Quick-Start Protocol March 2015

QuantiNova™ Probe RT-PCR Kit

The QuantiNova Probe RT-PCR Kit should be stored immediately upon receipt at –30 to –15°C in a constant-temperature freezer and protected from light. QuantiNova Probe RT-PCR Master Mix can also be stored protected from light at 2–8°C for up to 12 months, depending on the expiration date.

Further information

- *QuantiNova Probe RT-PCR Kit Handbook*: **www.qiagen.com/handbooks**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: toll-free 00800-22-44-6000, or **www.qiagen.com/contact**

Notes before starting

- This protocol is optimized for quantification of RNA targets using TaqMan® probes in a singleplex or duplex 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, ViiA®7 and QuantStudio™ Real-Time PCR Systems.

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

- The QuantiNova Probe RT Mix contains HotStarRT-Script Reverse Transcriptase, for heat mediated activation of the reverse-transcription step; an RNase Inhibitor; and a DNase, for removing more than 90% of residual gDNA in the RNA preparation.
- The reference dye in QuantiNova Yellow Template Dilution Buffer allows tracking of pipetted samples in the qRT-PCR. When template is added to the blue QuantiNova Probe 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 the sample stability and qPCR.
- For the highest efficiency in real-time RT-PCR using TaqMan probes, amplicons should ideally be 60–150 bp in length.
- Before performing duplex analyses, choose suitable combinations of reporter dyes and quenchers that are compatible with the detection optics of your real-time cycler. We strongly recommend using non-fluorescent quenchers.
- 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 5 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 4 µM probe in TE buffer. Alternatively, it may be preferable to prepare the reaction mix with separate primer and probe solutions.
- 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. It is detected as a 200 bp internal control (IC) in the yellow channel on the Rotor-Gene Q or in the VIC®/HEX dye channel on other real-time PCR instruments, using the QuantiNova IC Probe Assay (cat. no. 20581). 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.

1. Thaw QuantiNova Probe RT-PCR Master Mix, QuantiNova Yellow Template Dilution Buffer, template RNA, QuantiNova Internal Control RNA (optional), primers, probes, QN ROX Reference Dye (if required) and RNase-free water. Mix the individual solutions.
2. Prepare a reaction mix according to Table 1. Due to the 2-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 1. Reaction mix setup

Component	96-well block, Rotor-Gene	384-well block	Final concentration
2x Probe RT-PCR Master Mix	10 µl	5 µl	1x
QN ROX Reference Dye (AB instruments only)	1 µl/0.1 µl*	0.5 µl/0.05 µl*	1x
QN Probe RT-Mix	0.2 µl	0.1 µl	1x
20x primer–probe mix 1	1 µl	0.5 µl	0.8 µM forward primer 1 0.8 µM reverse primer 1 0.2 µM TaqMan probe 1
20x primer–probe mix 2† (or QuantiNova IC Probe Assay†)	1 µl	0.5 µl	0.8 µM forward primer 2 0.8 µM reverse primer 2 0.2 µM TaqMan probe 2
QN IC RNA (optional)	1 µl	1 µl	1x
RNase-free water	Variable	Variable	-
Template RNA (added at step 4)	Variable	Variable	≤400 ng/reaction
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.

† If using the QN IC RNA to monitor RT-PCR amplification, please add 2 µl or 1 µl of the 10x QuantiNova IC Probe Assay.

3. Mix the reaction thoroughly and dispense appropriate volumes into PCR tubes, PCR capillaries or wells of a PCR plate.
4. Add template RNA (≤400 ng – 100 fg per reaction, depending on target transcript abundance) to the individual PCR tubes, capillaries or wells containing the reaction mix.
5. Program the real-time cycler according to Table 2.

   Note: Data acquisition should be performed during the combined annealing/extension step.
6. Place the PCR tubes or plates in the real-time cycler and start the cycling program.

Table 2. Cycling conditions

Step	Time	Temperature	Ramp rate
RT-step	10 min	45°C	Maximal/fast mode
PCR initial heat activation	5 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 RNA.

7. For interpretation of the QuantiNova IC Probe Assay results, please refer to the quick-start protocol *QuantiNova Internal Control RNA and Assay*.