Quick-Start Protocol July 2015

QuantiNova™ SYBR Green RT-PCR Kit

The QuantiNova SYBR Green RT-PCR Kit should be stored immediately upon receipt at –30 to –15°C in a constant-temperature freezer and protected from light. QuantiNova SYBR Green 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 SYBR Green RT-PCR Kit Handbook: **www.qiagen.com/HB-1973**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- This protocol is optimized for quantification of RNA targets by real-time one-step RT-PCR using SYBR Green I detection 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 used as a 20x concentrated solution for a 1x reaction when using an

instrument requiring a high ROX dye concentration. For instruments requiring a low ROX dye concentration, use the dye as a 200x concentrate.

- The QuantiNova SYBR Green RT Mix contains HotStaRT-Script reverse transcriptase that is almost inactive at room temperature, and an RNase inhibitor.
- 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 SYBR Green 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 or qPCR.
- For the highest efficiency in real-time PCR, 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 mix containing target-specific primers for each target. A 20x primer mix consists of 10 µM forward primer and 10 µM reverse primer in TE buffer. Alternatively, it may be preferable to prepare the reaction mix with separate primer 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 amplicon using the Ctrl_QNIC_1_SG QuantiTect® Primer Assay (QT02589307). Before use, add 180 µl (20 µl tube) or 900 µl (100 µl tube) of RNase-free water to the QN IC RNA provided in the kit and mix thoroughly by vortexing.

1. Thaw QuantiNova SYBR Green RT-PCR Master Mix, QuantiNova Yellow Template Dilution Buffer, template RNA, QuantiNova Internal Control RNA (optional), primers, 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.

Table1. Reaction mix setup

Component	96-well block, Rotor-Gene	384-well block	Final concentration
2x SYBR Green 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 SYBR Green RT-Mix	0.2 µl	0.1 µl	1x
20x primer mix (or Ctrl_QNIC_1_SG QuantiTect Primer Assay†)	1 µl†	0.5 µl†	0.5 µM forward primer 0.5 µM reverse primer
QN IC RNA (optional)	1 µl	1 µl	1x
RNase-free water	Variable	Variable	–
Template RNA (added at step 4)	Variable	Variable	≤200 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, add 2 µl (for 96-well) or 1 µl (for 384-well) of the 10x Ctrl_QNIC_1_SG QuantiTect Primer 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 (200 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	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	10 s*	60°C	Maximal/fast mode
Number of cycles	40†		

*If your cycler does not accept this short time for data acquisition, choose the shortest acceptable time.
† The number of cycles depends on the amount of template RNA.

7. For interpretation of the QuantiNova Internal Control results, please refer to the *QuantiNova Internal Control RNA and Assay Quick-Start Protocol*.

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