December 2021

QuantiNova® LNA® PCR Assay Handbook for the QIAcuity® System

For highly sensitive, real-time RT-PCR detection of mRNA and lncRNA using QIAcuity EG PCR Kit ®

Sample to Insight

QIAGEN

Contents

Kit Contents

Assay (1 single tube containing dried primer mix)	Catalog no. (650/200 reactions)*	Catalog no. (2500/750 reactions)†
QuantiNova LNA PCR Assay‡	249990	249992
QuantiNova LNA PCR Custom Assay‡	249910	249911
QuantiNova LNA PCR Reference Assay‡	249920	249921

* Sufficient for 650 reactions using the QIAcuity Nanoplate 8.5k and 200 reactions using the QIAcuity Nanoplate 26k.

† Sufficient for 2500 reactions using the QIAcuity Nanoplate 8.5k and 750 reactions using the QIAcuity Nanoplate 26k.

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

Product	Catalog no.
QuantiTect Reverse Transcription Kit (for 10, 50, or 200 reactions)	205311, 205313, or 205314
QIAcuity EG PCR Kit (for 100, 500, or 2500 reactions)	250111, 250112, or 250113

Shipping and Storage

The QuantiNova LNA PCR Assays are shipped at room temperature (15–25°C). Upon receipt, store QuantiNova LNA PCR Assays at 2–8°C for short-term storage (up to 6 months) or at –30 to –15°C in a constant-temperature freezer for long-term storage (up to 1 year). After reconstitution, store 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, without showing any reduction in performance and quality, until the date indicated on the label.

Intended Use

The QuantiNova LNA PCR Assays 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 are tested against predetermined specifications to ensure consistent product quality.

Introduction

QuantiNova LNA PCR Assays provide highly sensitive and accurate locked nucleic acid (LNA)–enhanced digital PCR (dPCR) 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 dPCR amplification using EvaGreen® for detection.

To obtain optimal results in dPCR, the use of QuantiNova LNA PCR Assay products in combination with the QuantiTect Reverse Transcription Kit and the QIAcuity EG PCR Kit is recommended. The QuantiNova Reverse Transcription Kit is not recommended.

In dPCR, the QuantiTect Reverse Transcription Kit is recommended for cDNA synthesis. The kit is designed for use as part of 2-step RT-PCR (Figure 1) and provides high cDNA yields for sensitive quantification of even low-abundance transcripts.

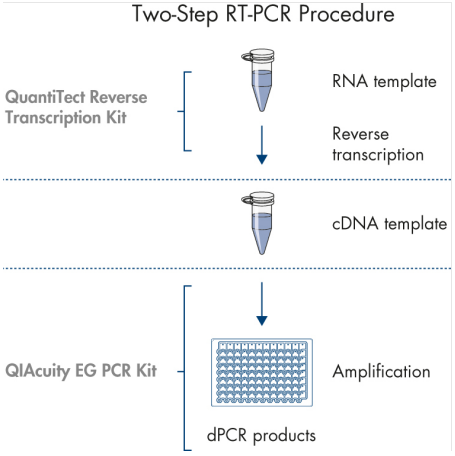

Figure 1. QIAcuity workflow. Two-step RT-PCR procedure.

The QIAcuity EG PCR Kit provides the highest specificity in dPCR 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.

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
QuantiTect Reverse Transcription Kit	Buffer and kit work best in dPCR with EvaGreen detection. Do not use QuantiNova Reverse Transcription Kit.
QuantiNova Internal Control (QN IC) RNA	Synthetic transcript for monitoring successful reverse transcription
QIAcuity EG PCR Kit	A ready-to-use Master Mix for conducting dPCR runs on the QIAcuity instrument

Principle and procedure

The QuantiNova LNA PCR Assays and the QIAcuity instrument form 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 dPCR 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 formats.
- **LNA-enhanced dPCR 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 Assays and the outstanding performance of the QIAcuity EG PCR Kit offer solutions for both high-throughput mRNA/lncRNA expression profiling and for quantification of individual mRNAs/lncRNAs.

High specificity and sensitivity in dPCR 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 QIAcuity EG 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 on the QIAcuity instrument.

3x QIAcuity EG PCR Master Mix

The 3x QIAcuity EG PCR Master Mix includes QuantiNova DNA Polymerase. The dPCR-optimized Master Mix ensures ultrafast amplification in dPCR, with high specificity and sensitivity.

The additive Q-Bond allows short cycling times. 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 stabilizes the complex and improves 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.

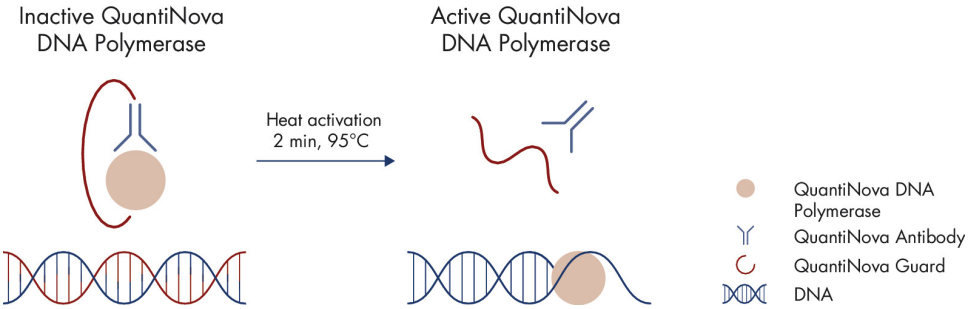

Figure 2. Principle of the novel QuantiNova hot-start mechanism. At ambient temperature, the QuantiNova DNA Polymerase is kept inactive by QuantiNova Antibody and QuantiNova Guard, until the initial heat activation step.

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.

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.

- QuantiTect Reverse Transcription Kit (cat. no. 205311, 205313, or 205314) and QIAcuity EG PCR Kit (cat. no. 250111, 250112, or 250113)
- 10x QuantiNova IC SYBR® Green Assay (**geneglobe.qiagen.com**, cat. no. QT02589307)
- QIAcuity Nanoplate (cat. no. 250001, 250011, or 250021)
- 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 (included with the nanoplate kits, or sold separately as cat. no. 250099)
- Real-time PCR instrument

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.

Protocol: 2-Step RT-PCR

cDNA synthesis

For cDNA synthesis, the use of the QuantiTect Reverse Transcription Kit is critical for obtaining optimal results with QuantiNova LNA PCR Assays. 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 dPCR using the QIAcuity dPCR platform, the use of the QIAcuity EG PCR Kit is critical for obtaining optimal results with QuantiNova LNA PCR assays.

Important points before starting

- A fluorescent reference dye is provided as a component of the QIAcuity EG PCR Master Mix, for reliable detection of proper partition filling in the dPCR plates.
- After using the QuantiTect 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 QIAcuity EG PCR Kit.
- Always start with the cycling conditions and primer concentrations specified in this protocol.

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.

Note: The stock concentration of the reconstituted QuantiNova LNA PCR Assays is 10x. In the dPCR reaction using the QIAcuity EG PCR Kit, half of the assay concentration is used; therefore, the final assay concentration in the reaction setup is 0.5x.

Procedure

1. Thaw 3x QIAcuity EG PCR Master Mix, template cDNA, 10x QuantiNova LNA PCR Assay, and RNase-free water. Mix the individual solutions.
2. Prepare a reaction mix according to Table 2. Reaction setup for QuantiNova LNA PCR Assays

	Volume/reaction		
Component	**Nanoplate 8.5k (24-well, 96-well)**	**Nanoplate 26k (24-well)**	**Final concentration**
3x EvaGreen PCR Master Mix (green channel)	4 µl	13.3 µl	1x
QuantiNova LNA PCR Assay (10x)	0.6 µl	2 µl	**0.5x***
RNase-free water	Variable	Variable	
Template cDNA (added at step 4)	Variable†	Variable†	≤10% of total reaction volume
Total reaction volume	**12 µl**	**40 µl**	

* Assay concentration in dPCR is half in comparison to qPCR use, therefore final concentration is 0.5x.

† Appropriate template amount depends on various parameters. For detailed information, please refer to the *QIAcuity Application Guide*.

3. . Due to the hot-start, it is not necessary to keep samples on ice during reaction setup or while programming the QIAcuity dPCR instrument.

Table 2. Reaction setup for QuantiNova LNA PCR Assays

	Volume/reaction		
Component	**Nanoplate 8.5k (24-well, 96-well)**	**Nanoplate 26k (24-well)**	**Final concentration**
3x EvaGreen PCR Master Mix (green channel)	4 µl	13.3 µl	1x
QuantiNova LNA PCR Assay (10x)	0.6 µl	2 µl	**0.5x***
RNase-free water	Variable	Variable	
Template cDNA (added at step 4)	Variable†	Variable†	≤10% of total reaction volume
Total reaction volume	**12 µl**	**40 µl**	

* Assay concentration in dPCR is half in comparison to qPCR use, therefore final concentration is 0.5x.

† Appropriate template amount depends on various parameters. For detailed information, please refer to the *QIAcuity Application Guide*.

4. Vortex the reaction mix.
5. Dispense appropriate volumes of the reaction mix, which contains all components except the template, into the wells of a standard PCR plate. Then, add template cDNA into each well that contains the reaction mix.

   Note: The appropriate amounts of reaction mix and template DNA depends on various parameters. Please refer to the *QIAcuity Application Guide* for details.

   Note: For 2-step RT-PCR, use the QuantiTect Reverse Transcription Kit for the first step to synthesize the cDNA.

   The volume of the cDNA added (from the undiluted reverse-transcription reaction) should not exceed 10% of the final PCR volume if using the QuantiTect Reverse Transcription Kit. If using another RT Kit, it should not exceed 5%.
6. Transfer the contents of each well of the standard PCR plate to the wells of the QIAcuity Nanoplate.
7. Seal the QIAcuity Nanoplate properly using the QIAcuity Nanoplate Seal provided in the QIAcuity Nanoplate Kits.

   Note: For exact sealing procedure, please see the *QIAcuity User Manual*, **www.qiagen.com/HB-2717**.

Thermal cycling and imaging conditions

1. In the QIAcuity Software Suite or on the QIAcuity instrument, under the dPCR parameters, set the cycling conditions according to Table 3.

Table 3. 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
3-step cycling (40 cycles)				
Denaturation	15 s	95°C	Maximal/fast mode	
Annealing	15 s	55°C	Maximal/fast mode	
Extension	15 s	72°C	Maximal/fast mode	
Cooling down	5 min	40°C		

2. Still under the dPCR parameters in the QIAcuity Software Suite or on the QIAcuity instrument, activate the green channel and deactivate the other channels in **Imaging**.
3. Place the QIAcuity Nanoplate in the QIAcuity instrument and start the run.

Data analysis

1. To set up a plate layout according to the experimental design, open the QIAcuity Software Suite and define the reaction mixes, samples, and controls. Plate layout can be defined before or after the nanoplate run.

   Note: Refer to the *QIAcuity User Manual* for details on setting up the plate layout.
2. After the run is completed, the raw data is automatically sent to the QIAcuity Software suite.
3. For data analysis, open the QIAcuity Software Suite and select the individual nanoplate for the analysis in **Plate Overview** of the software suite.

   Note: Refer to the *QIAcuity Application Guide*, **www.qiagen.com/HB-2839**, and the *QIAcuity User Manual* for details on how to analyze absolute quantification data and gene expression data including normalization.

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 in 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 or protocols in this handbook (for contact information, visit **support.qiagen.com**).

	Comments and suggestions
No or low signal (low signal-to-noise)	
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) 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.
e) 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.
f) Insufficient number of cycles	Increase the number of cycles.
g) Generated signals are weak	RNA samples may contain PCR inhibitors. Further purification or an alternative RNA extraction method may be necessary. Check positive controls.
High concentration values detected 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.

Appendix: 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 minimal 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 plasticware 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

* 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.

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.

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.

* 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.

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, 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.

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 3). Any smearing of the RNA bands or shoulders on the RNA peaks indicate that degradation has occurred in the RNA sample.

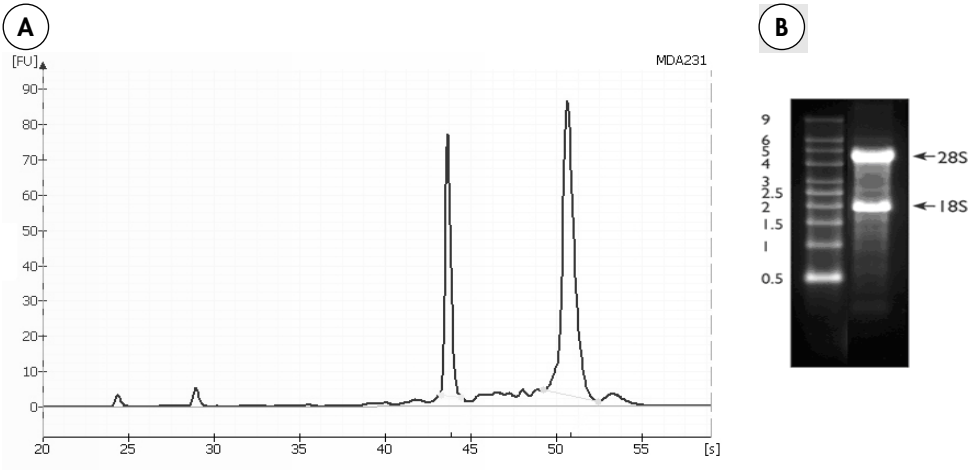

Figure 3. Ribosomal RNA integrity. A: Agilent Bioanalyzer electropherogram of high-quality total RNA showing sharp peaks for the 18S (left) and 28S (right) ribosomal RNA. Due to high quality of the RNA, peaks do not have shoulders (especially to the left of each peak). **B**: Agarose gel electrophoresis 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

The Quantiscript Reverse Transcriptase 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 dPCR using the QIAcuity EG 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.

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 4. 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 Services at **support.qiagen.com**.

Built-in visual control for correct pipetting

The 3x QIAcuity EG PCR Kit contains an inert blue dye that increases visibility in the tube or well without interfering with the PCR.

Normalization of mRNA/lncRNA concentration in quantitative gene expression 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.

Further guidance on normalization can also be found in GeneGlobe Data Analysis Center, **geneglobe.qiagen.com**.

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
QuantiTect Reverse Transcription Kit (50)	For 50 x 20 µl reactions: 100 µl 7x gDNA Wipeout Buffer, 50 µl Quantiscript Reverse Transcriptase, 200 µl 5x Quantiscript RT Buffer, 50 µl RT Primer Mix, 1.9 ml RNase-Free Water	205311
QuantiTect Reverse Transcription Kit (200)	For 200 x 20 µl reactions: 4 x 100 µl 7x gDNA Wipeout Buffer, 4 x 50 µl Quantiscript Reverse Transcriptase, 4 x 200	205313

Product	Contents	Cat. no.
	µl 5x Quantiscript RT Buffer, 4 x 50 µl RT Primer Mix, 4 x 1.9 ml RNase-Free Water	
QuantiTect Reverse Transcription Kit (400)	For 400 x 20 µl reactions: 800 µl 7x gDNA Wipeout Buffer, 400 µl Quantiscript Reverse Transcriptase, 1.6 ml 5x Quantiscript RT Buffer, 400 µl RT Primer Mix, 8 x 1.9 ml RNase-Free Water	205314
QIAcuity EG PCR Kit	For 100, 500, 2500 reactions: ready-to-use Master Mix for conducting dPCR runs on the QIAcuity instrument	250111 250112 250113
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
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	73504

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

Product	Contents	Cat. no.
	DNase I, DNase booster buffer, RNase-free buffers, RNase-free water	
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 (200 ml)	200 ml QIAzol Lysis Reagent	79306

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.

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
07/2020	Initial release
12/2021	Fixed an error in the second footnote of the "Kit Contents" table (changed "QIAcuity Nanoplate 8.5k" to "QIAcuity Nanoplate 26k" for 750 reactions). Deleted unavailable catalog numbers of QIAcuity Nanoplate. Updated the content description and catalog numbers of QuantiTect Reverse Transcription Kit products in the Ordering Information section.

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