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QuantiNova® Pathogen +IC Kit Handbook

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

Sample to Insight

QIAGEN

Contents

Kit Contents

QuantiNova Pathogen +IC Kit	**(100)**	**(500)**
Catalog no.	**208652**	**208654**
Number of reactions (20 µl/10 µl)	**100/200**	**500/1000**
QuantiNova Pathogen Master Mix, containing: QuantiNova DNA Polymerase, composed of: *Taq* DNA Polymerase, QuantiNova Antibody and QuantiNova Guard; HotStaRT-Script Reverse Transcriptase; QuantiNova Pathogen Buffer; dNTP mix (dATP, dCTP, dGTP, dTTP)	500 µl	2 x 1.25 ml
QuantiNova Yellow Template Dilution Buffer	500 µl	500 µl
QuantiNova Internal Control DNA	100 µl	250 µl
QuantiNova Internal Control RNA	100 µl	250 µl
QuantiNova IC Probe Assay	200 µl	1 ml
QuantiNova ROX™ Reference Dye	250 µl	1 ml
RNase-Free Water	1.9 ml	3 x 1.9 ml
QuantiTect® Nucleic Acid Dilution Buffer	1.5 ml	2 x 1.5 ml
Quick-Start Protocol	1	1

Storage

QuantiNova Pathogen +IC 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 quality control label inside the kit box or on the kit envelope). QuantiNova Pathogen Master Mix, QuantiNova Yellow Template Dilution Buffer and QuantiNova ROX Reference Dye can also be stored protected from light at 2–8°C for up to 12 months, depending on the expiry date.

If desired, QuantiNova ROX Reference Dye can be added to QuantiNova Pathogen Master Mix for long-term storage. For details, see "Adding ROX dye to the RT-PCR master mix", page 16.

Intended Use

The QuantiNova Pathogen +IC 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.

Quality Control

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

Product Information

The QuantiNova Pathogen +IC Kit contains:

4 x QuantiNova Pathogen 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.
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.
QuantiNova Pathogen 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 ultrapure quality.

Other components

Component	Description
QuantiNova Internal Control DNA	Synthetic DNA fragment for monitoring successful extraction and/or amplification.
QuantiNova Internal Control RNA	Synthetic transcript for monitoring successful extraction and/or reverse transcription/amplification.
QuantiNova IC Probe Assay	Primers and probe mix labelled with MAX™ dye to detect the QuantiNova Internal Control DNA and RNA.
QuantiNova 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.
QuantiTect Nucleic Acid Dilution Buffer	Proprietary buffer formulation for dilution and storage of nucleic acid standards.
RNase-Free Water	Ultrapure quality, PCR-grade.

Introduction

The QuantiNova Pathogen + IC Kit provides highly sensitive and rapid real-time PCR and one-step RT-PCR analysis of both pathogen DNA and RNA in an easy-to-handle format. The kit is compatible with dual-labeled probes e.g., TaqMan® probes. High specificity and sensitivity in real-time RT-PCR are achieved by a novel two-phase hot-start procedure. For high in-process safety during pathogen detection, each kit contains reagents for the simultaneous detection of user-defined targets and the QuantiNova Internal Control (QuantiNova IC).

The newly formulated QuantiNova Pathogen Master Mix contains both the QuantiNova DNA Polymerase and the HotStaRT-Script Reverse Transcriptase. This enables the simultaneous detection of pathogen DNA and RNA using the same procedure.

The HotStaRT-Script Reverse Transcriptase associates with an 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, and QuantiNova Guard, a novel additive. 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, as well as Q-Bond, an additive in the buffer that enables short cycling steps without loss of PCR sensitivity and efficiency.

The QuantiNova IC is detected in the same tube as the pathogen target(s) during multiplex PCR or RT-PCR, and is used to test for successful amplification (e.g., exclusion of PCR inhibitors). Alternatively, the QuantiNova IC can be added to the sample prior to nucleic

acid purification to control both the efficiency of the purification process and the PCR or RT-PCR amplification. The artificial synthetic template for the QuantiNova IC is provided as both DNA and RNA. The QuantiNova Pathogen +IC Kit includes the QuantiNova Internal Control Probe Assay (primer/probe set). For use as an amplification control, a 100-fold dilution of the internal control DNA or RNA template should be added directly to the reaction mix. For use as an extraction control, a 10-fold dilution of the internal control DNA or RNA template should be added to the sample (e.g., the lysate) prior to purification. The QuantiNova Internal Control Probe Assay design eliminates the need to optimize multiplex detection for the pathogen targets. The use of one universal exogenous internal control for all pathogen assays allows parallel read-out of different pathogen targets and easy implementation of new pathogen assays.

The QuantiNova Pathogen +IC Kit has been optimized for use with TaqMan probes in multiplex real-time PCR or one-step RT-PCR detection of one or more pathogen nucleic acid targets and the QuantiNova IC (altogether, up to 4 assays). Real-time PCR and one-step RT-PCR can be run simultaneously using the same protocol.

The kit has been optimized for use with most real-time cyclers. The QuantiNova 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

PCR and one-step RT-PCR

The QuantiNova Pathogen +IC Kit contains a highly concentrated 4x Master Mix, which allows use of larger volumes of template in order to increase assay sensitivity. Use of the QuantiNova Pathogen Master Mix allows both reverse transcription and PCR to take place in a single tube. All reagents required for both reactions are contained in the Master Mix, so there is no need to open the tube once the reverse transcription reaction has been started. There is no need to set up the reaction on ice, and the whole reaction can be left for up to 4 h at 4°C or 1 h at room temperature without any loss of performance.

QuantiNova Pathogen Master Mix

The components of the QuantiNova Pathogen Master Mix include HotStarRT-Script Reverse Transcriptase, QuantiNova DNA Polymerase, QuantiNova Pathogen Buffer and dNTPs. The optimized master mix ensures fast real-time PCR or RT-PCR amplification with high specificity and sensitivity.

HotStarRT-Script Reverse Transcriptase

The QuantiNova Pathogen Master Mix contains HotStarRT-Script Reverse Transcriptase for heat-mediated activation of the reverse transcription step. 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).

Novel, antibody-mediated hot-start mechanism

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 RT-PCR products and primer–dimers during reaction setup, reverse transcription 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 reverse transcription and within 2 minutes of raising the temperature to 95°C, the QuantiNova Antibody and Guard are denatured and the QuantiNova DNA Polymerase is activated, enabling PCR amplification (Figure 1). This two-phase hot-start enables rapid and convenient room-temperature setup and allows both steps to be performed sequentially in a single tube.

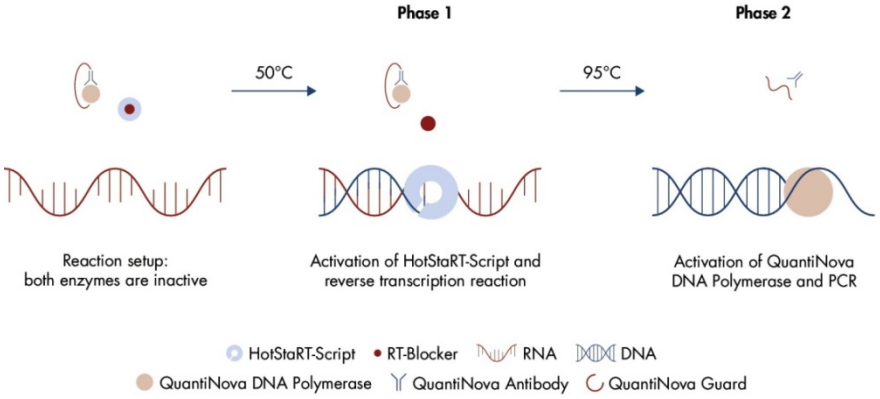

Figure 1. Principle of the novel QuantiNova two-phase hot-start mechanism. At ambient temperature the HotStaRT-Script is inhibited by the RT-Blocker and the QuantiNova DNA Polymerase is kept inactive by QuantiNova Antibody and QuantiNova Guard. At 50°C, the RT is activated while the QuantiNova DNA polymerase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

QuantiNova Pathogen Buffer

QuantiNova Pathogen 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 Pathogen 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 Pathogen 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 1 h at room temperature without impairing the performance of the subsequent reaction.

Built-in visual control for correct pipetting

The master mix supplied with the QuantiNova Pathogen +IC 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 Internal Control

For increased in-process safety in pathogen-detection assays, an internal positive control is detected in the same tube as the pathogen DNA or RNA targets during multiplex RT-PCR. The QuantiNova IC is available both as a synthetic DNA or RNA with a unique and artificial sequence that can optionally be used to monitor successful amplification. The QuantiNova IC DNA and RNA are 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 sample material or lysis and purification steps during nucleic acid isolation.

The QuantiNova IC DNA and RNA can be used optionally. It can be added either during reaction setup (amplification control) or directly to the sample lysate to monitor purification and amplification.

Please note that adding the internal control DNA or RNA to the reaction mix as an amplification control will result in positive signals in NTCs. These signals serve as a reference to assure that the internal control has been successfully amplified. C_T shifts between the template + QuantiNova IC samples compared to the QuantiNova IC only samples indicate inhibition in the PCR or RT-PCR. If the internal control signal is detected in the NTCs, but not in sample reactions, this may indicate the presence of inhibitors or other issues with the sample reaction.

If the QuantiNova IC has been added to the sample lysate to determine purification efficiency, no internal control signals will be detected in the NTCs. To create a reference for successful amplification of the internal control, a separate internal control reaction can be

prepared by adding 1µl of a 1:100 dilution of the internal control to the reaction mix instead of sample RNA. For guidelines on data interpretation of pathogen and internal control detection, see "Optimization, Analysis and Interpretation of the QuantiNova Internal Control", page 26.

QuantiNova Internal Control Probe Assay

The QuantiNova Internal Control Probe Assay allows simultaneous amplification of the internal control DNA or RNA and user-defined pathogen targets in multiplex real-time PCR or one-step RT-PCR using sequence-specific probes. The pre-mixed QuantiNova Internal Control Probe Assay contains a forward and reverse primer and a TaqMan probe for detection of the internal control template, and has been specifically optimized to prevent interference with target primers. The primer and probe sequences for the detection of the QuantiNova IC 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 QuantiNova IC DNA and RNA are detected as a 200 bp amplicon. The QuantiNova Internal Control Probe Assay contained in the kit employs MAX NHS Ester as a reporter dye. 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. To guarantee optimal performance of multiplex amplification of the pathogen target and the QuantiNova IC, we recommend designing the pathogen target assay to follow certain specifications. These specifications are in accordance with general recommendations for the design of optimal real-time PCR and RT-PCR primers and probes, and are therefore likely to already apply for the majority of proven and established (e.g., literature-derived) real-time RT-PCR assays. For more details on pathogen target assay design, see "Appendix C: Assay Design and Optimization", page 33.

In case the MAX dye is not suitable for the intended application, the QuantiNova Internal Control Probe Assay is also available separately as the QuantiNova Internal Control Probe

Assay Red 650 (cat. no. 205824). The QuantiNova Internal Control Probe Assay Red 650 contains primers and probe with the same sequence as the QuantiNova Internal Control Probe Assay but employs a Cy®5 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.

QuantiTect Nucleic Acid Dilution Buffer

QuantiTect Nucleic Acid Dilution Buffer is intended for dilution of nucleic acids used to generate standard curves or as positive controls in real-time PCR or RT-PCR. The buffer stabilizes RNA and DNA standards during dilution and reaction setup and prevents loss of nucleic acids on plastic surfaces, such as tubes or pipet tips. The buffer is ready to use and is free of RNases and DNases. Proper use of the buffer enables safe and accurate dilution of the small amounts of nucleic acids typically used as standards for analysis of pathogen nucleic acids. Aliquots of diluted standards can be stored in QuantiTect Nucleic Acid Dilution Buffer at –15 to –30°C for up to 6 months. Avoid repeated freezing and thawing.

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 due to slight variations in well 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 Pathogen +IC Kit is provided with a separate tube of QuantiNova ROX Reference Dye. ROX dye should be used as a 20x concentrated solution 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.

Refer to Table 1 for details on real-time cyclers that require low or high ROX concentrations. If desired, QuantiNova ROX Reference Dye can be added to the QuantiNova Pathogen Master Mix for long-term storage (Table 2). For details, see "Adding ROX dye to the master mix", page 16.

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 Pathogen +IC Kit, QuantiNova ROX Reference Dye can be added to QuantiNova Pathogen Master Mix for long-term storage, if desired. For information on the concentration of ROX required for Applied Biosystems instruments, refer to Table 1. For reaction setups with master mix that already contains a high concentration of added QuantiNova ROX Reference Dye, refer to "Appendix A: Reaction Setup Using Master Mix Containing High Concentration of ROX", page 31."

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

Volume of QuantiNova Pathogen Master Mix (without QN ROX dye)	Volume of ROX dye for high ROX concentration/low ROX concentration
0.5 ml	100/10 µl
1.25 ml	250/25 µl

Important points before starting

- The following protocols are optimized for detection of DNA and RNA targets using TaqMan probes in a multiplex reaction with any real-time cycler and conditions 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 used as a 20x concentrated solution 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.
- You may use the same protocol for both DNA and RNA targets. For DNA targets, the 10 min reverse transcription step is optional.
- The dye in QuantiNova Yellow Template Dilution Buffer allows tracking of pipetted samples in the RT-PCR. When template is added to the blue QuantiNova Pathogen 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 or buffer) 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

* Example: Add 0.5 µl Yellow Template Dilution Buffer to a 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.

(using template and water or buffer) to obtain a final concentration of 1x QuantiNova Yellow Template Dilution Buffer. The buffer does not affect sample stability or RT-PCR.

- For the highest efficiency in real-time PCR or RT-PCR using TaqMan probes, amplicons should ideally be 60–150 bp in length.
- 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 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.
- 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; always readjust the threshold value for analysis of every run.
- The use of the QuantiNova IC DNA and RNA is optional.

Protocol: Multiplex Real-Time PCR and RT-PCR Using the QuantiNova Internal Control as an Extraction Control

Important points before starting

- **Pre-dilute the QuantiNova IC DNA and RNA 1:10 in QuantiTect Nucleic Acid Dilution Buffer before use as extraction control.**
- After pre-dilution of the QuantiNova IC in QuantiTect Nucleic Acid Dilution buffer, add the resulting highly concentrated internal control solution to the sample lysate at a ratio of 0.1 µl per 1 µl elution volume. As an example, if the QIAamp® MinElute® Virus Kit is eluted using 50 µl Buffer AVE, then 5 µl of the (1:10) pre-diluted internal control should be added to the sample lysate.

  Note: When the internal control is used as extraction control, no internal control should be added to the reaction mixture.
- If the QuantiNova IC has been added to the sample lysate as a purification control, no internal control signals will be detected in the NTCs. As a reference for successful amplification of the internal control, a separate, individual internal control reaction can be prepared by adding 1µl of a 1:100 dilution of the internal control to the reaction mix instead of the sample.
- The internal control should be added only to the lysis buffer and sample mixture. Do not add the internal control directly to the sample material as it might contain active nucleases which are rendered inactive only upon addition of the lysis buffer. Once the internal control has been added, immediately proceed with purification. Generally, the quantity of internal control to be added during purification depends only on the elution volume. Successful purification and amplification should result in a C_T value of the internal control of 27±3 depending on the cycler used.
- For further information about the QuantiNova IC, please refer to page 12.

Procedure

1. Thaw the QuantiNova IC DNA or RNA and QuantiTect Nucleic Acid Dilution Buffer. Mix the individual solutions.
2. Pre-dilute the QuantiNova IC DNA or RNA 1:10 in QuantiTect Nucleic Acid Dilution Buffer before use. If QuantiNova IC RNA is used to monitor the complete RT-PCR procedure, a separate internal control DNA is not required.
3. Add the resulting highly concentrated internal control solution to the sample lysate at a ratio of 0.1 µl per 1 µl elution volume.
4. Perform nucleic acid extraction as usual.
5. Thaw the QuantiNova Pathogen Master Mix, QuantiNova Yellow Template Dilution Buffer, template DNA and/or RNA, QuantiNova Internal Control Probe Assay, primers, probes, QN ROX Reference Dye (if required) and RNase-free water. Mix the individual solutions.

   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 cycle.

Table 3. Reaction setup

Component	Volume/reaction		
	96-well block	**384-well block**	**Final concentration**
QuantiNova Pathogen Master Mix, 4x	5 µl	2.5 µl	1 x
QN ROX Reference Dye (AB instruments only)*	1 µl/0.1 µl	0.5 µl/0.05 µl	1 x
20x primer–probe mix† (for each of up to 3 targets)	1 µl	0.5 µl	0.8 µM forward primer 0.8 µM reverse primer 0.25 µM TaqMan probe
QuantiNova IC Probe Assay 10x‡	2 µl	1 µl	1 x
RNase-Free Water	Variable	Variable	–
Template DNA or RNA (added at step 8)	Variable	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.

‡ Detects both QuantiNova DNA and RNA Internal Control.

6. Mix the reaction thoroughly and dispense appropriate volumes into PCR tubes or wells of a PCR plate.
7. Set up a separate, individual internal control reaction, by diluting QuantiNova IC DNA or RNA 1:100 in QuantiTect Nucleic Acid Buffer. Add 1µl to the reaction mix instead of sample.
8. Add template to the individual PCR tubes or wells containing the reaction mix.
9. Program the real-time cycler according to the program outlined in Table 4.

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

Table 4. Cycling conditions

Step	Time	Temperature	Ramp rate
RT-step (optional in case of DNA template)	10 min	50°C	Maximal/fast mode
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	30 s	60°C	Maximal/fast mode
Number of cycles	**40***		

* The number of cycles depends on the amount of template.

Protocol: Multiplex Real-Time PCR and RT-PCR Using the QuantiNova Internal Control as Amplification Control

Important points before starting

- **Pre-dilute the QuantiNova IC DNA and RNA 1:100 in QuantiTect Nucleic Acid Dilution Buffer before use as amplification control.**
- If QuantiNova IC RNA is used to monitor the complete RT-PCR procedure, a separate internal control DNA is not required.
- The QuantiNova IC template and QuantiNova IC probe assay are added to the reaction mix. The addition of the internal control DNA or RNA to the reaction mix as an amplification control will result in positive signals in NTCs. These signals serve as a reference to assure that the internal control has been successfully amplified. C_T shifts between the template + QuantiNova IC samples compared to the QuantiNova IC only samples indicate inhibition in the PCR or RT-PCR. If the internal control signal is detected in the NTCs, but not in sample reactions, this may indicate the presence of inhibitors or other issues with the sample reaction.

Procedure

1. Thaw QuantiNova Pathogen Master Mix, QuantiNova Yellow Template Dilution Buffer, template DNA and/or RNA, QuantiNova IC DNA or RNA (optional), QuantiNova Internal Control Probe Assay (optional), primers, probes, ROX Reference Dye (if required) and RNase-free water. Mix the individual solutions.

   Prepare a reaction mix according to Table 5. 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 5. Reaction setup

Component	Volume/reaction		
	96-well block	**384-well block**	**Final concentration**
QuantiNova Pathogen Master Mix, 4x	5 µl	2.5 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 3 targets)	1 µl	0.5 µl	0.8 µM forward primer 0.8 µM reverse primer 0.25 µM TaqMan probe
QuantiNova IC Probe Assay 10x‡	2 µl	1 µl	1x
QN IC DNA or RNA (optional)	1 µl	1 µl	1 x
RNase-Free Water	Variable	Variable	
Template DNA or RNA (added at step 3)	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.

‡ Detects both QuantiNova DNA and RNA Internal Control.

2. Mix the reaction thoroughly and dispense appropriate volumes into PCR tubes or wells of a PCR plate.
3. Add template to the individual PCR tubes or wells containing the reaction mix.
4. Program the real-time cycler according to the program outlined in Table 6.

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

Table 6. Cycling conditions

Step	Time	Temperature	Ramp rate
RT-step (optional in case of DNA template)	10 min	50°C	Maximal/fast mode
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	30 s	60°C	Maximal/fast mode
Number of cycles	**40***		

* The number of cycles depends on the amount of template.

Optimization, Analysis and Interpretation of the QuantiNova Internal Control

To analyze the QuantiNova internal control with the QuantiNova Pathogen +IC Kit, add the appropriate volume of 10x QuantiNova IC Probe Assay to the reaction. Signal detection is performed on the filter/channel for HEX/VIC of your real-time PCR instrument.

In the case that MAX dye is not suitable for the intended application the QuantiNova Internal Control Probe Assay is also available separately as QuantiNova Internal Control Probe Assay Red 650 (cat. no. 205824). The QuantiNova Internal Control Probe Assay Red 650 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.

After amplification, perform data analysis as recommended for your real-time PCR instrument. The C_T value for the QuantiNova IC in the QuantiNova Pathogen +IC Kit depends on the real-time PCR instrument used and can be expected within a C_T range of 27±3. The C_T 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_T range above 32.

The purification efficiency for the internal control may vary, depending on the purification method used. In case of lower purification efficiency, increase the volume of internal control per sample to more than 0.1 µl per 1 µl elution volume to obtain a C_T value in the range of 27±3. Test the extraction of the internal control using a negative purification control.

Compare C_T values between the QuantiNova IC only and samples containing QuantiNova IC plus template. Consistent C_T values indicate successful RT-PCR and reliable results. A C_T difference >2 is likely to indicate inhibition or sample failure.

If a shifted C_T 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 using RNase-free water before repeating the experiment. This dilutes inhibitors present in the sample.

c. Consider repeating the extraction and avoid contamination or carry-over of inhibitors.

Alternatively, the MinElute PCR Purification Kit (cat. no. 28004) or the RNeasy® MinElute Cleanup Kit (cat. no. 74204) can be used to remove potential inhibitors and concentrate the 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. Be sure that the PCR step of your cycling conditions include 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 (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, probes and template nucleic acid. See "Appendix C: Assay Design and Optimization", page 33, 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 C: Assay Design and Optimization", page 33).
f)	Problems with starting template	Check the concentration, storage conditions and quality of the starting template (see "Appendix C: Assay Design and Optimization", page 33).
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.
h)	Insufficient number of cycles	Increase the number of cycles.

		Comments and suggestions
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 C: Assay Design and Optimization", page 33).
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.
Increased fluorescence or C_T 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.
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.

		Comments and suggestions
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.
b)	Carry-over contamination	If the negative control (without template RNA) shows a RT-PCR product or a smear, exchange all reagents. Use disposable pipet tips containing hydrophobic filters to minimize cross-contamination. Set up all reaction mixtures in an area separate from that used for RNA preparation or PCR product analysis.
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, page16.

Appendix A: Reaction Setup Using 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 16. 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 21) or Table 5 (page 24) should be used.

Table 7. Reaction setup

Component	Volume/reaction		
	96-well block	**384-well block**	**Final concentration**
QuantiNova Pathogen Master Mix, 4x after ROX addition*	6 µl	3 µl	1x
20x primer–probe mix† (for each of up to 3 targets)	1 µl	0.5 µl	0.8 µM forward primer 0.8 µM reverse primer 0.25 µM TaqMan probe
QuantiNova IC Probe Assay 10x‡	2 µl	1 µl	1x
QN IC DNA or RNA (optional)	1 µl	1 µl	1 x
RNase-Free Water	Variable	Variable	–
Template DNA or RNA	Variable	Variable	Variable
Total reaction volume	**20 µl**	**10 µl**	**1x**

* Contains a 1:20 dilution for high ROX instruments (i.e., ABI PRISM 7000, Applied Biosystems 7300, 7900 and StepOne Real-Time PCR Systems).

† 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.

‡ Detects both QuantiNova DNA and RNA Internal Control.

Appendix B: Cycling Adjustments when Using FRET Probes

This appendix is only relevant when using fluorescence resonance energy transfer (FRET) or hybridization probes. Real-time PCR with FRET probes, such as LightCycler hybridization probes, uses two labeled oligonucleotide probes that bind to the PCR product in a head-to-tail fashion. When the two probes bind, their fluorophores come into close proximity, allowing energy transfer from a donor fluorophore to an acceptor fluorophore. This causes fluorescence that is proportional to the amount of PCR product. FRET probes are not cleaved during the reaction, and can bind to a target again in the next PCR cycle. The optimal cycling conditions using FRET or hybridization probes include a 2-step cycling (Table 8). Adjust the annealing/extension temperature according to your assay.

Table 8. Cycling conditions for FRET or hybridization probes

Step	**Volume/reaction**		
	Time	Temperature	Final concentration
RT-step (optional in case of DNA template)	10 min	50°C	Maximal/fast mode
PCR initial heat activation	2 min	95°C	Maximal/fast mode
2-step cycling			
Denaturation	5 s	95°C	Maximal/fast mode
Annealing/Extension (data acquiring)	30 s	50–60°C	Maximal/fast mode
Number of cycles	**40***		

* The number of cycles depends on the amount of template.

Appendix C: Assay Design and Optimization

Important factors for successful quantitative, singleplex and duplex 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).

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 duplex RT-PCR, with minimal optimization.

Handling and storing primers and probes

Guidelines for handling and storing primers and probes are given in Table 9 below. For optimal results, we recommend only combining primers of comparable quality.

Table 9. 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 duplex assays, we recommend preparing 20x primer–probe mixes, each containing two primers and one probe for a particular target 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: 1 A_{260} unit = 20–30 µg/ml To check primer concentration, the molar extinction coefficient can be used: $A_{260} = \varepsilon_{260}$ x molar concentration of primer or probe If the ε_{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: ε_{260} = 0.89 x [(A x 15,480) + (C x 7340) + (G x 11,760) + (T x 8850)] Example Concentration of diluted primer: 1 µM = 1 x 10–6 M Primer length: 24 nucleotides with 6 each of A, C, G and T bases 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 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.

	Description
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.

Assay and Multiplex Assay Optimization

The QuantiNova Pathogen +IC Kit works well with most existing probe systems that have been designed using standard design methods. However, for optimal performance of a probe system in real-time RT-PCR, some considerations need to be made, including the quality of the primers and probes, the concentration of primers and probes and the annealing temperature and the choice of a compatible combination of reporter dyes (i.e., the fluorophores on the probes). Please read the following guidelines before starting.

- 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).
- Check the real-time cycler user manual for correct setup of the cycler. Be sure to activate the detector for each reporter dye used. For multiplex analysis setup the detection of multiple dyes from the same well.
- Check the functionality of each set of primers and probe. Check the functionality of each set of primers and probe in individual assays before combining the different sets in a multiplex assay. For multiplex analysis, the use of non-fluorescent quenchers (e.g., Black Hole Quencher® [BHQ] on TaqMan probes) is preferred over fluorescent quenchers (e.g., TAMRA™ fluorescent dye). For details about fluorescent dyes, see “Suitable Combinations of Reporter Dyes on the Rotor-Gene Q Instrument”, page 39.
- 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).

  Always start with the concentrations for primers and probes specified in the protocol. In some cases it may be preferable to test the optimal concentration for primers and probes.

We recommend testing combinations of different primers and probe concentrations using Table 10.

Table 10. Primers and probe concentration

	Final concentration			
Forward primer	0.3 µM	0.3 µM	0.8 µM	0.8 µM
Reverse primer	0.3 µM	0.8 µM	0.3 µM	0.8 µM
Probe concentration	0.25 µM			
Forward primer	0.3 µM	0.3 µM	0.8µM	0.8 µM
Reverse primer	0.3 µM	0.8 µM	0.3 µM	0.8 µM
Probe concentration	0.1µM			

- Always start with the cycling conditions specified in the protocol. Depending on the melting temperature of primers and probes, the annealing/extension temperature can be between 58°C and 62°C.
- When performing multiplex experiments with a high number of targets the annealing/extension time may be prolonged to 45 s.
- Optimal analysis settings (i.e., baseline settings and threshold values) for each reporter dye are a prerequisite for accurate quantification data. For details, refer to the manufacturer's instructions for your real-time cycler.

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

Multiplex real-time 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 11). 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 11. Dyes commonly used in multiplex real-time PCR on RotorGene-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.

Ordering Information

Product	Contents	Cat. no.
QuantiNova Pathogen +IC Kit (100)	For 100 x 20 µl reactions: 500 µl 4x QuantiNova Pathogen Master Mix, 100 µl QN IC RNA, 100 µl QN IC DNA, 200 µl QuantiNova IC Probe Assay, 500 µl QuantiNova Yellow Template Dilution Buffer, 250 µl QN ROX Reference Dye, 1.5 ml QuantiTect Nucleic Acid Dilution Buffer, 1.9 ml RNase-Free Water	208652
QuantiNova Pathogen +IC Kit (500)	For 500 x 20 µl reactions: 2 x 1.25 ml 4x QuantiNova Pathogen Master Mix , 250 µl QN IC RNA, 250 µl QN IC DNA, 1 ml QuantiNova IC Probe Assay, 500 µl QuantiNova Yellow Template Dilution Buffer, 1 ml QN ROX Reference Dye, 2 x 1.5 ml QuantiTect Nucleic Acid Dilution Buffer, 3 x 1.9 ml RNase-Free Water	208654
QuantiNova IC Probe Assay (200)	For 200 x 20 µl reactions: 400 µl primer/probe mix (10x), detecting internal control RNA/DNA, Max label	205813
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

Product	Contents	Cat. no.
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, 1.9 ml RNase-Free Water	205410
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
RNeasy MinElute Cleanup Kit (50)	50 RNeasy MinElute Spin Columns, Collection Tubes (1.5 ml and 2 ml), RNase-Free Reagents and Buffers	74204
QIAamp MinElute Virus Spin Kit (50)*	For 50 minipreps: 50 QIAamp MinElute Columns, QIAGEN Protease, carrier RNA, buffers, Collection Tubes (2 ml)	57704
Rotor-Gene Q 6-plex Platform	Real-time PCR cycler and High Resolution Melt analyzer with 6 channels (blue, green, yellow, orange, red, crimson), laptop computer, software, accessories: includes 1-year warranty on parts and labor, installation and training not included	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, training, 1-year warranty on parts and labor	9001532

* Other kit sizes and formats available; please inquire.

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