January 2017

# QuantiNova® Multiplex RT-PCR Handbook

For highly sensitive, ultrafast quantitative, multiplex real-time RT-PCR using sequence-specific probes

Sample to Insight

QIAGEN

# Contents

# Kit Contents

| **QuantiNova Multiplex RT-PCR Kit** | **(100)** | **(500)** | **(2500)** |
|---|---|---|---|
| **Catalog no.** | **208552** | **208554** | **208556** |
| **Number of reactions (20 µl/10 µl)** | **100/200** | **500/1000** | **2500/5000** |
| QuantiNova Multiplex RT-PCR Master Mix (contains: QuantiNova DNA Polymerase, which is composed of Taq DNA Polymerase, QuantiNova Antibody and QuantiNova Guard; QuantiNova MP RT-PCR Buffer; dNTP mix [dATP, dCTP, dGTP, dTTP]) | 0.5 ml | 2 x 1.3 ml | 10 x 1.3 ml |
| 100x QuantiNova RT Mix, containing: HotStaRT-Script Reverse Transcriptase, RNase Inhibitor | 20 µl | 100 µl | 5 x 100 µl |
| QuantiNova Yellow Template Dilution Buffer | 500 µl | 500 µl | 3 x 500 µl |
| QuantiNova Internal Control RNA | 20 µl | 100 µl | 3 x 100 µl |
| QN ROX™ Reference Dye | 250 µl | 1 ml | 3 x 1 ml |
| RNase-Free Water | 1.9 ml | 4 x 1.9 ml | 20 x 1.9 ml |
| Quick-Start Protocol QuantiNova Multiplex RT-PCR | 1 | 1 | 1 |
| Quick-Start Protocol QuantiNova IC & Assay | 1 | 1 | 1 |

# Storage

QuantiNova Multiplex RT-PCR Kits are shipped on dry ice. The kits should be stored immediately upon receipt at –15 to –30°C in a constant-temperature freezer and protected from light. When the kits are stored under these conditions and handled correctly, performance is guaranteed until the expiration date (see the kit label). QuantiNova Multiplex RT-PCR Master Mix, QuantiNova Yellow Template Dilution Buffer and QN ROX Reference Dye (protected from light) can also be stored at 2–8°C for up to 12 months, depending on the expiry date.

If desired, QN ROX Reference Dye can be added to 4x QuantiNova Multiplex RT-PCR Master Mix for long-term storage. For details, see “Adding ROX dye to the master mix”, page 13.

# Intended Use

The QuantiNova Multiplex RT-PCR Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention or treatment of a disease.

All due care and attention should be exercised in the handling of the products. 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.

**CAUTION: DO NOT add bleach or acidic solutions directly to the sample preparation waste.**

# Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of QuantiNova Multiplex RT-PCR Kit is tested against predetermined specifications to ensure consistent product quality.

# Product Information

The QuantiNova Multiplex RT-PCR Kit contains:

**4x QuantiNova Multiplex RT-PCR Master Mix**

| Component | Description |
|---|---|
| QuantiNova DNA Polymerase | QuantiNova DNA Polymerase is 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-minute, 95°C incubation step. |
| QuantiNova Multiplex RT-PCR Buffer | Contains Tris·HCl, KCl, $NH_4Cl$, $MgCl_2$ and additives enabling fast cycling, including Q-Bond®. |
| dNTP mix | Contains dATP, dCTP, dGTP and dTTP of ultra-pure quality. |

**100x QuantiNova Multiplex RT Mix**

| Component | Description |
|---|---|
| HotStarRT-Script Reverse Transcriptase | HotStarRT-Script Reverse Transcriptase is 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 | The RNase inhibitor is a recombinant mammalian protein that inhibits eukaryotic RNases, such as RNase A and B. |

**Other components**

| Component | Description |
|---|---|
| QuantiNova Internal Control RNA | Synthetic transcript for monitoring successful reverse transcription. |
| 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 | Ultra-pure quality, PCR-grade. |
| RNase-Free Water | Ultra-pure quality, PCR-grade. |

# Introduction

The QuantiNova Multiplex RT-PCR Kit provides highly sensitive and rapid real-time quantification of RNA targets in an easy-to-handle multiplex format. Depending on the real-time cycler used, up to 5 targets can be quantified simultaneously in the same well or tube. The kit can be used in real-time RT-PCR using various RNA targets such as total RNA from eukaryotes and prokaryotes, as well as poly(A)-RNA and in vitro-transcribed RNA. The kit is compatible with dual-labeled probes, e.g., TaqMan® probes. High specificity and sensitivity in multiplex real-time RT-PCR are achieved by the use of a novel two-phase hot-start procedure.

The HotStaRT-Script Reverse Transcriptase is associated with a RT-blocker, rendering the enzyme almost inactive at ambient temperature. This allows room-temperature setup of the RT-PCR reaction without the risk of primer–dimer formation by the reverse transcriptase. When starting the RT-PCR protocol with the RT step at 50°C, the inhibitor is released from the reverse transcriptase and cDNA synthesis is initiated. The second phase of the hot-start is achieved using QuantiNova DNA Polymerase, a novel hot-start enzyme, together with QuantiNova Guard and a specialized real-time RT-PCR buffer based on QIAGEN's proprietary multiplex PCR buffer technology. These unique components further improve the stringency of the antibody-mediated hot-start. The kit also features a built-in control for visual identification of correct template addition and Q-Bond, an additive in the RT-PCR buffer that enables short cycling steps without loss of PCR sensitivity and efficiency. The QuantiNova Internal Control (QN IC) RNA can be optionally used to monitor successful reverse transcription. The QuantiNova 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 ethylene diaminetetraacetic acid (EDTA) may remain from the lysis and purification steps during the RNA isolation procedure.

The kits have been optimized for use with any real-time cycler. The QN ROX Reference Dye is provided in a separate tube and can be added if using a cycler that requires ROX as a passive reference dye.

## Principle and Procedure

### One-step RT-PCR

Use of 4x QuantiNova Multiplex RT-PCR Master Mix together with QuantiNova Multiplex RT Mix allows both reverse transcription and PCR to take place in a single tube. All reagents required for both reactions are added at the beginning, so there is no need to open the tube once the reverse transcription reaction has been started. There is also no need to set up the reaction on ice, and the whole reaction can be left for up to 2 hours at room temperature without any loss of performance.

### QuantiNova Multiplex RT Mix

The QuantiNova Multiplex RT Mix contains HotStarRT-Script Reverse Transcriptase for heat-mediated activation of the reverse transcription step, and an RNase inhibitor. The HotStaRT-Script Reverse Transcriptase is associated with a RT-blocker, rendering the enzyme almost inactive at ambient temperature. This allows RT-PCR reaction setup at room temperature without the risk of primer–dimer formation by the reverse transcriptase. Upon starting the RT-PCR protocol with the RT step at 50°C, the inhibitor is released from the reverse transcriptase and cDNA synthesis is initiated (Figure 1).

### 4x QuantiNova Multiplex RT-PCR Master Mix

The components of the 4x QuantiNova Multiplex RT-PCR Master Mix include QuantiNova DNA Polymerase and QuantiNova Multiplex RT-PCR Buffer and dNTPs. The optimized master mix ensures fast real-time RT-PCR amplification with high specificity and sensitivity.

### Novel, antibody-mediated hot-start mechanism

QuantiNova DNA Polymerase is provided in an inactive state and has no enzymatic activity at ambient temperature. This prevents the formation and extension of nonspecifically annealed

primers during reaction setup and the first denaturation step, leading to high PCR specificity and accurate quantification. At low temperatures, the QuantiNova DNA Polymerase is kept in an inactive state by the QuantiNova Antibody and a novel additive, QuantiNova Guard, which stabilizes the complex. This improves the stringency of the hot-start.

Within two minutes of raising the temperature to 95°C, the QuantiNova Antibody and QuantiNova Guard are denatured and QuantiNova DNA Polymerase is activated, enabling PCR amplification (Figure 1). The hot-start enables reactions to be set up rapidly and conveniently at room temperature. Furthermore, the real-time PCR sample can be stored after setup at room temperature for up to 2 hours without impairing the performance of the subsequent reaction.

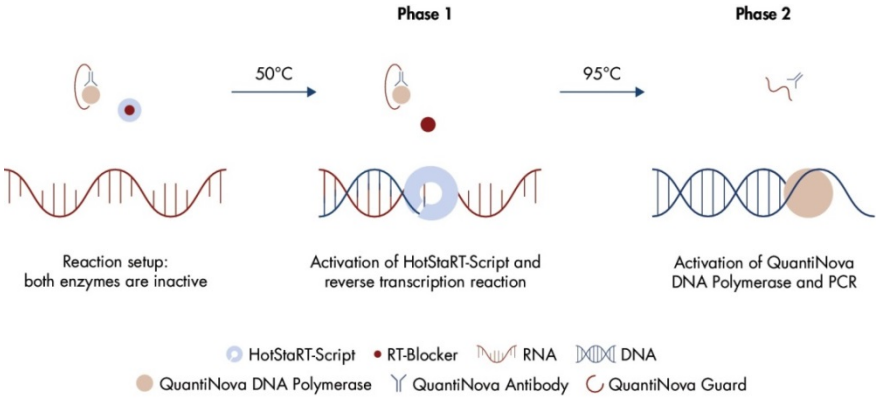



**Figure 1. Principle of the novel QuantiNova two-phase hot-start mechanism.** At ambient temperature the HotStaRT-Script is inhibited by the RT-Blocker and the QuantiNova DNA Polymerase is kept inactive by QuantiNova Antibody and QuantiNova Guard. At 50°C, the RT is activated, while the QuantiNova DNA Polymerase remains inactive. At 95°C, the RT enzyme is denatured and the DNA polymerase is activated.

**Built-in visual control for correct pipetting**

The master mix supplied with the QuantiNova Multiplex RT-PCR Kit contains an inert blue dye that does not interfere with the PCR or RT-PCR, but increases visibility in the tube or well. QuantiNova Yellow Template Dilution Buffer contains an inert yellow dye. When the template nucleic acid, diluted with the QuantiNova Yellow Template Dilution Buffer, is added to the master mix, the color of the solution changes from blue to green, providing a visual indication of correct pipetting. The use of the QuantiNova Yellow Template Dilution Buffer is optional.

**QuantiNova Multiplex RT-PCR Buffer**

QuantiNova Multiplex RT-PCR Buffer is specifically designed to facilitate both efficient reverse transcription and fast real-time PCR using sequence-specific probes. The buffer additive, Q-Bond, allows for short cycling times. Q-Bond increases the affinity of the QuantiNova DNA Polymerase for short single-stranded DNA, reducing the time required for primer/probe annealing to a few seconds. In addition, the unique composition of the buffer supports the melting behavior of DNA, enabling short denaturation and annealing/extension times.

QuantiNova Multiplex RT-PCR Buffer is also based on the unique QIAGEN PCR buffer system. The buffer contains a balanced combination of KCl and $NH_4Cl$, which promotes a high ratio of specific to nonspecific primer binding during the annealing step of each PCR cycle. This creates stringent primer annealing conditions, leading to increased PCR specificity. When using this buffer, primer annealing is only marginally influenced by the $MgCl_2$ concentration, so optimization by titration of $Mg^{2+}$ is not required.

The buffer also contains Factor MP, which facilitates multiplex PCR. This synthetic factor increases the local concentration of primers and probes at the DNA template and stabilizes specifically bound primers and probes, allowing efficient annealing and extension. The combination of these various components of QuantiNova Multiplex RT-PCR Buffer prevents multiple amplification reactions from affecting each other.

The composition of the novel RT stabilizing buffer allows room-temperature RT-PCR reaction setup without the need for cooling. The reaction can be stored for up to 4 h at 4°C or 2 h at room temperature without impairing the performance of the subsequent reaction.

**QuantiNova Internal Control RNA**

The QN IC RNA is a synthetic RNA that can optionally be 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 ethylene diaminetetraacetic acid (EDTA) may remain from the lysis and purification steps during the RNA isolation procedure.

The primer and probe sequences for the detection of the QN IC RNA have been bioinformatically validated for non-homology 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.

The QN IC RNA is detected as a 200 bp amplicon. For probe-based detection, use the QuantiNova Internal Control Probe Assay. The assay is available with MAX™ as a reporter dye (cat. no. 205813). With excitation/emission maxima of 524/557 nm, the MAX dye has a spectral profile allowing detection in the same channel as HEX™, JOE®, or VIC®, and therefore can be used with most real-time cyclers.

In case the MAX dye is not suitable for the intended application, the QuantiNova Internal Control Probe Assay is also available as QuantiNova Internal Control Probe Assay Red 650 (cat. no. 205824). The QuantiNova Internal Control Probe Assay Red 650 employs a Cy5® analogue as a reporter dye. With excitation/emission maxima of 646/664 nm, the dye has a spectral profile allowing detection in the same channel as Cy5, and therefore can be used with most real-time cyclers.

The QN IC RNA can be optionally added to the experimental RNA sample. Additionally, a no-template RNA control sample, which exclusively contains the QN IC RNA, should be set up. $C_q$ shifts between the template RNA+QN IC RNA samples compared to the QN IC RNA only samples and between the different template RNA containing samples indicate inhibition in the RT-PCR.

**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 since it is not involved in the reaction and has an emission spectrum different from fluorescent dyes commonly used for probes.

The use of ROX dye is necessary for instruments from Applied Biosystems. The QuantiNova Multiplex RT-PCR Kit is provided with a separate tube of QN ROX Reference Dye. It can be added to the real-time RT-PCR if using a real-time cycler that uses ROX as a passive reference dye. The QN ROX Reference Dye should be diluted 1:20 in the 1x real-time RT-PCR sample when used on instruments requiring a high ROX concentration and 1:200 for those instruments requiring a low ROX concentration. Refer to Table 1 for details on real-time cyclers that require low or high ROX concentrations. If desired, QN ROX Reference Dye can be added to 4x QuantiNova Multiplex RT-PCR Master Mix for long-term storage (Table 2). For details, see “Adding ROX dye to the master mix”, page 13.

**Table 1. Real-time cyclers requiring high/low concentrations of ROX dye**

| High ROX dye concentration (1:20 dilution of QN ROX Reference Dye in 1x reaction) | Low ROX dye concentration (1:200 dilution of QN ROX Reference Dye in 1x reaction) |
|---|---|
| ABI PRISM® 7000 | Applied Biosystems 7500 |
| Applied Biosystems 7300 | Applied Biosystems ViiA7™ |
| Applied Biosystems 7900 | Applied Biosystems QuantStudio® Systems |
| Applied Biosystems StepOne™ | |
| Applied Biosystems StepOne Plus | |

## Adding ROX dye to the master mix

If only using cyclers from Applied Biosystems with the QuantiNova Multiplex RT-PCR Kit, QuantiNova ROX Reference Dye can be added to QuantiNova Multiplex 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 1, page 13. For reaction setups with master mix that already contains a high concentration of added QuantiNova ROX Reference Dye, refer to "Appendix D: Reaction Setup Using a Master Mix Containing a High Concentration of ROX", page 32."

**Table 2. Addition of QuantiNova ROX Reference Dye to master mix**

| Volume of QuantiNova Multiplex RT-PCR Master Mix (without QN ROX dye) | Volume of ROX dye for high ROX concentration/low ROX concentration |
|---|---|
| 0.5 ml | 100/10 µl |
| 1.3 ml | 250/25 µl |

## Sequence-specific probes

The *QuantiNova Multiplex RT-PCR Kit Handbook* contains protocols optimized for use with TaqMan probes. Other type of probes can be used, however attention should be payed to

certain factors such as data acquisition points or combinations of suitable dyes and quenchers. Please follow the recommendations of your probe or instrument provider.

For more details on commonly used dyes, sequence-specific probes, and their design and handling, see Appendix A and B, page 26 and 27 respectively.

# Protocol: Singleplex and Multiplex Real-Time RT-PCR Using Dual-Labeled Probes

This protocol is for use with the QuantiNova Multiplex RT-PCR Kit and dual-labeled probes (e.g., TaqMan probes) on any cycler.

## Important points before starting

- This protocol is optimized for quantification of RNA targets in a multiplex format (up to 5-plex), using TaqMan probes with any real-time cycler and condition for fluorescence normalization. ROX dye is required for various cyclers 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, ViiA7 and QuantStudio Real-Time PCR Systems.

  **High concentration of ROX dye**: ABI PRISM 7000, Applied Biosystems 7300, 7900 and StepOne Real-Time PCR Systems.

- QuantiNova ROX Reference Dye is provided as a separate tube of passive reference dye for normalization of fluorescent signals on all real-time cyclers from Applied Biosystems. ROX dye should be diluted 1:20 for a 1x reaction when using an instrument requiring a high ROX dye concentration. For instruments requiring a low ROX dye concentration, dilute the dye 1:200 for a 1x reaction.
- The QuantiNova Multiplex 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
- Check the functionality of each set of primers and probes in individual assays before combining the different sets in a multiplex assay.

- Perform appropriate controls for evaluating the performance of your multiplex assays (e.g., amplifying each target individually and comparing the results with those for the multiplex assay).
- Before performing multiplex analyses, choose suitable combinations of reporter dyes and quenchers that are compatible with multiplex analysis using the detection optics of your real-time cycler. We recommend using dual-labeled probes with non-fluorescent quenchers.
- Some real-time cyclers require you to perform a calibration procedure for each reporter dye. Check whether the reporter dyes you selected for your multiplex assay are part of the standard set of dyes already calibrated on your cycler. If they are not, perform a calibration procedure for each dye before using them for the first time (for details, refer to the manufacturer's instructions for your real-time cycler).
- For information on suitable combinations of dyes for multiplex PCR using the Rotor-Gene Q, see Appendix A (page 26).
- Optimal analysis settings (i.e., baseline settings and threshold values) for each reporter dye are a prerequisite for accurate quantification data. For details, check the literature from the manufacturer of your real-time cycler.
- For the highest efficiency in real-time RT-PCR using TaqMan probes, amplicons should ideally be 60–150 bp in length.
- Check the concentration and integrity of primers and probes before starting. For details, see Appendix B, page 27.
- For ease of use, we recommend preparing a 20x primer–probe mix containing target-specific primers and probes for each target. A 20x primer–probe mix consists of 16 µM forward primer, 16 µM reverse primer and 5 µM probe in TE buffer. Alternatively, it may be preferable to prepare the reaction mix with separate primer and probe solutions.
- Always start with the cycling conditions and primer concentrations specified in this protocol.
- The PCR section of the RT-PCR protocol must start with an initial incubation step of 2 min at 95°C to activate the QuantiNova DNA Polymerase.

- 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.
- The QuantiNova Internal Control RNA (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. Using the QuantiNova IC Probe Assay, which needs to be ordered separately (cat. no. 205813), the IC is detected as a 200 bp amplicon in the yellow channel on the Rotor-Gene Q or in the VIC/HEX dye channel on other real-time PCR instruments. Alternatively, it can be detected in the red channel on the Rotor-Gene Q or in the Cy5 dye channel on other real-time PCR instruments, using the QuantiNova IC Probe Assay Red 650 (cat. no. 205824). 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.
- The QN IC RNA (optional) is added to the experimental RNA sample. An additional no-template RNA control sample, containing only QN IC RNA, should be set up. $C_q$ shifts >2 between the template RNA+QN IC RNA compared to the QN IC RNA only samples and between different template RNA containing samples indicate inhibition of RT-PCR.
- The reference dye in QuantiNova Yellow Template Dilution Buffer allows tracking of pipetted samples in the qPCR. When template is added to the blue QuantiNova Multiplex RT-PCR Master Mix, the color changes from blue to green. The use of this buffer is optional. It is provided as a 100x concentrate and should be diluted (using water) to obtain a 1x final concentration within the sample*. To generate a template dilution series, e.g., for absolute quantification or determination of PCR efficiency, dilute the 100x concentrate (using template and water) to obtain a final concentration of 1x QuantiNova Yellow Template Dilution Buffer. The buffer does not affect sample stability and qPCR results.

* Example: add 0.5 µl Yellow Template Dilution Buffer to 50 µl sample, which can be used as template in various PCR runs regardless of the volume added to each reaction. If pipetting volumes are too small to handle, Yellow Template Dilution Buffer can be pre-diluted using sterile water. In this example, 5 µl of 1:10 pre-diluted Yellow Template Dilution Buffer could be added.

## Procedure

1. Thaw 4x QuantiNova Multiplex RT-PCR Master Mix, QuantiNova Yellow Template Dilution Buffer, template RNA, QN IC RNA (optional), primers, probes, QN ROX Reference Dye (if required) and RNase-free water. Thawing of the QuantiNova MP RT Mix is not required. Mix the individual solutions. Put QuantiNova Multiplex Reverse Transcription Mix on ice.
2. Prepare a reaction mix according to Table 3.

   Due to the two-phase hot-start of both the RT and the PCR reactions, it is not necessary to keep samples on ice during reaction setup or while programming the real-time cycler.

**Table 3. Reaction setup**

| | Volume/reaction | | |
|---|---|---|---|
| **Component** | **96-well block, Rotor-Gene Q** | **384-well block** | **Final concentration** |
| 4x QuantiNova Multiplex RT-PCR Master Mix | 5 µl | 2.5 µl | 1x |
| 100x Multiplex Reverse Transcription Mix | 0.2 µl | 0.1 µl | 1x |
| QN ROX Reference Dye (AB instruments only) | 1 µl/0.1 µl* | 0.5 µl/0.05 µl* | 1x |
| 20x primer-probe mix† (for each of up to 5 targets) | 1 µl | 0.5 µl | 0.8 µM forward Primer<br>0.8 µM reverse Primer<br>0.25 µM TaqMan Probe |
| QN IC RNA (optional) | 1 µl | 1 µl | |
| Template RNA (added at step 4) | Variable | Variable | ≤800 ng/ reaction |
| RNase-Free Water | Variable | Variable | |
| **Total reaction volume** | **20 µl** | **10 µl** | |

*Results in a 1:20 dilution for high ROX dye cyclers (i.e., ABI PRISM 7000, Applied Biosystems 7300, 7900 and StepOne Real-Time PCR Systems) and a 1:200 dilution for low ROX dye cyclers (i.e., Applied Biosystems 7500 and ViiA7 Real-Time PCR Systems) in the final 1x reaction.

† A 20x primer–probe mix consists of 16 µM forward primer, 16 µM reverse primer and 5 µM probe in TE buffer for each target. Primers and probes can either be pre-mixed and added simultaneously or primer-probe mixes for each target can be added separately. If concentration of primer-probe mix(es) differ, the respective added volume needs to be adjusted to achieve a final concentration of 0.8 µM for each primer and 0.25 µM for each probe.
When using the 10x QuantiNova IC Probe Assay (cat. no. 205813 or 205824) add 2 µl (96-well) or 1 µl (384-well) respectively.

3. Mix the reaction mix thoroughly and dispense appropriate volumes into PCR tubes or wells of a PCR plate.
4. Add template RNA (≤800 ng/reaction) to the individual PCR tubes or wells containing the reaction mix.
5. Program your real-time cycler according to the program outlined in Table 4.

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

**Table 4. Cycling conditions**

| Step | Time | Temperature | Ramp rate | Additional comments |
|---|---|---|---|---|
| Reverse transcription | 10 min | 50°C | Maximal/fast mode | HotStaRT-Script Reverse Transcriptase is activated |
| PCR initial activation step | 2 min | 95°C | Maximal/fast mode | QuantiNova DNA Polymerase is heat-activated |
| **2-step cycling** | | | | |
| Denaturation | 5 s | 95°C | Maximal/fast mode | |
| Combined annealing/extension | 30 s* | 60°C | Maximal/fast mode | Perform fluorescence data collection |
| **Number of cycles** | **35–40** | | | The number of cycles depends on the amount of template DNA |

* 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).

6. Place the PCR tubes or plates in the real-time cycler and start the cycling program.
7. Perform data analysis.

   **Note**: Before performing data analysis, select the analysis settings for each probe (i.e., baseline settings and threshold values). Optimal analysis settings are a prerequisite for accurate quantification data.

# Analysis and Interpretation of the Internal Control Assay Results

To analyze the QN IC RNA with the QuantiNova Multiplex RT-PCR Kit, add the appropriate volume of 2 µl (96-well protocol) or 1 µl (384-well protocol) of 10x QuantiNova IC Probe Assay (available separately as cat. no. 205813) to the sample. Signal detection is performed on the filter/channel for HEX/VIC of your real-time PCR instrument. Alternatively, use the QuantiNova Internal Control Probe Assay Red 650 (cat. no. 205824). This assay contains primers and probe with the same sequence as the QuantiNova Internal Control Probe Assay but employs a reporter dye with excitation/emission maxima of 646/664 nm that can be detected in the Cy5 channel.

1. After amplification, perform data analysis as recommended for your real-time PCR instrument. The $C_q$ value for the QN IC RNA in the QuantiNova Multiplex RT-PCR Kit depends on the real-time PCR instrument used and can be expected within a $C_q$ range of 27±3. The $C_q$ value on the Rotor-Gene Q can be expected within a range of 24±3. You can also decide to dilute the QuantiNova IC further to meet your own validation criteria. We recommend avoiding a $C_q$ range above 32.

2. Compare $C_q$ values between the QN IC RNA only and 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 $C_q$ of >2 appears, indicating inhibition or failure of a specific sample, we recommend the following:

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

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

c. 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.

# 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. Make sure that the PCR step of your cycling conditions includes the initial step for activation of the 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 cycling program includes the QuantiNova DNA Polymerase activation step (95°C for 2 min) as described in the protocols. |
| c) | Pipetting error or missing reagent | Check the concentrations and storage conditions of the reagents, including primers, probes and template nucleic acid. See "Appendix B: Assay Design and Optimization", page 27, for details on evaluating the concentration of primers and probes. Repeat the PCR. Use the provided QuantiNova Yellow Template Dilution Buffer to prevent errors during reaction setup. |
| d) | Wrong or no detection step | Ensure that fluorescence detection takes place during the combined annealing/extension step when using hybridization probes. |
| e) | Primer or probe concentration not optimal | Use optimal primer concentrations. For TaqMan assays, use each primer at 0.8 µM. In most cases, a probe concentration of 0.25 µM provides satisfactory results. Check the concentrations of primers and probes by spectrophotometry (see "Appendix B: Assay Design and Optimization", page 27). |
| f) | Problems with starting template | Check the concentration, storage conditions and quality of the starting template (see "Appendix B: Assay Design and Optimization", page 27). |
| g) | 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. If necessary, make new serial dilutions of template nucleic acid from the stock solutions. Repeat the PCR using the new dilutions. |

| | | Comments and suggestions |
|---|---|---|
| h) | Insufficient number of cycles | Increase the number of cycles. |
| i) | Reaction volume too high | 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. |
| j) | RT-PCR product too long | Increase the annealing/extension time. |
| k) | Primer design not optimal | For optimal results, RT-PCR products should be between 60 and 150 bp. RT-PCR products should not exceed 300 bp. |
| l) | Probe design not optimal | If the amplification reaction was successful, there may be a problem with the probe. Review the probe design guidelines (see "Appendix B: Assay Design and Optimization", page 27). |
| m) | Wrong detection channel/filter chosen | Ensure that the correct detection channel is activated or the correct filter setting is chosen for the reporter dye. |
| n) | No detection activated | Check that fluorescence detection was activated in the cycling program. |
| o) | Probe synthesis not optimal | Check the quality of dual-labeled probes by incubation with DNase I. A correctly synthesized probe, containing both fluorophore and quencher, will show a significant increase in fluorescence after DNase I incubation. |
| p) | Primers degraded | Check for possible degradation of primers on a denaturing polyacrylamide gel. |
| q) | Incorrect temperature for RT reaction | We recommend performing the RT reaction at 50°C; however, if this temperature does not yield satisfactory results, the temperature can be adjusted between 42°C and 50°C. |
| r) | Incorrect ratio of QuantiNova RT Mix to QuantiNova RT-PCR Master Mix | If not using the standard reaction volumes, ensure that the volume of QuantiNova RT Mix is changed proportionately so that the ratio of QuantiNova Multiplex RT Mix to QuantiNova Multiplex RT-PCR Master Mix remains the same. |
| s) | Fluorescence crosstalk | Check that the reporter dyes used in your assay are suitable for multiplex analysis on your instrument. Run appropriate controls to estimate potential crosstalk effects. |
| **Increased fluorescence or $C_q$ value for "No Template" control** | | |
| a) | Contamination of reagents | Discard all the components of the assay (e.g., master mix, primers, and probes). Repeat the assay using new components. |
| b) | Contamination during reaction setup | Take appropriate precautions during reaction setup, such as using aerosol-barrier pipet tips. |
| c) | Minimal probe degradation, leading to sliding increase in fluorescence | Check the amplification plots, and adjust the threshold settings. |

| | | Comments and suggestions |
|---|---|---|
| **High fluorescence in "No Reverse Transcription" control** | | |
| a) | Contamination of RNA sample with genomic DNA | Preferably design primers and/or probes that span exon-exon boundaries, so that only cDNA targets can be amplified and detected. Alternatively, treat the RNA sample with DNase to remove contaminating genomic DNA. |
| **Varying fluorescence intensity** | | |
| a) | Contamination of real-time cycler | Decontaminate the real-time cycler according to the manufacturer's instructions. |
| b) | Real-time cycler no longer calibrated | Recalibrate the real-time cycler according to the manufacturer's instructions. |
| **All cycler systems** | | |
| a) | 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** | | |
| a) | ΔRn values unexpectedly too high or too low | The concentration of the QN ROX Reference Dye is incorrect. To choose the right ROX concentration for your cycler, refer to Table 2, page 13. |
| **Differences in $C_q$ values or in PCR efficiencies between a multiplex assay and the corresponding singleplex assays** | | |
| a) | Wrong cycling conditions | Always start with the optimized cycling conditions specified in the protocols. Be sure that the 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) | Analysis settings (e.g., threshold and baseline) | Check the analysis settings (threshold and baseline settings) for each reporter dye. Repeat analysis using optimal settings for each reporter dye. |
| c) | Imprecise spectral separation of reporter dyes | Since multiplex assays use multiple probes, each with a fluorescent dye, the increased fluorescent background may affect the shape of the amplification plots obtained with some real-time cyclers. This may lead to differences in $C_q$ values of up to 5% between the multiplex assay and the corresponding singleplex assays; this can usually be avoided by using optimal threshold settings. If using the ABI PRISM 7700, perform analysis with and without spectral compensation. |
| **No linearity in ratio of $C_q$ value/crossing point to log of the template amount** | | |
| a) | Template amount too high | When signals are coming up at very early $C_q$ values, adjust the analysis settings accordingly. |
| b) | Template amount too low | Increase template amount if possible. Note that detection of very low starting copy numbers may not be in the linear range of a standard curve. |

# Appendix A: Suitable Combinations of Reporter Dyes on the Rotor-Gene Q Instrument

Multiplex real-time (RT-)PCR requires the simultaneous detection of up to five different fluorescent reporter dyes. For accurate detection, the fluorescence spectra of the dyes should be well separated or exhibit only minimal overlap (Table 5). For up to 4-plex analysis we recommend using the core channels: Green, Yellow, Orange and Red. If a higher multiplex degree (5-plex, 6-plex) is performed, extend the spectral range to blue channel and/or crimson channel. These channels require less frequently used fluorophores which will not be detected on all commonly used real-time PCR instruments.

**Note**: To find out which reporter dyes can be used in multiplex analyses, if using other real-time PCR instruments, please refer to the user manual or the manufacturer's instructions for your real-time cycler.

**Table 5. Dyes commonly used in multiplex real-time PCR on Rotor-Gene Q**

| Channel | Excitation (nm) | Detection (nm)* | Examples of fluorophores detected |
|---|---|---|---|
| Blue | 365±20 | 460±20 | Marina Blue®, Edans, Bothell Blue, Alexa Fluor® 350, AMCA-X, ATTO 390 |
| Green | 470±10 | 510±5 | FAM™, Alexa Fluor 488 |
| Yellow | 530±5 | 557±5 | JOE, VIC, HEX, TET™, CAL Fluor® Gold 540, Yakima Yellow® |
| Orange | 585±5 | 610±5 | ROX, CAL Fluor Red 610, Cy3.5, Texas Red®, Alexa Fluor 568 |
| Red | 625±10 | 660±10 | Cy5, Quasar® 670, LightCycler Red 640, Alexa Fluor 633 |
| Crimson | 680±5 | 712 high pass | Quasar 705, LightCycler Red 705, Alexa Fluor 680 |

* Emission spectra may vary depending on the buffer conditions.

# Appendix B: Assay Design and Optimization

Important factors for successful quantitative, singleplex and multiplex real-time RT-PCR include the design of optimal primer pairs and probes, the use of appropriate primer and probe concentrations and the correct storage of primers and probes.

## Assay design

Guidelines for the optimal design of primers and probes are given below. It is particularly important to minimize nonspecific annealing of primers and probes. This can be achieved through careful assay design.

### $T_m$ of primers for TaqMan assays

- Use specialized design software (e.g., Primer Express® Software) to design primers and probes.
- $T_m$ of all primers should be 58–62°C and within 2°C of each other.
- $T_m$ of probes should be 8–10°C higher than the $T_m$ of the primers.
- Avoid a guanidine at the 5' end of probes, next to the reporter, since this causes quenching.
- Avoid runs of 4 or more of the same nucleotide, especially of guanidine.
- Choose the binding strand so that the probe has more C than G bases.
- All assays should be designed using the same settings to ensure that they will work optimally under the same cycling conditions (60°C annealing/extension).
- Primers and probes should be supplied from an established oligonucleotide manufacturer. Primers should be of standard quality, and probes should be HPLC purified. Lyophilized primers and probes should be dissolved in TE buffer to provide a stock solution of 100 µM; concentration should be checked by spectrophotometry (see Table 6. Primer and probe stock solutions and primer-probe mixes should be stored in aliquots at –20°C. Probe stock solutions should be protected from exposure to light.

### Primer sequence

- Length: 18–30 nucleotides.
- GC content: 30–70%.
- Always check the specificity of primers by performing a BLAST® search (**www.ncbi.nlm.nih.gov/blast**). Ensure that primer sequences are unique for your template sequence.
- Check that primers and probes are not complementary to each other.
- Try to avoid highly repetitive sequences.
- Avoid complementarity of two or three bases at the 3' ends of primer pairs to minimize primer–dimer formation.
- Avoid mismatches between the 3' end of primers and the template sequence.
- Avoid runs of three or more Gs and/or Cs at the 3' end. Avoid complementary sequences within a primer sequence and between the primer pair.

### Product size

Ensure that the length of RT-PCR products is 60–150 bp. Some longer amplicons may amplify efficiently in multiplex RT-PCR, with minimal optimization.

### Handling and storing primers and probes

Guidelines for handling and storing primers and probes are given in Table 6 below. For optimal results, we recommend only combining primers of comparable quality.

**Table 6. Guidelines for handling and storing primers and probes**

| | Description |
|---|---|
| Storage buffer | Lyophilized primers should be dissolved in a small volume of low-salt buffer to give a concentrated stock solution (e.g., 100 µM). We recommend using TE (10 mM Tris.HCl, 1 mM EDTA, pH 8.0) for standard primers and probes labeled with most fluorescent dyes. However, probes labeled with fluorescent dyes such as Cy3, Cy3.5, Cy5 and Cy5.5 should be stored in TE buffer, pH 7.0, since they tend to degrade at higher pH. |
| Storage | Primers should be stored in sterile, nuclease-free TE buffer in small aliquots at –20°C. Standard primers are stable under these conditions for at least 1 year. Fluorescently labeled probes are usually stable under these conditions for at least 6–9 months. Repeated freeze–thaw cycles should be avoided, since they may lead to degradation. For easy and reproducible handling of primer–probe sets used in multiplex assays, we recommend preparing 20x primer–probe mixes, each containing primers and probes for particular targets at the suggested concentrations (see protocols). |
| Dissolving primers and probes | Before opening a tube containing lyophilized primer or probe, centrifuge the tube briefly to collect all material at the bottom of the tube. To dissolve the primer or the probe, add the required volume of sterile, nuclease-free TE buffer, mix and leave for 20 minutes to allow the primer or probe to completely dissolve. Mix again and determine the concentration by spectrophotometry as described below. We do not recommend dissolving primers and probes in water. They are less stable in water than in TE buffer and some may not dissolve easily in water. |
| Concentration | Spectrophotometric conversion for primers and probes:<br>1 $A_{260}$ unit = 20–30 µg/ml<br>To check primer concentration, the molar extinction coefficient can be used:<br>$A_{260} = \varepsilon_{260}$ x molar concentration of primer or probe<br>If the $\varepsilon_{260}$ value is not given on the data sheet supplied with the primers or probes, it can be calculated from the primer sequence using the following formula:<br>$\varepsilon_{260}$ = 0.89 x [(A x 15,480) + (C x 7340) + (G x 11,760) + (T x 8850)]<br>Example<br>Concentration of diluted primer: 1 µM = 1 x 10–6 M<br>Primer length: 24 nucleotides with 6 each of A, C, G and T bases<br>Calculation of expected $A_{260}$: 0.89 x [(6 x 15,480) + (6 x 7340) + (6 x 11,760) + (6 x 8850)] x (1 x 10–6) = 0.232<br>The measured $A_{260}$ should be within +/– 30% of the theoretical value. If the measured $A_{260}$ is very different to the theoretical value, we recommend recalculating the concentration of the primers or probes, or having the primers or probes resynthesized. For probes, the fluorescent dye does not significantly affect the $A_{260}$ value. |

| | |
|---|---|
| Primer quality | The quality of 18–30 mers can be checked on a 15% denaturing polyacrylamide gel; a single band should be seen. Please contact QIAGEN Technical Services for a protocol. |
| Probe quality | The quality of the fluorescent label and the purity of TaqMan probes can be determined by comparing fluorescence before and after DNase digestion. Incubate probes with or without 5 units DNase at 37°C for 1 hour. A significant difference in fluorescence following DNase treatment should be detectable. |

# Appendix C: Generating Color Compensation Files on LightCycler Systems

The LightCycler 2.0 system has detection channels that allow detection of multiple reporter dyes in the same capillary. However, even when reporter dyes with well separated emission spectra are used, each reporter dye will be detected by more than one detection channel. Therefore, multiplex assay results will be inaccurate unless a correction is made. This is achieved by using a color compensation file that contains information that corrects the crosstalk between the detection channels. The LightCycler 480 system can also use a color compensation file to correct the crosstalk between detection channels. However, if the multiplex assay uses reporter dyes with widely separated emission spectra (e.g., FAM and Cy5), it may not be necessary to use a color compensation file. Color compensation files can be generated before or after carrying out a multiplex assay and can be stored for later use. For detailed information, please refer to the manual of the corresponding instrument.

# Appendix D: Reaction Setup Using a Master Mix Containing a High Concentration of ROX

This appendix is only relevant for a reaction setup using a master mix containing a high concentration of ROX that has been added according to Table 2, page 13. When using a master mix containing low concentration of ROX, the volume of ROX added is negligible and the standard reaction setup as described in Table 3 (page 19) should be used.

**Table 7. Reaction setup**

| | Volume/reaction | | |
|---|---|---|---|
| **Component** | **96-well block, Rotor-Gene** | **384-well block** | **Final concentration** |
| 4x QuantiNova Multiplex RT-PCR Master Mix (containing high ROX) | 6 µl | 3 µl | 1x |
| 20x primer-probe mix* (for each of up to 5 targets) | 1 µl | 0.5 µl | 0.8 µM forward primer<br>0.8 µM reverse primer<br>0.25 µM TaqMan probe |
| QN Multiplex RT-Mix | 0.2 µl | 0.1 µl | 1x |
| Template RNA (added at step 4) | 1 µl | Variable | ≤800 ng/reaction |
| RNase-Free Water | Variable | Variable | – |
| **Total reaction volume** | **20 µl** | **10 µl** | |

* A 20x primer–probe mix consists of 16 µM forward primer, 16 µM reverse primer and 5 µM probe in TE buffer for each target. Primers and probes can either be pre-mixed and added simultaneously, or primer-probe mixes for each target can be added separately. If concentration of primer-probe mix(es) differ, the respective added volume needs to be adjusted, to achieve a final concentration of 0.8 µM for each primer and 0.25 µM for each probe. When using the 10x QuantiNova IC Probe Assay (cat. no. 205813 or 205824) add 2 µl (96-well) or 1 µl (384-well) respectively.

# Appendix E: Direct Multiplex RT-PCR from Single or Multiple Cells

This protocol describes the accelerated and streamlined real-time RT-PCR analysis of cultured cells. By eliminating the need for RNA purification, the protocol allows real-time multiplex RT-PCR to be carried out directly from cell lysates. The protocol can be applied to single cells as well as to multiple cells (up to 2000 cells per sample). For a dedicated protocol from single cells isolated with the QIAscout™ platform please visit **www.qiagen.com/HB-2313**.

Cell wash

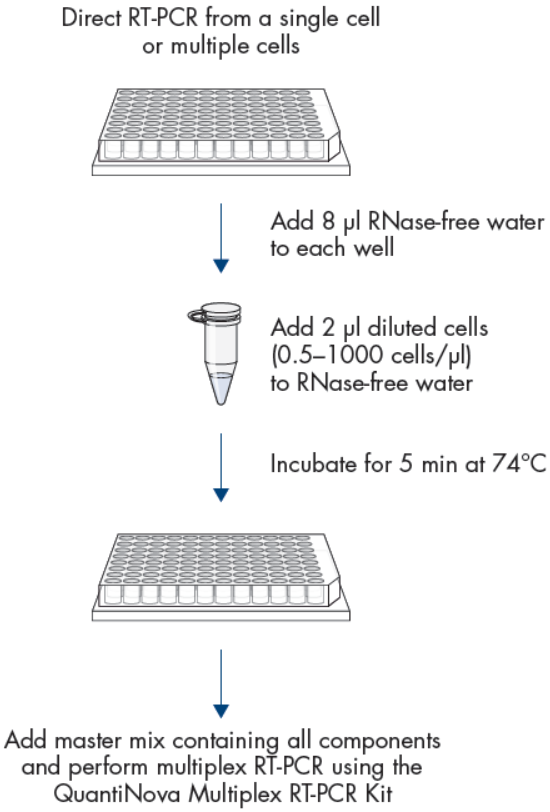
1. Cultivate cells under the standard culture conditions for the cell line being used.
2. Wash the cells with cell-culture medium to remove extracellular material released by living cells, and intracellular material released by any dead, lysed cells. Removal of such materials is important, since they can interfere with quantification by real-time RT-PCR. Additionally, remnants from any enzymatic treatment of cells (e.g., by trypsine for cell detachment) will be washed away.
3. Determine the cell density using a standard cell counting method (e.g., cell counter or counting chamber).
4. Dilute the cells with cell-culture medium to adjust the desired amount of cells per PCR well (appropriate number of cells: 1000–0.5 cell/µl).

Cell lysis and RT-PCR

1. Add 8 µl RNase-free water per well to a 96-well real-time PCR plate. This circumvents the need to transfer the cell lysate to a new reaction plate or tube.
2. Transfer 2 µl of diluted cells to the 8 µl pre-aliquoted RNase free water, vortex and spin down briefly. To check that no contaminating template is introduced by the cell culture medium, we recommend using a no cell template control containing 2 µl cell culture medium and 8 µl RNase free water.
3. Incubate the plate for 5 min at 74°C, either in the real-time PCR cycler or in any suitable end-point PCR cycler.
4. Centrifuge the plate briefly, proceed to step 5.

   **Note**: If a pause in the procedure is required, store the plates containing the lysates at 4–10°C for no more than 30 minutes.
5. Set up the real-time, one-step RT-PCR in a final volume of 20 µl using Table 3, page 19. The template volume is 10 µl (8 µl water plus 2 µl cell dilution).
6. To monitor successful reverse transcription and amplification, we strongly recommend adding 1 µl of QN IC RNA to the reaction. The QN IC RNA should not be pre-incubated with the cells. We suggest the addition of the QN IC RNA to the QuantiNova Multiplex RT-PCR master mix, resulting in positive signals for the QN IC RNA also in the "no cell" control.

   **Note**: For cell numbers up to 100 cells/reaction, we recommend further diluting the previously 1:10 diluted QN IC RNA by a factor of 1:100, resulting in an overall dilution of 1:1000. For cell numbers between 100 and 2000, we recommend further diluting previously 1:10 diluted QN IC RNA by a factor of 1:10, resulting in an overall dilution of 1:100.


Direct RT-PCR from a single cell
or multiple cells
Add 8 µl RNase-free water
to each well
Add 2 µl diluted cells
(0.5–1000 cells/µl)
to RNase-free water
Incubate for 5 min at 74°C
Add master mix containing all components
and perform multiplex RT-PCR using the
QuantiNova Multiplex RT-PCR Kit

# Ordering Information

| Product | Contents | Cat. no. |
|---|---|---|
| QuantiNova Multiplex RT-PCR Kit (100) | For 100 x 20 µl reactions: 0.5 ml 4x QuantiNova Multiplex RT-PCR Master Mix, 20 µl QuantiNova Multiplex RT-Mix, 20 µl QuantiNova IC RNA, 500 µl QuantiNova Yellow Template Dilution Buffer, 250 µl QN ROX Reference Dye, 1.9 ml RNase-Free Water | 208552 |
| QuantiNova Multiplex RT-PCR Kit (500) | For 500 x 20 µl reactions: 2 x 1.3 ml 4x QuantiNova Multiplex RT-PCR Master Mix, 100 µl QuantiNova Multiplex RT-Mix, 100 µl QuantiNova IC RNA, 500 µl QuantiNova Yellow Template Dilution Buffer, 1 ml QN ROX Reference Dye, 4 x 1.9 ml RNase-Free Water | 208554 |
| QuantiNova Multiplex RT-PCR Kit (2500) | For 2500 x 20 µl reactions: 10 x 1.3 ml 4x QuantiNova Multiplex RT-PCR Master Mix, 5 x 100 µl QuantiNova Multiplex RT-Mix, 3 x 100 µl QuantiNova IC RNA, 3 x 500 µl QuantiNova Yellow Template Dilution Buffer, 3 x 1 ml QN ROX Reference Dye, 20 x 1.9 ml RNase-Free Water | 208556 |
| QuantiNova IC Probe Assay (200) | For 200 x 20 µl reactions: 400 µl primer/probe mix (10x), detecting IC RNA | 205813 |

| Product | Contents | Cat. no. |
|---|---|---|
| QuantiNova IC Probe Assay Red 650 (500) | For 500 x 20 µl reactions: 1000 µl primer/probe mix (10x), detecting internal control RNA/DNA Cy5 analogue label | 205824 |
| QuantiNova Probe PCR Kit (100)* | For 100 x 20 µl reactions: 1 ml 2x QuantiNova Probe PCR Master Mix, 250 µl QN ROX Reference Dye, 500 µl QuantiNova Yellow Template Dilution Buffer, 1.9 ml Water | 208252 |
| QuantiNova SYBR Green PCR Kit (100)* | For 100 x 20 µl reactions: 1 ml 2x QuantiNova SYBR Green PCR Master Mix , 250 µl QN ROX Reference Dye, 500 µl QuantiNova Yellow Template Dilution Buffer, 1.9 ml Water | 208052 |
| **For RNA cleanup and concentration with small elution volumes** | | |
| RNeasy MinElute Cleanup Kit (50) | 50 RNeasy MinElute Spin Columns, Collection Tubes (1.5 ml and 2 ml), RNase-Free Reagents and Buffers | 74204 |
| **RNeasy Mini Kit – for purification of total RNA from animal cells, animal tissues and yeast, and for RNA cleanup** | | |
| RNeasy Mini Kit (50) | For 50 RNA minipreps: RNeasy Mini Spin Columns, Collection Tubes, RNase-Free Water and Buffers | 74104 |
| **For purification of total RNA from all types of tissue using gDNA Eliminator Solution** | | |
| RNeasy Plus Universal Mini Kit (50) | For 50 RNA minipreps: RNeasy Mini Spin Columns, gDNA Eliminator Solution, Collection Tubes, RNase-Free Water and Buffers | 73404 |

| Product | Contents | Cat. no. |
|---|---|---|
| **AllPrep® DNA/RNA Mini Kit – for simultaneous purification of genomic DNA and total RNA from the same cell or tissue sample** | | |
| AllPrep DNA/RNA Mini Kit (50) | 50 AllPrep DNA Mini Spin Columns, 50 RNeasy Mini Spin Columns, Collection Tubes, RNase-Free Reagents and Buffers | 80204 |
| **Instruments** | | |
| Rotor-Gene Q 6-plex Platform | Real-time PCR instrument with 6 channels (blue, green, yellow, orange, red, crimson) including, laptop computer, software, accessories | 9001590 |
| QIAgility® System HEPA/UV (incl. PC) | Robotic workstation for automated PCR setup (with UV light and HEPA filter), notebook computer and QIAgility Software: includes installation and training, 1-year warranty on parts and labor | 9001532 |

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