SYBR Green PCR Buffer or QuantiNova SYBR Green RT-PCR Buffer	Contains Tris·Cl , KCl, NH_4Cl, $MgCl_2$, and additives enabling fast cycling, including Q-Bond®
dNTP mix	Contains dATP, dCTP, dGTP, and dTTP of ultrapure quality
Fluorescent dye	SYBR Green I
QN ROX™ Reference Dye	Optimized concentration of fluorescent dye for normalization of fluorescent signals on all instruments from Applied Biosystems®
QuantiNova Yellow Template Dilution Buffer	Ultrapure quality, PCR-grade dilution buffer
HotStarRT-Script Reverse Transcriptase	A modified form of a recombinant 77 kDa reverse transcriptase. It is provided in an inactive state and has minimal enzymatic activity at ambient temperature. The enzyme is activated during the reverse transcription step at 50°C.
RNase inhibitor	A recombinant mammalian protein that inhibits eukaryotic RNases, such as RNase A and B
DNase	RNase-free DNase
RNase-free water	Ultrapure quality, PCR-grade water

Principle and procedure

The QuantiNova LNA PCR System is a unique system for mRNA and lncRNA profiling that offers the best combination of performance and ease-of-use tools on the mRNA and lncRNA real-time PCR market.

- **cDNA synthesis:** The first-strand cDNA synthesis reaction provides the template for all real-time mRNA/lncRNA PCR assays. This saves precious sample, reduces technical variation, consumes less reagents, and saves time in the laboratory. The same cDNA synthesis can be used across all assay and panel formats.
- **LNA-enhanced PCR amplification**: 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 result is exceptional sensitivity and specificity with extremely low background, enabling accurate quantification of very low levels of mRNA/lncRNA.

The QuantiNova LNA PCR System and the outstanding performance of the QuantiNova SYBR Green PCR Kit offer solutions for both high-throughput mRNA/lncRNA expression profiling and for quantification of individual mRNAs/lncRNAs.

High specificity and sensitivity in real-time PCR are achieved by a hot-start procedure. This allows room-temperature setup of the PCR reaction without the risk of primer–dimer formation. The hot start is achieved using QuantiNova DNA Polymerase, which is a novel hot-start enzyme, and the additive QuantiNova Guard. These unique components further improve the stringency of the antibody-mediated hot start.

The QuantiNova SYBR Green PCR Kit and QuantiNova SYBR Green RT-PCR Kit also features a built-in control for visual identification of correct template addition, as well as Q-Bond, an additive in the PCR buffer that enables short cycling steps without loss of PCR sensitivity and efficiency. The kit has been optimized for use with any real-time cycler. ROX Reference Dye is provided in a separate tube and can be added if your cycler requires ROX as a passive reference dye.

LNA technology

LNAs are a class of high-affinity RNA analogs in which the ribose ring is locked in the ideal conformation for Watson-Crick binding. As a result, LNA oligonucleotides exhibit unprecedented thermal stability when hybridized to a complementary DNA or RNA strand. Since LNA oligonucleotides typically consist of a mixture of LNA plus either DNA or RNA, it is possible to optimize the sensitivity and specificity by varying the LNA content of the oligonucleotide. Incorporation of LNA into oligonucleotides has been shown to improve sensitivity and specificity for many hybridization-based technologies (e.g., PCR, microarray, *in situ* hybridization).

For each incorporated LNA monomer, the melting temperature (T_m) of the duplex increases by 2–8°C. When applying this in designing primers for qPCR, it results in assays with increased binding affinity and enhanced sensitivity.

In addition, LNA oligonucleotides can be made shorter than traditional DNA or RNA oligonucleotides and still retain a high T_m. This is of advantage when the oligonucleotides are used for designing primers that precisely should amplify a specific region of a transcript. This is useful for detecting specific isoforms or SNPs. Furthermore, intelligent placement of LNA within the primers can increase the T_m between perfect match and mismatch targets, enabling better discrimination between closely related sequences even with single nucleotide differences.

By varying the number of LNAs incorporated, the primer T_m can be adjusted so that all qPCR primers have the optimal T_m for the specific qPCR cycling conditions irrespective of the target GC content. T_m normalization is especially important for AT-rich transcripts, where it is challenging to design DNA primers with sufficient binding affinity.

Important Notes

Preparing a workspace free of DNA contamination

For accurate and reproducible PCR results, it is important to avoid contamination of the assay with foreign DNA. Any DNA contamination will artificially inflate the detection signal, yielding skewed gene expression profiles and false positive signals. The most common sources of DNA contamination are the products of previous experiments spread into the air of the working environment. To set up and maintain a working environment free of DNA contamination, follow the guidelines below.

- Wear gloves throughout the procedure. Use only fresh PCR-grade reagents (water) and labware (tips and tubes).
- Physically separate the workspaces used for PCR setup and post-PCR processing or non-PCR operations. Prior to each usage, decontaminate the PCR workspace and labware (pipettor barrels, tube racks, etc.) with UV light (to render any contaminating DNA ineffective in PCR through the formation of thymidine dimers) or with 10% bleach (to chemically inactivate and degrade any DNA).
- Close all tubes containing PCR products once you are finished adding or removing volumes. Before discarding any labware (tips or tubes) containing PCR products or other DNA, treat with 10% bleach.
- Do not leave labware (tubes and tip boxes) exposed to the air for long periods of time.

General remarks on handling RNA

Handling RNA

Ribonucleases (RNases) are very stable and active enzymes that generally do not require cofactors to function. Since RNases are difficult to inactivate, and even min amounts are sufficient to degrade RNA, do not use any plasticware or glassware without first eliminating possible RNase contamination. We recommend that you take care to avoid inadvertently introducing RNases into the RNA sample during or after the purification procedure. To create

and maintain an RNase-free environment, the following precautions must be taken during the pretreatment and usage of both disposable and nondisposable vessels and solutions while working with RNA.

General handling

Proper microbiological, aseptic technique should always be used when working with RNA. Hands and dust particles may carry bacteria and molds and are the most common sources of RNase contamination. Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase contamination from the surface of the skin or from dusty laboratory equipment. Change gloves frequently and keep tubes closed whenever possible.

Disposable plasticware

The use of sterile, disposable polypropylene tubes is recommended throughout the procedure. These tubes are generally RNase-free and do not require pretreatment to inactivate RNases.

Nondisposable plasticware

Nondisposable plasticware should be treated before use to ensure that it is RNase-free. Plasticware should be thoroughly rinsed with 0.1 M NaOH,* 1 mM EDTA,* followed by RNase-free water. Alternatively, chloroform-resistant plasticware can be rinsed with chloroform* to inactivate RNases.

Glassware

Glassware should be treated before use to ensure that it is RNase-free. Glassware used for RNA work should be cleaned with a detergent,* thoroughly rinsed, and oven baked at 240°C for 4 h or more (overnight, if more convenient) before use. Autoclaving alone will not fully inactivate many RNases. Alternatively, glassware can be treated with DEPC* (diethyl pyrocarbonate). Fill glassware with 0.1% DEPC (0.1% in water), allow to stand overnight (12 h) at 37°C, and then autoclave or heat to 100°C for 15 min to eliminate residual DEPC.

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate data sheets (SDSs), available from the product supplier.

Solutions

Solutions (water and other solutions) should be treated with 0.1% DEPC.* DEPC is a strong but not absolute inhibitor of RNases. It is commonly used at a concentration of 0.1% to inactivate RNases on glass or plasticware or to create RNase-free solutions and water. DEPC inactivates RNases by covalent modification. Add 0.1 ml DEPC to 100 ml of the solution to be treated, and then shake vigorously to bring the DEPC into solution. Let the solution incubate for 12 h at 37°C. Autoclave for 15 min to remove any trace of DEPC. DEPC will react with primary amines and cannot be used directly to treat Tris* buffers. DEPC is highly unstable in the presence of Tris buffers and decomposes rapidly into ethanol and CO_2. When preparing Tris buffers, treat water with DEPC first, and then dissolve Tris to make the appropriate buffer. Trace amounts of DEPC will modify purine residues in RNA by carbethoxylation. Carbethoxylated RNA is translated with very low efficiency in cell-free systems. However, its ability to form DNA:RNA or RNA:RNA hybrids is not seriously affected, unless a large fraction of the purine residues has been modified. Residual DEPC must always be eliminated from solutions or vessels by autoclaving or heating to 100°C for 15 min.

RNA preparation, quantification, and quality control

The most important prerequisite for any gene expression analysis experiment is consistent, high-quality RNA from every experimental sample. Residual traces of proteins, salts or other contaminants may degrade the RNA or decrease the efficiency of enzyme activities necessary for optimal reverse transcription and real-time PCR performance. RNA quantification and quality control.

For best results from the QuantiNova LNA PCR Assays and Panels, all RNA samples should also demonstrate consistent quality according to the following criteria for concentration and purity determined, as determined by UV spectrophotometry and gel electrophoresis.

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate data sheets (SDSs), available from the product supplier.

Concentration and purity determined by UV spectrophotometry

The concentration and purity of RNA should be determined by measuring the absorbance in a spectrophotometer. Prepare dilutions and measure absorbance in 10 mM Tris·Cl, pH 8.0. The spectral properties of nucleic acids are highly dependent on pH. An absorbance reading of 1.0 at 260 nm in a 1 cm detection path corresponds to an RNA concentration of 40 µg/ml.

- *A*260:*A*230 ratio should be greater than 1.7
- *A*260:*A*280 ratio should be 1.8 to 2.0
- Concentration determined by *A*260 should be >40 µg/ml
- Ribosomal RNA band integrity

Run an aliquot of each RNA sample on a denaturing agarose gel, the Agilent® Bioanalyzer using an RNA 6000 Nano LabChip®, the QIAxpert Instrument (cat. no. 9002340), or the QIAxcel (cat. no. 9001941 or 9002123). Verify that there are sharp bands/peaks present for both the 18S and 28S ribosomal RNAs (Figure 1). Any smearing of the RNA bands or shoulders on the RNA peaks indicate that degradation has occurred in the RNA sample.

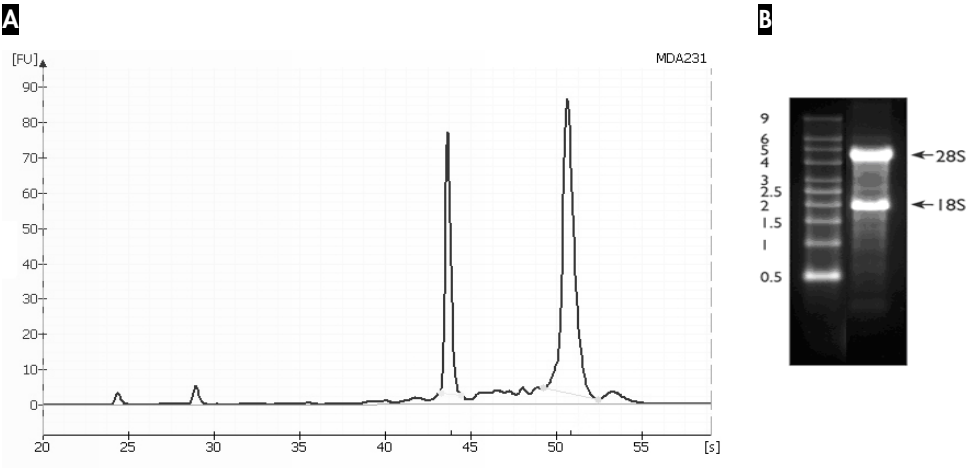


Figure 1. Ribosomal RNA integrity. A: Agilent Bioanalyzer electropherogram of high-quality total RNA showing sharp peaks for the 18S (left) and 28S (right) ribosomal RNA. Due to high quality of the RNA, peaks do not have shoulders (especially to the left of each peak). **B**: Agarose gel electrophoresis shows sharp bands (especially at the bottom of each band) for 28S and 18S ribosomal RNA.

Genomic DNA contamination

Unwanted signals generated by contaminating genomic DNA in a sample is limited by the design of QuantiNova LNA PCR assays. If the gene structure allows, QuantiNova LNA PCR assays make use of primers that target different exons while spanning a large intron in the mRNA (intron-spanning assay). GeneGlobe® indicates if intron-spanning assay design is not possible by the warning "Important: this assay may detect gDNA".

To remove genomic DNA contamination from your RNA samples, we strongly recommend RNA purification using the RNeasy®/RNeasy Plus products, including the optional on-column DNase digestion step.

Starting RNA amounts

Two-step RT-PCR protocol: The QuantiNova Reverse Transcription Enzyme has a high affinity for RNA and is optimized for efficient and sensitive cDNA synthesis from 10 pg to 5 µg of RNA. For the subsequent qPCR using the QuantiNova SYBR Green PCR Kit, we recommend to use ≤ 100 ng cDNA per reaction.

For successful results, we recommend that first-time users start with 0.5–2 µg of total RNA. It is important to use a consistent amount of total RNA for all reactions in a single experiment.

One-step RT-PCR protocol: Using the QuantiNova SYBR Green RT-PCR Kit, we recommend to use 100 fg to ≤ 200 ng per reaction, depending on target transcript abundance per reaction.

Recommended RNA preparation methods

High-quality total RNA for your real-time PCR experiment should be prepared using one of the methods described below, depending on the biological sample. For optimal results, RNA samples should be eluted in RNase-free water.

Important: Do not use DEPC-treated water.

Table 2. Recommended RNA preparation methods

Sample type	Recommendation
Cultured cells	RNeasy Plus Mini Kit (cat. no. 74134)
Tissue samples	Use the RNeasy Plus Mini Kit for RNA purification. **Note**: Some tissues, such as spleen and thymus, contain very high amounts of DNA, which will overload the gDNA Eliminator spin column. For these tissues, we recommend using the RNeasy Mini Kit (cat. no. 74104) in combination with the RNase-Free DNase Set (cat. no. 79254).
Formalin-fixed paraffin-embedded (FFPE) samples	RNeasy FFPE Kit (cat. no. 73504)
Small samples yielding <100 ng total RNA	RNeasy Plus Micro Kit (cat no. 74034)
Whole blood samples	PAXgene® Blood RNA Kit (see www.qiagen.com) or the QIAamp® RNA Blood Mini Kit (cat. no. 52304)
Total RNA isolated using a phenol-based method (e.g., QIAzol® Lysis Reagent, TRIzol® Reagent, RNAzol® Reagent)	Purify further using the RNeasy Mini Kit. **Important**: Perform the on-column DNase digestion step described in the *RNeasy Mini Handbook*, **www.qiagen.com/HB-0435**.
Other biological samples	Contact QIAGEN Technical Service

2x QuantiNova SYBR Green PCR Master Mix

The components of 2x QuantiNova SYBR Green PCR Master Mix include QuantiNova DNA Polymerase and QuantiNova SYBR Green PCR Buffer. The optimized Master Mix ensures ultrafast real-time PCR amplification with high specificity and sensitivity.

QuantiNova SYBR Green PCR Buffer is specifically designed for ultrafast, real-time PCR using SYBR Green for detection . The buffer additive Q-Bond allows short cycling times on any real-time cycler and a combined annealing/extension step of only 10 seconds. In addition, the unique composition of the buffer supports the melting behavior of DNA, enabling short denaturation and annealing/extension times.

QuantiNova DNA Polymerase is provided in an inactive state and has no enzymatic activity at ambient or higher temperatures. The enzyme remains completely inactive during the reverse transcription reaction and does not interfere with it. The antibody-mediated hot-start mechanism prevents the formation and extension of nonspecific PCR products and primer–dimers during reaction setup and the first denaturation step. Therefore, this mechanism allows higher PCR specificity and accurate quantification. At low temperatures, the QuantiNova DNA Polymerase is kept in an inactive state by the QuantiNova Antibody and Guard, which stabilize the complex and improve the stringency of the hot start.

After the reverse transcription, and within 2 min 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 2). The hot start enables rapid and convenient room-temperature setup. After setup, the PCR can be stored for up to 2 h at room temperature or up to 24 h at 2–8°C without impairing the performance of the subsequent reaction.

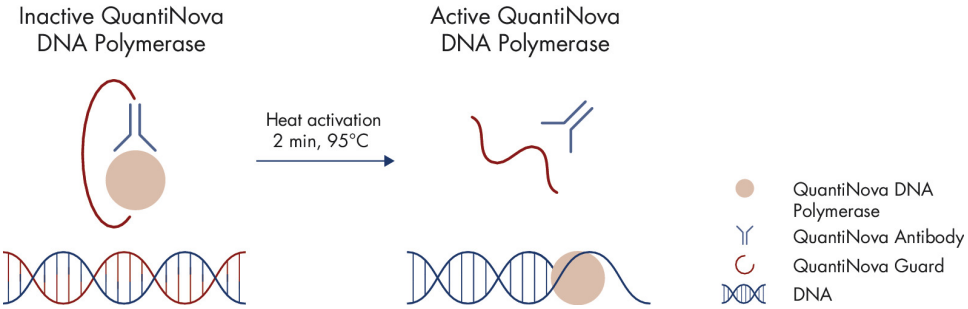


Figure 2. Principle of the novel QuantiNova hot-start mechanism. At ambient temperature, the QuantiNova DNA Polymerase is kept inactive by QuantiNova Antibody and QuantiNova Guard, until the initial heat activation step.

Built-in visual control for correct pipetting

The 2x QuantiNova SYBR Green PCR Master Mix contains an inert blue dye that increases visibility in the tube or well without interfering with the PCR.

Normalization of mRNA/lncRNA quantitative PCR experiments

The purpose of normalization is to remove technical and biological intersample variation that is not related to the biological changes under investigation. Proper normalization is critical for the correct analysis and interpretation of results from real-time PCR experiments. Most commonly, stably expressed reference genes are used for normalization.

In general, it is recommended to test several endogenous control candidates (reference genes) before setting up the actual mRNA/lncRNA expression analysis. These candidates should be chosen among genes that can be expected to be stably expressed over the whole range of samples being investigated. They could be stably expressed mRNA or stably expressed lncRNAs that are selected based on literature or preexisting data (e.g., next-generation sequencing or qPCR panel screening).

The QuantiNova LNA PCR system offers validated reference assays for a number of different RNAs that tend to be stably expressed and are therefore often good candidates for reference genes.

All reference gene candidates should be empirically validated for each study. One option for normalizing data from PCR panels for profiling a large number of mRNAs/lncRNAs is to normalize against the global mean – the average of all expressed mRNAs/lncRNAs. This can be a good option in samples with a high call rate (expressed genes) but should be used with caution in samples with low call rates. It is also not a good option in samples for which the general gene expression level is changed. Further guidance on normalization can also be found in GeneGlobe Data Analysis Center.

Plate layout for QuantiNova LNA PCR Focus panels

QuantiNova LNA PCR Focus panels are provided in 96-well plates, 384-well plates, or Rotor-Discs (Figure 3 to Figure 5). QuantiNova LNA PCR Focus panels in 96-well plates contain assays for 84 pathway- or disease-focused genes and 5 reference genes. In addition, 1 well contains a genomic DNA control, 3 wells contain QuantiNova Internal Control (QN IC) RNA assays, and 3 wells contain positive PCR controls (Figure 3).

QuantiNova LNA PCR Focus panels are available in 384-well plates (4 x 96) options. The 384 (4 x 96) option contains 4 replicate primer assays for each of 84 pathway- or disease-focused genes and 4 replicate primer assays for each of 5 reference genes. In addition, 4 wells contain genomic DNA controls, 12 wells contain QN IC RNA assays, and 12 wells contain positive PCR controls (Figure 4).

QuantiNova LNA PCR Focus panels in the Rotor-Disc 100 format contain primer assays for 84 pathway- or disease-focused genes and 5 reference genes. In addition, 1 well contains a genomic DNA control, 3 wells contain QN IC RNA assays, and 3 wells contain a positive PCR control. Wells 97–100 of the Rotor-Disc 100 do not contain assays (Figure 5). During the procedure, Master Mix is added to these wells for balance, but the wells are not used for analysis.

Custom QuantiNova LNA PCR panels contain assays defined by the customer. The plate layout and targeted genes are detailed in the product sheet provided.

Definitions of controls in QuantiNova LNA Panels

Assays for 5 reference genes included in the panels enable normalization of data. The genomic DNA control (GDC) is an assay that specifically detects nontranscribed genomic DNA contamination with a high level of sensitivity. QuantiNova Internal Control (QN IC) RNA is an assay that tests the efficiency of the reverse-transcription reaction performed with the QuantiNova Reverse Transcription Kit by detecting template synthesized from the kit's built-in external RNA control. The positive PCR control (PPC) consists of a predispensed artificial DNA sequence and the assay that detects it. This control tests the efficiency of the polymerase chain reaction itself. Controls provided in replicates can be used to test for interwell, intraplate consistency.

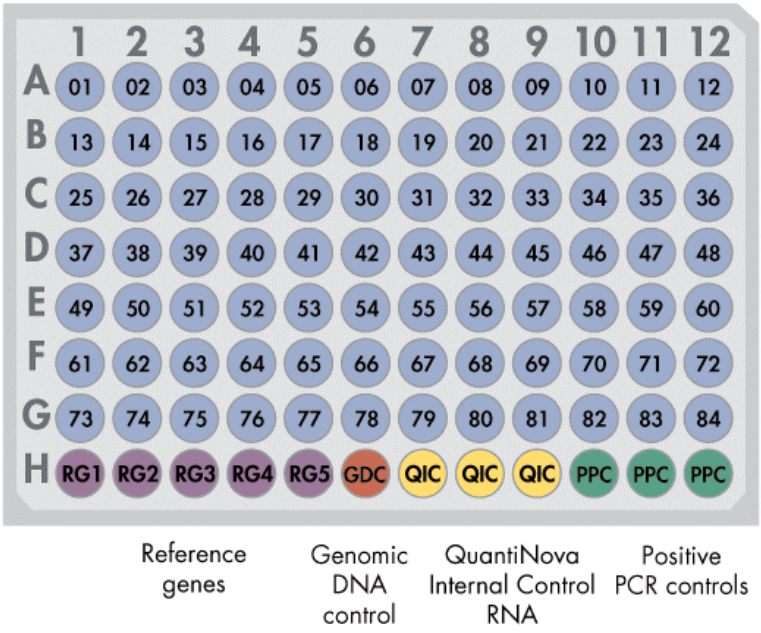


Figure 3. QuantiNova LNA PCR Focus panels, formats A, C, D, F, and H. Wells A1–G12 each contain a real-time PCR assay for a pathway/disease/functionally related gene. Wells H1–H5 contain a reference gene panel to normalize panel data (RG1–RG5). Well H6 contains a genomic DNA control (GDC). Wells H7–H9 contain replicate QuantiNova Internal Control RNA assays (QIC). Wells H10–H12 contain replicate positive PCR controls (PPC).

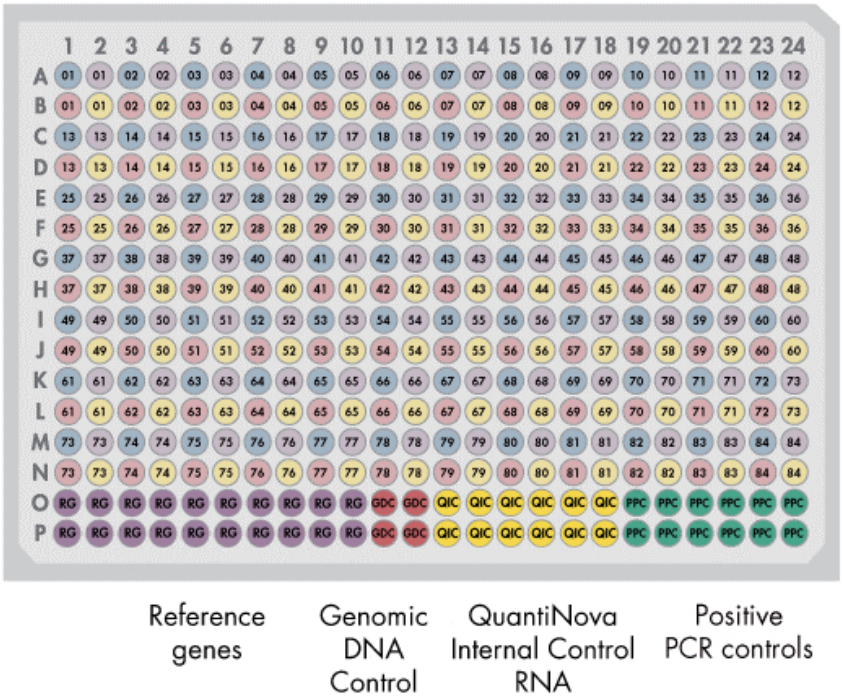


Figure 4. QuantiNova LNA PCR Focus panels, formats E and G, 384 (4 x 96) option layout. QuantiNova LNA PCR Focus panels with the 384 (4 x 96) option include 4 replicates of the same assays as provided in the 96-well format shown in Figure 3.

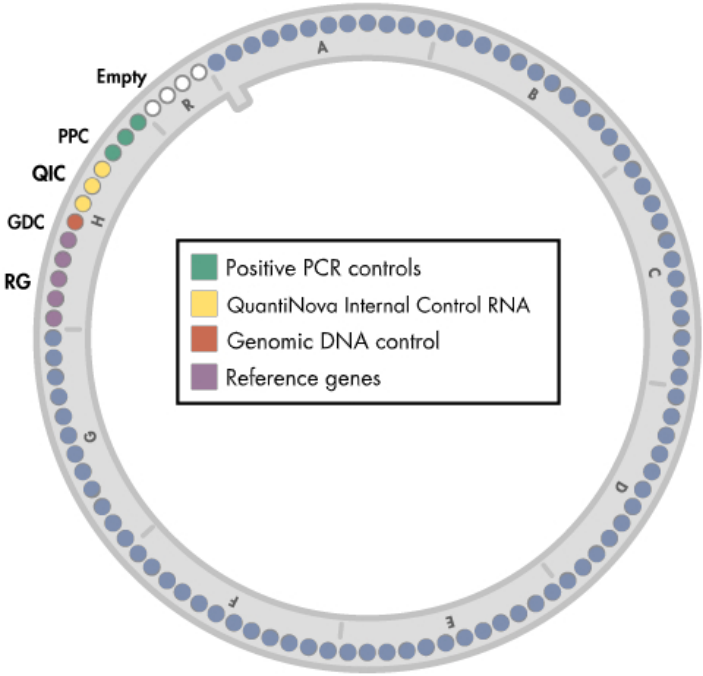


Figure 5. QuantiNova LNA PCR Focus panels, format R layout. Wells 1–84 each contain a real-time PCR assay for a pathway/disease/functionally related gene. Wells 85–89 contain a reference gene panel to normalize panel data. Well 90 contains a genomic DNA control. Wells 91–93 contain replicate QuantiNova Internal Control RNA assays (QIC). Wells 94–96 contain replicate positive PCR controls. Wells 97–100 are empty.

Passive reference dye

For certain real-time cyclers, the presence of ROX passive reference dye in real-time PCR compensates for non-PCR-related variations in fluorescence detection. Fluorescence from ROX dye does not change during the course of real-time PCR but provides a stable baseline to which PCR-related fluorescent signals are normalized. Thus, ROX dye compensates for differences in fluorescence detection between wells due to slight variations in reaction volume or to differences in well position. ROX dye does not interfere with real-time PCR, because it is not involved in the reaction and has an emission spectrum different from the FAM™ dye as well as the HEX™ dye.

The use of ROX dye is necessary for instruments from Applied Biosystems. The QuantiNova SYBR Green PCR Kit includes a separate tube of ROX Reference Dye, which can be added to the real-time PCR if you are using a real-time cycler that uses ROX as a passive reference dye. For instruments requiring a high concentration of ROX dye, use the ROX Reference Dye as a 20x concentrate. For instruments requiring a low concentration of ROX dye, use the dye as a 200x concentrate. Refer to Table 3 for details on real-time cyclers that require low or high ROX concentrations.

Table 3. Real-time cyclers requiring high/low concentrations of ROX

High ROX concentration (ROX Reference Dye to be used at a 20x dilution)	**Low ROX concentration (ROX Reference Dye to be used at a 200x dilution)**
ABI PRISM® 7000	Applied Biosystems 7500
Applied Biosystems 7300	Applied Biosystems ViiA® 7
Applied Biosystems 7900	Applied Biosystems QuantStudio® Systems
Applied Biosystems StepOne®	
Applied Biosystems StepOne Plus	

Adding ROX dye to the QuantiNova SYBR Green PCR or the QuantiNova SYBR Green RT-PCR Master Mix

If only using cyclers from Applied Biosystems with the QuantiNova SYBR Green PCR or the QuantiNova SYBR Green RT-PCR Kit, QuantiNova ROX Reference Dye can be added to the 2x QuantiNova SYBR Green PCR or the 2x QuantiNova SYBR Green RT-PCR Master Mix for long-term storage, if desired. For information on the concentration of ROX required for Applied Biosystems instruments, refer to Table 4. For reaction setups with a Master Mix that already contains a high concentration of added QuantiNova ROX Reference Dye, refer to the "Appendix: Reaction Setup Using Master Mix that Contains a High Concentration of ROX", page 50.

Table 4. Addition of QN ROX Reference Dye to Master Mix

Volume of 2x QuantiNova SYBR Green PCR Master Mix or 2x QuantiNova SYBR Green RT-PCR Master Mix (w/o QN ROX Reference Dye)	Volume of QN ROX Reference Dye for high ROX concentration/low ROX concentration
1 ml	100 µl/10 µl
1.7 ml	170 µl/17 µl

Equipment and Reagents to Be Supplied by User

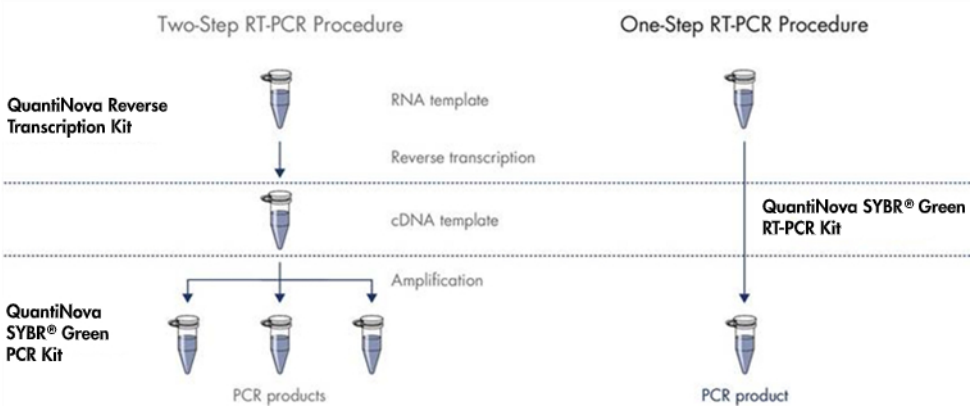
When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs) available from the product supplier.

- QuantiNova Reverse Transcription Kit and QuantiNova SYBR Green PCR Kit or QuantiNova SYBR Green RT-PCR Kit
- 10x QuantiNova IC SYBR Green Assay (**geneglobe.qiagen.com**, cat. no. QT02589307)
- Purified RNA samples
- High-quality nuclease-free water (do not use DEPC-treated water)
- Multichannel pipettor
- Single-channel pipettor (if using QuantiNova LNA PCR Assays)
- Nuclease-free plastic tubes (for 20 µl reactions)
- Nuclease-free PCR tubes or plates for use with individual assays
- Nuclease-free aerosol-barrier pipette tips
- Ice
- PCR cycler, heating block or water bath (capable of reaching 95°C)
- Vortexer
- Microcentrifuge and plate centrifuge
- Sealing foils for PCR plates
- Real-time PCR instrument

Description of Protocols

This handbook contains 2 protocols:

- "Protocol: 2-Step RT-PCR Protocol" details cDNA synthesis by reverse transcription followed by real-time PCR using the QuantiNova LNA PCR (single tube) assay or panel product.
- "Protocol: 1-Step RT-PCR Protocol" details the combination of cDNA synthesis by reverse transcription and real-time PCR using the QuantiNova LNA PCR (single tube) assay or panel product.



Protocol: 2-Step RT-PCR Protocol

cDNA synthesis

For cDNA synthesis the use of the QuantiNova Reverse Transcription Kit is critical for obtaining optimal results with QuantiNova LNA PCR Assays or Panels. Please follow the instructions provided in the QuantiNova Reverse Transcription Kit Handbook and use the QuantiNova Internal Control (QN IC) RNA template.

QuantiNova LNA PCR single-tube assays

For quantitative real-time PCR, the use of the QuantiNova SYBR Green PCR Kit is critical for obtaining optimal results with QuantiNova LNA PCR assays.

Important points before starting

- This protocol is optimized for the detection of mRNA/lncRNA targets with any real-time cycler and conditions for fluorescence normalization. ROX dye is required at the following concentrations:
    - **No requirement for ROX dye**: Rotor-Gene®, Bio-Rad® CFX, Roche® LightCycler® 480, and Agilent Technologies Mx instruments.
    - **Low concentration of ROX dye**: Applied Biosystems 7500, ViiA 7, and QuantStudio Real-Time PCR Systems.
    - **High concentration of ROX dye**: ABI PRISM 7000, Applied Biosystems 7300, 7900, and StepOne Real-Time PCR Systems.

- The ROX Reference Dye should be used as a 20x concentrated solution for a 1x reaction when using an instrument requiring a high ROX dye concentration. For instruments requiring a low ROX dye concentration, use the dye as a 200x concentrate.
- The 2x QuantiNova SYBR Green PCR Master Mix contains the QuantiNova DNA Polymerase, which is inactive at room temperature. The PCR protocol must start with an initial incubation step of 2 min at 95°C to activate the QuantiNova DNA Polymerase.
- Always start with the cycling conditions and primer concentrations specified in this protocol.
- After using the QuantiNova Reverse Transcription Kit, cDNA should be diluted (1:10 to 1:100) and an aliquot of the reaction should be used for subsequent amplification with the QuantiNova SYBR Green PCR Kit. If using the QuantiNova IC RNA to control for absence of inhibitors, this should be detected using the respective QuantiNova IC assay. For detection with the QuantiNova SYBR Green PCR Kit, use the appropriate volume of 10x QuantiNova IC SYBR Green Assay.
- The QN IC RNA is added to the experimental RNA sample. An additional no-template RNA control sample, which only contains the QN IC RNA, should also be set up.

Things to do before starting

- Resuspend the QuantiNova LNA PCR Assay: Centrifuge the tube before opening it for the first time. Add 440 µl nuclease-free water (for QuantiNova LNA PCR Assay for 200 reactions) or 1650 µl nuclease-free water (for QuantiNova LNA PCR Assay for 750 reactions) to the tube and leave at room temperature for 20 min. Vortex and briefly centrifuge.

Procedure

1. Thaw 2x QuantiNova SYBR Green PCR Master Mix, template cDNA, QN ROX Reference Dye (if required), 10x QuantiNova LNA PCR Assay, 10x QuantiNova IC SYBR Green Assay and RNase-free water. Mix the individual solutions.
2. Prepare a reaction mix according to Table 5. Due to the hot start, it is not necessary to keep samples on ice during reaction setup or while programming the real-time cycler.

Table 5. Reaction mix setup for QuantiNova LNA PCR Assays

Component	Volume/reaction	Final concentration
2x QuantiNova SYBR Green PCR Master Mix	10 µl	1x
ROX Reference Dye (ABI instruments only)	1 µl/0.1 µl*	1x
10x QuantiNova LNA PCR Assay *or* 10x QuantiNova LNA PCR Reference Assay *or* 10x QuantiNova IC SYBR Green Assay	2 µl	1x
cDNA template (added at step 4)	Variable	≤10% of total reaction volume
RNase-free water	Variable	–
Total reaction volume	20 µl	–

* Use ROX Reference Dye as a 20x concentrate for cyclers requiring a high ROX dye concentration (i.e., ABI PRISM 7000, Applied Biosystems 7300, 7900, and StepOne Real-Time PCR Systems) and as a 200x concentrate for cyclers requiring a low ROX dye concentration (i.e., Applied Biosystems 7500, ViiA 7 and QuantStudio Real-Time PCR Systems). Adjust the amount of RNase-free water accordingly.

3. Mix the reaction mix thoroughly and dispense appropriate volumes into PCR vessels or plates.
4. Add template cDNA (≤10% of total reaction volume) to the individual PCR vessels or wells that contain the reaction mix.

5. Program the real-time cycler according to Table 6.

   Note: Data acquisition should be performed during the annealing/extension step.

Table 6. PCR cycling conditions for QuantiNova LNA PCR Assays

Step	Time	Temperature	Ramp rate	Additional comments
PCR initial heat activation	2 min	95°C	Maximal/fast mode	QuantiNova DNA Polymerase is activated by this heating step
2-step cycling				
Denaturation	5 s	95°C	Maximal/fast mode	
Combined annealing/extension	10 s*	60°C	Maximal/fast mode	Perform fluorescence data collection
Number of cycles	45			
Melting curve analysis†				

* If your cycler does not accept this short time for data acquisition, choose the shortest acceptable time (e.g., 31 s annealing/extension for the ABI PRISM 7000 or Applied Biosystems 7300).

† Melting curve analysis is an analysis step built into the software of real-time cyclers. To perform the analysis, follow instructions provided by the supplier.

6. Place the PCR vessels or plates in the real-time cycler and start the cycling program.
7. Perform melting curve analysis of the PCR product(s).

   We strongly recommend routinely performing this analysis, which is built into the software of real-time cyclers, to verify the specificity and identity of PCR products.

   Optional: Check the specificity of PCR product(s) by agarose gel electrophoresis
8. Perform the data analysis using the software supplied with your real-time PCR instrument to obtain raw C_q values (C_p or C_t, depending on PCR instrument). If you are using an ABI instrument, please note that it is not recommended to use auto C_q settings. If you are using a Roche LC480 instrument, we recommend analysis using the second derivative method. For further details, see "Single-Tube Assays: Analysis and Interpretation of Internal Control Assay Results", page 44.

QuantiNova LNA PCR 96-well and 384-well panels

For quantitative real-time PCR, the use of the QuantiNova SYBR Green PCR Kit is critical for obtaining optimal results with QuantiNova LNA PCR panels.

Important points before starting

- For 96-well block cyclers, we recommend a final reaction volume of 20 µl. For 384-well block cyclers, we recommend a final reaction volume of 10 µl.
- This protocol is optimized for the detection of mRNA/lncRNA targets with any real-time cycler and conditions for fluorescence normalization. ROX dye is required at the following concentrations:
  - **No requirement for ROX dye**: Rotor-Gene, Bio-Rad CFX, Roche LightCycler 480, and Agilent Technologies Mx instruments.
  - **Low concentration of ROX dye**: Applied Biosystems 7500, ViiA 7, and QuantStudio Real-Time PCR Systems.
  - **High concentration of ROX dye**: ABI PRISM 7000, Applied Biosystems 7300, 7900, and StepOne Real-Time PCR Systems.
- The ROX Reference Dye should be used as a 20x concentrated solution for a 1x reaction when using an instrument requiring a high ROX dye concentration. For instruments requiring a low ROX dye concentration, use the dye as a 200x concentrate.
- The 2x QuantiNova SYBR Green PCR Master Mix contains the QuantiNova DNA Polymerase, which is inactive at room temperature. The PCR protocol must start with an initial incubation step of 2 min at 95°C to activate the QuantiNova DNA Polymerase.
- Ensure that the QN IC RNA was added to the experimental RNA samples.

Procedure

1. Thaw 2x QuantiNova SYBR Green PCR Master Mix, template cDNA, QN ROX Reference Dye (if required), and RNase-free water. Mix the individual solutions.
2. When using the QuantiNova Reverse Transcription Kit, add 90 µl RNase-free water to each 20 µl reverse transcription reaction to dilute the cDNA. Mix by pipetting up and down several times.
3. Prepare a Master Mix for 1 sample according to Table 7 or for more than 1 sample according to Table 8 or Table 9. Due to the hot start, it is not necessary to keep samples on ice during reaction setup or while programming the real-time cycler.

   Note: Save the remaining volume of the cDNA synthesis reaction at –15 to –30°C, for potential quality control analysis.

Table 7. Master Mix setup for QuantiNova LNA PCR Panels for 1 sample

Component	96-well panels	384-well panels	Final concentration
2x QuantiNova SYBR Green PCR Master Mix	1000 µl	2000 µl	1x
ROX Reference Dye (ABI instruments only)	100 µl/10 µl*	200 µl/20 µl*	1x
Diluted cDNA template	100 µl	100 µl	–
RNase-free water	Variable	Variable	–
Total Master Mix volume	2000 µl†	4000 µl†	–

* Use ROX Reference Dye as a 20x concentrate for cyclers requiring a high ROX dye concentration (i.e., ABI PRISM 7000, Applied Biosystems 7300, 7900, and StepOne Real-Time PCR Systems) and as a 200x concentrate for cyclers requiring a low ROX dye concentration (i.e., Applied Biosystems 7500, ViiA 7, and QuantStudio Real-Time PCR Systems). Adjust the amount of RNase-free water accordingly.

† Total Master Mix volume includes a reserve to compensate for pipetting variations.

Table 8. Master Mix setup for QuantiNova LNA PCR Flexible Panels and Custom Panels for more than 1 sample per 96-well plate/Rotor-Disc 100

Component	2 samples (48 wells per sample)	4 samples (24 wells per sample)	8 samples (12 wells per sample)	Final concentration
2x QuantiNova SYBR Green PCR Master Mix	520 µl	280 µl	160 µl	1x
ROX Reference Dye (ABI instruments only)	52 µl/ 5.2 µl*	28 µl/ 2.8 µl*	16 µl/ 1.6 µl*	1x
Diluted cDNA template	100 µl	100 µl	100 µl	–
RNase-free water	Variable	Variable	Variable	–
Total Master Mix volume	2 x 1040 µl†	4 x 560 µl†	8 x 320 µl†	–

* Use ROX Reference Dye as a 20x concentrate for cyclers requiring a high ROX dye concentration (i.e., ABI PRISM 7000, Applied Biosystems 7300, 7900, and StepOne Real-Time PCR Systems) and as a 200x concentrate for cyclers requiring a low ROX dye concentration (i.e., Applied Biosystems 7500, ViiA 7 and QuantStudio Real-Time PCR Systems). Adjust the amount of RNase-free water accordingly.

† Each Master Mix includes a reserve of at least 4 single reaction volumes (80 µl for 96-well plates and 40 µl for 384-well plates) to compensate for pipetting variations.

Table 9. Master Mix setup for QuantiNova LNA PCR Flexible Panels and Custom Panels for more than 1 sample per 384-well plate

Component	2 samples (192 wells per sample)	4 samples (96 wells per sample)	8 samples (48 wells per sample)	16 samples (24 wells per sample)	Final concentration
2x QuantiNova SYBR Green PCR Master Mix	1000 µl	500 µl	260 µl	140 µl	1x
ROX Reference Dye (ABI instruments only)	100 µl/ 10 µl*	50 µl/5 µl*	26 µl/2.6 µl*	14 µl/1.4 µl*	1x
Diluted cDNA template	100 µl	100 µl	100 µl	100 µl	–
RNase-free water	Variable	Variable	Variable	Variable	–
Total Master Mix volume	2 x 2000 µl†	4 x 1000 µl†	8 x 520 µl†	16 x 280 µl†	–

* Use ROX Reference Dye as a 20x concentrate for cyclers requiring a high ROX dye concentration (i.e., ABI PRISM 7000, Applied Biosystems 7300, 7900, and StepOne Real-Time PCR Systems) and as a 200x concentrate for cyclers requiring a low ROX dye concentration (i.e., Applied Biosystems 7500, ViiA 7 and QuantStudio Real-Time PCR Systems). Adjust the amount of RNase-free water accordingly.

† Each Master Mix includes a reserve of at least 4 single reaction volumes (80 µl for 96-well plates and 40 µl for 384-well plates) to compensate for pipetting variations.

4. Mix the reaction mix thoroughly and dispense 20 µl per well (for 96-well formats) or 10 µl per well (for 384-well formats) into the PCR plates.

   Note: The experiment can be paused at this point. Store the reactions protected from light at 2–8°C for up to 24 h.

5. Seal the plates. Carefully vortex it to dissolve the primers (optional). Briefly centrifuge the plates at room temperature. Wait 5 min while the primers dissolve in the reaction mix.

6. Program the real-time cycler according to Table 10.

   Note: Data acquisition should be performed during the annealing/extension step.

7. Place the plates into the real-time cycler and start the cycling program.

Table 10. PCR cycling conditions for QuantiNova LNA PCR Panels

Step	Time	Temperature	Ramp rate	Additional comments
PCR initial heat activation	2 min	95°C	Maximal/ fast mode	QuantiNova DNA Polymerase is activated by this heating step
2-step cycling				
Denaturation	5 s	95°C	Maximal/ fast mode	
Combined annealing/extension	10 s*	60°C	Maximal/ fast mode	Perform fluorescence data collection
Number of cycles	45			
Melting curve analysis†				

* If your cycler does not accept this short time for data acquisition, choose the shortest acceptable time (e.g., 31 s annealing/extension for the ABI PRISM 7000 or Applied Biosystems 7300).

† Melting curve analysis is an analysis step built into the software of real-time cyclers. To perform the analysis, follow instructions provided by the supplier.

8. Perform melting curve analysis of the PCR product(s).

   We strongly recommend routinely performing this analysis, which is built into the software of real-time cyclers, to verify the specificity and identity of PCR products.

   Optional: Check the specificity of PCR product(s) by agarose gel electrophoresis.

9. Perform the initial data analysis using the software supplied with your real-time PCR instrument to obtain raw C_q values (C_p or C_t, depending on PCR instrument). If you are using an ABI instrument, please note that it is not recommended to use auto C_q settings. If you are using a Roche LC480 instrument, we recommend analysis using the second derivative method. For further details, see "96-Well and 384-Well Panels: Analysis of mRNA/lncRNA Quantitative PCR Experiments", page 46.

Protocol: 1-Step RT-PCR Protocol

QuantiNova LNA PCR single-tube assays

For 1-step RT-PCR, the use of the QuantiNova SYBR Green RT-PCR Kit is critical for obtaining optimal results with QuantiNova LNA PCR assays.

Important points before starting

- This protocol is optimized for the detection of mRNA/lncRNA targets with any real-time cycler and conditions for fluorescence normalization. ROX dye is required at the following concentrations:
  - **No requirement for ROX dye**: Rotor-Gene, Bio-Rad CFX, Roche LightCycler 480, and Agilent Technologies Mx instruments.
  - **Low concentration of ROX dye**: Applied Biosystems 7500, ViiA 7, and QuantStudio Real-Time PCR Systems.
  - **High concentration of ROX dye**: ABI PRISM 7000, Applied Biosystems 7300, 7900, and StepOne Real-Time PCR Systems.
- The ROX Reference Dye should be used as a 20x concentrated solution for a 1x reaction when using an instrument requiring a high ROX dye concentration. For instruments requiring a low ROX dye concentration, use the dye as a 200x concentrate.
- The QuantiNova SYBR Green RT Mix contains HotStarRT-Script Reverse Transcriptase for heat-mediated activation of the reverse-transcription step, and an RNase inhibitor.
  Note: Although the included RNase inhibitor effectively reduces the risk of RNA degradation, template RNA of high quality and purity should be used, and any contamination should be prevented to ensure reliable qRT-PCR results.
- Always start with the cycling conditions and primer concentrations specified in this protocol.

- The QuantiNova Internal Control (QN IC) RNA is an internal amplification control used to test successful reverse transcription/amplification. It is intended to report instrument or chemistry failures, errors in assay setup and the presence of inhibitors. For detection with the QuantiNova SYBR Green PCR Kit, use the appropriate volume of 10x QuantiNova IC SYBR Green Assay.
- The QN IC RNA is added to the experimental RNA sample. An additional no-template RNA control sample, which only contains the QN IC RNA, should also be set up.
- Before use, add 180 µl (or 900 µl) of RNase-free water to 20 µl (or 100 µl) of QN IC RNA provided in the kit and mix thoroughly by vortexing.

Things to do before starting

- Resuspend the PCR assays: Centrifuge the tube before opening it for the first time. Add 440 µl nuclease-free water (for QuantiNova LNA PCR Assay for 200 reactions) or 1650 µl nuclease-free water (for QuantiNova LNA PCR Assay for 750 reactions) to the tube and leave at room temperature for 20 min. Vortex and briefly centrifuge.

Procedure

1. Thaw 2x QuantiNova SYBR Green RT-PCR Master Mix, template RNA (QN IC RNA; optional), 10x QuantiNova LNA PCR Assay, QN ROX Reference Dye (if required) and RNase-free Water. Thawing of the QuantiNova RT Mix is not required. Mix the individual solutions.
2. Prepare a reaction mix according to Table 11. Due to the 2-phase hot start of both the RT reaction and the PCR reaction, it is not necessary to keep samples on ice during reaction setup or while programming the real-time cycler.

Table 11. One-step Reaction mix setup for QuantiNova LNA PCR Assays

Component	Volume/reaction	Final concentration
2x QuantiNova SYBR Green RT-PCR Master Mix	10 µl	1x
ROX Reference Dye (ABI instruments only)	1 µl/0.1 µl*	1x
QN SYBR Green RT-Mix	0.2 µl	1x
10x QuantiNova LNA PCR Assay *or* 10x QuantiNova LNA PCR Reference Assay *or* 10x QuantiNova IC SYBR Green Assay	2 µl	1x
Diluted QN IC RNA (optional)	1 µl	1x
RNA template (added at step 4)	Variable	≤10% of total reaction volume
RNase-free water	Variable	–
Total reaction volume	20 µl	–

* Use ROX Reference Dye as a 20x concentrate for cyclers requiring a high ROX dye concentration (i.e., ABI PRISM 7000, Applied Biosystems 7300, 7900, and StepOne Real-Time PCR Systems) and as a 200x concentrate for cyclers requiring a low ROX dye concentration (i.e., Applied Biosystems 7500, ViiA 7 and QuantStudio Real-Time PCR Systems). Adjust the amount of RNase-free water accordingly.

3. Mix the reaction mix thoroughly and dispense appropriate volumes into PCR vessels or plates.
4. Add template RNA (≤200 ng – 100 fg per reaction, depending on target transcript abundance) to the individual PCR tubes or wells containing the reaction mix.
5. Program the real-time cycler according to Table 12.

   Note: Data acquisition should be performed during the annealing/extension step.

Table 12. Real-time cycler conditions

Step	Time	Temperature	Ramp rate	Additional comments
Reverse transcription	10 min	50°C	Maximal/ fast mode	HotStarRT-Script Reverse Transcriptase is activated, residual gDNA is removed
PCR initial activation step	2 min	95°C	Maximal/ fast mode	QuantiNova DNA Polymerase is activated
2-step cycling				
Denaturation	5 s	95°C	Maximal/ fast mode	
Combined annealing/ extension	10 s*	60°C	Maximal/ fast mode	Perform fluorescence data collection
Number of cycles	45			
Melting curve analysis†				

* If your cycler does not accept this short time for data acquisition, choose the shortest acceptable time (e.g., 31 s annealing/extension for the ABI PRISM 7000 or Applied Biosystems 7300).

† Melting curve analysis is an analysis step built into the software of real-time cyclers. To perform the analysis, follow instructions provided by the supplier.

6. Place the PCR vessels or plates in the real-time cycler and start the cycling program.
7. Perform melting curve analysis of the PCR product(s).

   We strongly recommend routinely performing this analysis, which is built into the software of real-time cyclers, to verify the specificity and identity of PCR products.

   Optional: Check the specificity of PCR product(s) by agarose gel electrophoresis.

   Perform the data analysis using the software supplied with your real-time PCR instrument to obtain raw C_q values (C_p or C_t, depending on PCR instrument). If you are using an ABI instrument, please note that it is not recommended to use auto C_q settings. If you are using a Roche LC480 instrument, we recommend analysis using the second derivative method. For further details, see "Single-Tube Assays: Analysis and Interpretation of Internal Control Assay Results", page 44.

QuantiNova LNA PCR 96-well and 384-well panels

For 1-step RT-PCR, the use of the QuantiNova SYBR Green RT-PCR Kit is critical for obtaining optimal results with QuantiNova LNA PCR assays.

Important points before starting

- For 96-well block cyclers, we recommend a final reaction volume of 20 µl. For 384-well block cyclers, we recommend a final reaction volume of 10 µl.
- This protocol is optimized for the detection of mRNA/lncRNA targets with any real-time cycler and conditions for fluorescence normalization. ROX dye is required at the following concentrations:
  - **No requirement for ROX dye**: Rotor-Gene, Bio-Rad CFX, Roche LightCycler 480, and Agilent Technologies Mx instruments.
  - **Low concentration of ROX dye**: Applied Biosystems 7500, ViiA 7, and QuantStudio Real-Time PCR Systems.
  - **High concentration of ROX dye**: ABI PRISM 7000, Applied Biosystems 7300, 7900, and StepOne Real-Time PCR Systems.
- The ROX Reference Dye should be used as a 20x concentrated solution for a 1x reaction when using an instrument requiring a high ROX dye concentration. For instruments requiring a low ROX dye concentration, use the dye as a 200x concentrate.
- The QuantiNova SYBR Green RT Mix contains HotStarRT-Script Reverse Transcriptase for heat-mediated activation of the reverse-transcription step and an RNase inhibitor. **Note:** Although the included RNase inhibitor effectively reduces the risk of RNA degradation, template RNA of high quality and purity should be used, and any contamination should be prevented to ensure reliable qRT-PCR results.
- Always start with the cycling conditions and primer concentrations specified in this protocol.

Procedure

1. Thaw 2x QuantiNova SYBR Green RT-PCR Master Mix, template RNA (QN IC RNA; optional), 10x QuantiNova LNA PCR Assay, QN ROX Reference Dye (if required) and RNase-free Water. Thawing of the QuantiNova RT Mix is not required. Mix the individual solutions.
2. Prepare a Master Mix for 1 sample according to Table 13 or, for more than 1 sample, according to Table 14 or Table 15. Due to the hot start, it is not necessary to keep samples on ice during reaction setup or while programming the real-time cycler.

Table 13. One-step Master Mix setup for QuantiNova LNA PCR Panels for 1 sample

Component	96-well panels	384-well panels	Final concentration
2x QuantiNova SYBR Green RT-PCR Master Mix	1000 µl	2000 µl	1x
ROX Reference Dye (ABI instruments only)	100 µl/10 µl*	200 µl/20 µl*	1x
QN SYBR Green RT-Mix	20 µl	40 µl	1x
Diluted QN IC RNA (optional)	100 µl	400 µl	1x
RNA template	Variable	Variable	≤10% of total Master Mix volume
RNase-free water	Variable	Variable	–
Total Master Mix volume	2000 µl†	4000 µl†	–

* Use ROX Reference Dye as a 20x concentrate for cyclers requiring a high ROX dye concentration (i.e., ABI PRISM 7000, Applied Biosystems 7300, 7900, and StepOne Real-Time PCR Systems) and as a 200x concentrate for cyclers requiring a low ROX dye concentration (i.e., Applied Biosystems 7500, ViiA 7, and QuantStudio Real-Time PCR Systems). Adjust the amount of RNase-free water accordingly.

† Total Master Mix volume includes a reserve to compensate for pipetting variations.

Table 14. One-step Master Mix setup for QuantiNova LNA PCR Flexible Panels and Custom Panels for more than 1 sample per 96-well plate/Rotor-Disc 100

Component	2 samples (48 wells per sample)	4 samples (24 wells per sample)	8 samples (12 wells per sample)	Final concentration
2x QuantiNova SYBR Green RT-PCR Master Mix	520 µl	280 µl	160 µl	1x
ROX Reference Dye (ABI instruments only)	52 µl/ 5.2 µl*	28 µl/ 2.8 µl*	16 µl/ 1.6 µl*	1x
QN SYBR Green RT-Mix	10.4 µl	5.6 µl	3.2 µl	1x
Diluted QN IC RNA (optional)	52 µl	28 µl	16 µl	1x
RNA template	Variable	Variable	Variable	≤10% of Master Mix volume
RNase-free water	Variable	Variable	Variable	–
Total Master Mix volume	2 x 1040 µl†	4 x 560 µl†	8 x 320 µl†	–

* Use ROX Reference Dye as a 20x concentrate for cyclers requiring a high ROX dye concentration (i.e., ABI PRISM 7000, Applied Biosystems 7300, 7900, and StepOne Real-Time PCR Systems) and as a 200x concentrate for cyclers requiring a low ROX dye concentration (i.e., Applied Biosystems 7500, ViiA 7, and QuantStudio Real-Time PCR Systems). Adjust the amount of RNase-free water accordingly.

† Each Master Mix includes a reserve of at least 4 single reaction volumes (80 µl for 96-well plates and 40 µl for 384-well plates) to compensate for pipetting variations.

Table 15. One-step Master Mix setup for QuantiNova LNA PCR Flexible Panels and Custom Panels for more than 1 sample per 384-well plate

Component	2 samples (192 wells per sample)	4 samples (96 wells per sample)	8 samples (48 wells per sample)	16 samples (24 wells per sample)	Final concentration
2x QuantiNova RT-PCR Master Mix	1000 µl	500 µl	260 µl	140 µl	1x
ROX Reference Dye (ABI instruments only)	100 µl/ 10 µl*	50 µl/ 5 µl*	26 µl/ 2.6 µl*	14 µl/ 1.4 µl*	1x
QN SYBR Green RT-Mix	20 µl	10 µl	5.2 µl	2.8 µl	
Diluted QN IC RNA (optional)	200 µl	100 µl	52 µl	28 µl	1x
RNA template	Variable	Variable	Variable	Variable	≤10% of Master Mix volume
RNase-free water	Variable	Variable	Variable	Variable	–
Total Master Mix volume	2 x 2000 µl†	4 x 1000 µl†	8 x 520 µl†	16 x 280 µl†	–

* Use ROX Reference Dye as a 20x concentrate for cyclers requiring a high ROX dye concentration (i.e., ABI PRISM 7000, Applied Biosystems 7300, 7900, and StepOne Real-Time PCR Systems) and as a 200x concentrate for cyclers requiring a low ROX dye concentration (i.e., Applied Biosystems 7500, ViiA 7, and QuantStudio Real-Time PCR Systems). Adjust the amount of RNase-free water accordingly.

† Each Master Mix includes a reserve of at least 4 single reaction volumes (80 µl for 96-well plates and 40 µl for 384-well plates) to compensate for pipetting variations.

3. Mix the reaction mix thoroughly and dispense 20 µl per well (for 96-well formats) or 10 µl per well (for 384-well formats) into the PCR plates.

   Note: The experiment can be paused at this point. Store the reactions protected from light at 2–8°C for up to 24 h.

4. Seal the plates. Carefully vortex it to dissolve the primers (optional). Briefly centrifuge the plates at room temperature. Wait 5 min while the primers dissolve in the reaction mix.

5. Program the real-time cycler according to Table 16.

   Note: Data acquisition should be performed during the annealing/extension step.

Table 16. Real-time cycling conditions for QuantiNova LNA PCR Panels

Step	Time	Temperature	Ramp rate	Additional comments
Reverse transcription	10 min	50°C	Maximal/fast mode	HotStarRT-Script Reverse Transcriptase is activated
PCR initial heat activation	2 min	95°C	Maximal/fast mode	
2-step cycling				
Denaturation	5 s	95°C	Maximal/fast mode	
Combined annealing/extension	10 s*	60°C	Maximal/fast mode	Perform fluorescence data collection
Number of cycles	45			
Melting curve analysis†				

* If your cycler does not accept this short time for data acquisition, choose the shortest acceptable time (e.g., 31 s annealing/extension for the ABI PRISM 7000 or Applied Biosystems 7300).

† Melting curve analysis is an analysis step built into the software of real-time cyclers. To perform the analysis, follow instructions provided by the supplier.

6. Perform melting curve analysis of the PCR product(s).

   We strongly recommend routinely performing this analysis, which is built into the software of real-time cyclers, to verify the specificity and identity of PCR products.

   Optional: Check the specificity of PCR product(s) by agarose gel electrophoresis.

Perform the initial data analysis using the software supplied with your real-time PCR instrument to obtain raw C_q values (C_p or C_t, depending on PCR instrument). If you are using an ABI instrument, not that it is not recommended to use auto C_q settings. If you are using a Roche LC480 instrument, we recommend analysis using the 2nd derivative method. For further details, see "96-Well and 384-Well Panels: Analysis of mRNA/lncRNA Quantitative PCR Experiments", page 46.

Single-Tube Assays: Analysis and Interpretation of Internal Control Assay Results

- The QuantiNova Internal Control (QN IC) RNA template is a synthetic RNA that can be optionally used to monitor successful reverse transcription. The QN IC RNA is intended to report instrument or chemistry failures, errors in assay setup, and the presence of inhibitors. Inhibitors such as phenol, ethanol, sodium dodecyl sulfate (SDS) or ethylenediaminetetraacetic acid (EDTA) may remain from the lysis and purification steps of the RNA-isolation procedure.
- The primer sequences for the detection of the QN IC RNA have been bioinformatically validated for nonhomology against hundreds of eukaryotic and prokaryotic organisms. Additionally, they have been experimentally tested against a multitude of human, mouse, and rat RNA samples from multiple tissues and cell lines.
- After using the QuantiNova Reverse Transcription Kit, cDNA should be diluted (1:10 to 1:100) and an aliquot of the reaction should be used for subsequent amplification with the QuantiNova SYBR Green PCR Kit. If using the QuantiNova IC RNA to control for absence of inhibitors, this should be detected using the respective QuantiNova IC assay. For detection with the QuantiNova SYBR Green PCR Kit, use the appropriate volume of 10x QuantiNova IC SYBR Green Assay.
- An additional no-template RNA control sample, which only contains the QN IC RNA, should also be processed in reverse transcription and subsequent qPCR. C_q shifts >2 between the template RNA + QN IC RNA, compared to the QN IC RNA–only samples, and between the different template RNA-containing samples, indicate inhibition of the RT-PCR.

Procedure

1. After amplification, perform data analysis as recommended for your real-time PCR instrument.
2. Compare C_q values between the QN IC RNA only and the samples containing QN IC RNA plus template RNA.

   Consistent C_q values indicate successful RT-PCR and reliable results. A C_q difference >2 is likely to indicate inhibition or sample failure.
3. If a shifted C_q >2 appears, indicating inhibition or failure of a specific sample, we recommend the following:

   3a. Check the equipment for accurate performance and repeat sample/experiment to rule out pipetting or handling errors.

   3b. Dilute the affected template RNA using RNase-free water before repeating the experiment. This dilutes the inhibitors that are present in the sample.

   3c. Consider repeating the RNA extraction and avoid contamination or carry-over of inhibitors (e.g., use an appropriate RNeasy Kit). Alternatively, the RNeasy MinElute® Cleanup Kit (cat. no. 74204) can be used to remove potential inhibitors and concentrate the RNA template.

96-Well and 384-Well Panels: Analysis of mRNA/lncRNA Quantitative PCR Experiments

Data collection and analysis is an important part of the qPCR experiment. Proper collection data analysis will ensure your data are reliable and that you draw the correct conclusions to your experiment. In GeneGlobe Data Analysis Center, QIAGEN offers an online data analysis software that is specifically designed for analyzing data from the QuantiNova LNA PCR products. A wizard will guide you through the pre-processing steps including the normalization. In the section below is a step-by-step guideline on how to collect data and prepare them for upload to the GeneGlobe data analysis tool.

Procedure

1. Calculate the threshold cycle (C_q) for each well using the real-time cycler software, as described in steps 2–4.

   If using the Roche LightCycler 480, there are 2 options for data analysis:

   - Use the second derivate max setting (in this case there is no need to calculate the C_q)
   - Use "Fit Points" (in this case the C_q should be defined manually as described in step 3)

2. Define the baseline by choosing the automated baseline option if the cycler has the adaptive baseline function. If the cycler does not have the adaptive baseline function, set the baseline manually.

   To set the baseline manually, use the linear view of the amplification plots to determine the earliest visible amplification. Set the cycler to use the readings from cycle number 2 through 2 cycles before the earliest visible amplification, but no more than cycle 15. The earliest amplification will usually be visible between cycles 14 and 18.

3. Manually define the threshold by using the log view of the amplification plots. Choose a threshold value above the background signal but within the lower one-third to lower one-half of the linear phase of the amplification plot.

   Ensure that the threshold values are the same across all runs in the same analysis. The absolute position of the threshold is less critical than its consistent position across panels. If the RNA sample is of sufficient quality and the cycling program has been carried out correctly and the threshold values have been defined correctly, then the value of C_q^{PPC} should be <25 when using the 2-step RT-PCR protocol and <26 when using the 1-step RT-PCR protocol for all panels or samples.

4. Export the C_q values for all wells to a blank Excel® spreadsheet. Data analysis can then be conducted at QIAGEN's GeneGlobe Data Analysis Center, which you can find by going to **geneglobe.qiagen.com/analyze**

   Visually inspect the plate after the run for any signs of evaporation from any of the wells. If evaporation is observed, note which wells are affected, because this may affect the results of data analysis.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: **www.qiagen.com/FAQ/FAQList.aspx**. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and/or protocols in this handbook or sample and assay technologies (for contact information, visit **www.qiagen.com**).

		Comments and suggestions
No signal or one or more signals detected late in PCR		
a)	Incorrect cycling conditions	Always start with the optimized cycling conditions specified in the protocols. Ensure that the PCR cycling conditions include the initial step for activation of QuantiNova DNA Polymerase (95°C for 2 min) and the specified times for denaturation and annealing/extension.
b)	QuantiNova DNA Polymerase not activated	Ensure that the PCR cycling program includes the QuantiNova DNA Polymerase activation step (2 min at 95°C) as described in the protocols.
c)	Pipetting error or missing reagent	Check the concentrations and storage conditions of the reagents, including primers and template nucleic acid. Repeat the PCR.
d)	Wrong or no detection step	Ensure that fluorescence detection takes place during the combined annealing/extension step.
e)	Problems with starting template	Check the concentration, storage conditions, and quality of the starting template. If necessary, make new serial dilutions of template nucleic acid from the stock solutions. Repeat the PCR using the new dilutions.
f)	Insufficient amount of starting template	Increase the amount of template, if possible. Ensure that sufficient copies of the target nucleic acids are present in your sample.
g)	Insufficient number of cycles	Increase the number of cycles.
h)	Reaction volume too high	For 96-well block cyclers, we recommend a final reaction volume of 20 µl. For 384-well block cyclers, we strongly recommend a final reaction volume of 10 µl.

		Comments and suggestions
i)	Generated signals are weak	RNA samples may contain PCR inhibitors. Further purification or an alternative RNA extraction method may be necessary. Check positive controls.
j)	Wrong detection channel/filter chosen	Ensure that the correct detection channel is activated and that the correct filter set is chosen for the reporter dye.
k)	No detection activated	Check that fluorescence detection was activated in the cycling program.
Increased fluorescence or C_q value for no-template control (NTC)		
a)	Contamination of reagents	Discard all the components of the assay (e.g., Master Mix and primers). Repeat the assay using new components.
b)	Contamination during reaction setup	Take appropriate precautions during reaction setup, such as using aerosol-barrier pipette tips.
Varying fluorescence intensity		
a)	Contamination of real-time cycler	Decontaminate the real-time cycler according to the manufacturer's instructions.
b)	Real-time cycler is no longer calibrated	Recalibrate the real-time cycler according to the manufacturer's instructions.
All cycler systems		
	Wavy curve at high template amounts for highly expressed targets	In the analysis settings, reduce the number of cycles used for background calculation (if your real-time cycler allows you to do so) or reduce the amount of template.
Applied Biosystems instruments only		
	ΔRn values unexpectedly too high or too low	In the analysis settings, reduce the number of cycles used for background calculation (if your real-time cycler allows you to do so) or reduce the amount of template. Check that the correct concentration of ROX was used.

Appendix: Reaction Setup Using Master Mix that Contains a High Concentration of ROX

Note: This appendix and Table 17 to Table 24 are only relevant for setting up reactions using a Master Mix to which a high concentration of ROX has already been added according to Table 4. When using a Master Mix containing a low concentration of ROX, the volume of ROX added is negligible, and the standard reaction setup as described in the protocols should be used.

Table 17. Reaction mix setup for QuantiNova LNA PCR Assays using a Master Mix that contains a high concentration of ROX dye

Component	Volume/reaction	Final concentration
2x QuantiNova SYBR Green PCR Master Mix with high ROX	11 µl	1x
10x QuantiNova LNA PCR Assay *or* 10x QuantiNova LNA PCR Reference Assay *or* 10x QuantiNova IC SYBR Green Assay	2 µl	1x
cDNA template	Variable	≤10% of total reaction volume
RNase-free water	Variable	–
Total reaction volume	**20 µl**	**–**

Table 18. Master Mix setup for QuantiNova LNA PCR Panels for 1 sample per 96-well plate/Rotor-Disc 100 using a Master Mix that contains a high concentration of ROX dye

Component	96-well panels	384-well panels	Final concentration
2x QuantiNova SYBR Green PCR Master Mix with high ROX	1100 µl	2200 µl	1x
Diluted cDNA template	Variable	Variable	≤10% of total Master Mix volume
RNase-free water	Variable	Variable	–
Total Master Mix volume	**2000 µl***	**4000 µl***	**–**

* Total Master Mix volume includes a reserve to compensate for pipetting variations.

Table 19. Master Mix setup for QuantiNova LNA PCR Flexible Panels and Custom Panels for more than 1 sample per 96-well plate/Rotor-Disc 100 using a Master Mix that contains a high concentration of ROX dye

Component	2 samples (48 wells per sample)	4 samples (24 wells per sample)	8 samples (12 wells per sample)	Final concentration
2x QuantiNova SYBR Green PCR Master Mix with high ROX	572 µl	308 µl	176 µl	1x
Diluted cDNA template	100 µl	100 µl	100 µl	–
RNase-free water	Variable	Variable	Variable	–
Total Master Mix volume	**2 x 1040 µl***	**4 x 560 µl***	**8 x 320 µl***	**–**

* Total Master Mix volume includes a reserve to compensate for pipetting variations.

Table 20. Master Mix setup for QuantiNova LNA PCR Flexible Panels and Custom panels for more than 1 sample per 384-well plate using a Master Mix that contains a high concentration of ROX dye

Component	2 samples (192 wells per sample)	4 samples (96 wells per sample)	8 samples (48 wells per sample)	16 samples (24 wells per sample)	Final concentration
2x QuantiNova SYBR Green PCR Master Mix with high ROX	1100 µl	550 µl	286 µl	154 µl	1x
Diluted cDNA template	100 µl	100 µl	100 µl	100 µl	–
RNase-free water	Variable	Variable	Variable	Variable	–
Total Master Mix volume	**2 x 2000 µl***	**4 x 1000 µl***	**8 x 520 µl***	**16 x 280 µl***	**–**

* Each Master Mix includes a reserve of at least 4 single reaction volumes (80 µl for 96-well plates and 40 µl for 384-well plates) to compensate for pipetting variations.

Table 21. One-step reaction mix setup for QuantiNova LNA PCR Assays using a Master Mix that contains a high concentration of ROX dye

Component	Volume/reaction	Final concentration
2x QuantiNova SYBR Green RT-PCR Master Mix with high ROX	11 µl	1x
QN SYBR Green RT-Mix	0.2 µl	1x
10x QuantiNova LNA PCR Assay *or* 10x QuantiNova LNA PCR Reference Assay *or* 10x QuantiNova IC SYBR Green Assay	2 µl	1x
Diluted QN IC RNA (optional)	1 µl	1x
RNA template	Variable	≤10% of total reaction volume
RNase-free water	Variable	–
Total reaction volume	**20 µl**	–

Table 22. One-step Master Mix setup for QuantiNova LNA PCR Panels for 1 sample that contains a high concentration of ROX dye

Component	96-well panels	384-well panels	Final concentration
2x QuantiNova SYBR Green RT-PCR Master Mix with high ROX	1100 µl	2200 µl	1x
QN SYBR Green RT-Mix	20 µl	40 µl	1x
Diluted QN IC RNA (optional)	100 µl	400 µl	1x
RNA template	Variable	Variable	≤10% of total Master Mix volume
RNase-free water	Variable	Variable	–
Total Master Mix volume	**2000 µl***	**4000 µl***	**–**

* Total Master Mix volume includes a reserve to compensate for pipetting variations.

Table 23. One-step Master Mix setup for QuantiNova LNA PCR Flexible Panels and Custom Panels for more than 1 sample per 96-well plate/Rotor-Disc 100 that contains a high concentration of ROX dye

Component	2 samples (48 wells per sample)	4 samples (24 wells per sample)	8 samples (12 wells per sample)	Final concentration
2x QuantiNova SYBR Green RT-PCR Master Mix with high ROX	572 µl	308 µl	176 µl	1x
QN SYBR Green RT-Mix	10.4 µl	5.6 µl	3.2 µl	1x
Diluted QN IC RNA (optional)	52 µl	28 µl	16 µl	1x
RNA template	Variable	Variable	Variable	≤10% of Master Mix volume
RNase-free water	Variable	Variable	Variable	–
Total Master Mix volume	**2 x 1040 µl***	**4 x 560 µl***	**8 x 320 µl***	**–**

* Each Master Mix includes a reserve of at least 4 single reaction volumes to compensate for pipetting variations.

Table 24. One-step Master Mix setup for QuantiNova LNA SYBR Green PCR flexible panels and custom panels for more than 1 sample per 384-well plate that contains a high concentration of ROX dye

Component	2 samples (192 wells per sample)	4 samples (96 wells per sample)	8 samples (48 wells per sample)	16 samples (24 wells per sample)	Final concentration
2x QuantiNova SYBR Green RT-PCR Master Mix with high ROX	1100 µl	550 µl	286 µl	154 µl	1x
QN SYBR Green RT-Mix	20 µl	10 µl	5.2 µl	2.8 µl	
Diluted QN IC RNA (optional)	200 µl	100 µl	52 µl	28 µl	1x
RNA template	Variable	Variable	Variable	Variable	≤10% of Master Mix volume
RNase-free water	Variable	Variable	Variable	Variable	–
Total Master Mix volume	**2 x 2000 µl***	**4 x 1000 µl***	**8 x 520 µl***	**16 x 280 µl***	**–**

* Each Master Mix includes a reserve of at least 4 single reaction volumes to compensate for pipetting variations.

Ordering Information

Product	Contents	Cat. no.
QuantiNova LNA PCR Assay (200)	Single primer designed for highly sensitive and rapid real-time quantification of single mRNA and lncRNA targets	249990
QuantiNova LNA PCR Assay (750)	Single primer designed for highly sensitive and rapid real-time quantification of single mRNA and lncRNA targets	249992
QuantiNova LNA PCR Custom Assay (200)	Single primer designed for highly sensitive and rapid real-time quantification of single mRNA and lncRNA targets	249910
QuantiNova LNA PCR Custom Assay (750)	Single primer designed for highly sensitive and rapid real-time quantification of single mRNA and lncRNA targets	249911
QuantiNova LNA PCR Reference Assay (200)	Single primer designed for highly sensitive and rapid real-time quantification of single mRNA and lncRNA targets	249920
QuantiNova LNA PCR Reference Assay (750)	Single primer designed for highly sensitive and rapid real-time quantification of single mRNA and lncRNA targets	249921

Product	Contents	Cat. no.
QuantiNova LNA PCR Focus Panels	Panels of primer designed for highly sensitive and rapid real-time quantification of mRNA and lncRNA targets in 96- or 384-well plates	249950
QuantiNova LNA PCR lncRNA Focus Panels	Panels of primer designed for highly sensitive and rapid real-time quantification of mRNA and lncRNA targets in 96- or 384-well plates	249951
QuantiNova LNA PCR Flexible Panels	Panels of primer designed for highly sensitive and rapid real-time quantification of mRNA and lncRNA targets in 96- or 384-well plates	249960
QuantiNova LNA PCR Custom Panels	Panels of primer designed for highly sensitive and rapid real-time quantification of mRNA and lncRNA targets in 96- or 384-well plates	249970
QuantiNova Reverse Transcription Kit (10)	For 10 x 20 µl reactions: 20 µl 8x gDNA removal mix, 10 µl reverse transcription enzyme, 40 µl reverse transcription mix (containing RT primers), 20 µl internal control RNA, 1.9 ml RNase-free water	205410
QuantiNova Reverse Transcription Kit (50)	For 50 x 20 µl reactions: 100 µl 8x gDNA removal mix, 50 µl reverse transcription enzyme, 200 µl reverse transcription mix (containing RT primers), 100 µl internal control RNA, 1.9 ml RNase-free water	205411

Product	Contents	Cat. no.
QuantiNova Reverse Transcription Kit (200)	For 200 x 20 µl reactions: 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 internal control RNA, 4 x 1.9 ml RNase-free water	205413
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
QuantiNova SYBR Green RT-PCR Kit (100)	For 100 x 20 µl reactions: 1 ml QuantiNova SYBR Green RT-PCR Master Mix, 20 µl QuantiNova SYBR Green RT Mix, 20 µl Internal Control RNA, 500 µl Yellow Template Dilution Buffer, 250 µl ROX Reference Dye, 1.9 µl RNase-free Water	208152

Product	Contents	Cat. no.
QuantiNova SYBR Green RT-PCR Kit (500)	For 500 x 20 µl reactions: 3 x 1.7 ml QuantiNova SYBR Green RT-PCR Master Mix, 100 µl QuantiNova SYBR Green 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	208154
QuantiNova SYBR Green RT-PCR Kit (2500)	For 2500 x 20 µl reactions: 15 x 1.7 ml QuantiNova SYBR Green RT-PCR Master Mix, 5 x 100 µl QuantiNova SYBR Green RT Mix, 2 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	208156
QuantiNova IC SYBR Green Assay (500)	QuantiTect Primer Assay for SYBR-based detection of QuantiNova Internal Control RNA, available via GeneGlobe (sufficient for approx. 500 x 20 µl reactions), for use with QuantiNova SYBR Green PCR Kit or QuantiNova SYBR Green RT-PCR Kit	QT02589307*
Related products		
RNeasy Plus Mini Kit (50)	For 50 minipreps: RNeasy Mini Spin Columns, gDNA Eliminator Spin Columns, collection tubes, RNase-free water and buffers	74134

* Available from **geneglobe.qiagen.com**

Product	Contents	Cat. no.
RNeasy Mini Kit (50)	50 RNeasy Mini Spin Columns, collection tubes (1.5 ml and 2 ml), RNase-free reagents and buffers	74104
RNeasy FFPE Kit (50)	50 RNeasy MinElute Spin Columns, collection tubes, proteinase K, RNase-free DNase I, DNase booster buffer, RNase-free buffers, RNase-free water	73504
RNeasy Plus Micro Kit (50)	For 50 micropreps: RNeasy MinElute Spin Columns, gDNA Eliminator Spin Columns, collection tubes, carrier RNA, RNase-free water and buffer	74034
QIAamp RNA Blood Mini Kit (50)	50 QIAamp Mini Spin Columns, 50 QIAshredder Spin Columns, collection tubes (1.5 ml and 2 ml), RNase-free reagents and buffers	52304
QIAzol Lysis Reagent (200ml)	200 ml QIAzol Lysis Reagent	79306

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Visit **www.qiagen.com/geneXpression** to find out more about standardized solutions for gene expression analysis – from RNA preparation to real-time RT-PCR.

Document Revision History

Date	Changes
12/2019	Initial release
12/2021	Updated the "Introduction" section, to say that "the QuantiNova Reverse Transcription Kit includes QuantiNova Internal Control (QN IC) RNA template to test successful reverse transcription and amplification". Changed the recommended volume per reaction (from "≤200 ng – 100 fg" to "100 fg to ≤ 200") in the One-step RT-PCR protocol (page 14). Corrected Table 16: Revised title and added the reverse transcription step. Updated the Ordering Information section. Editorial and layout changes.

Notes

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