

QuantiNova[®] Pathogen +IC Kit

The QuantiNova Pathogen +IC Kit (cat. nos. 208652, 208654 and 205824) should be stored immediately upon receipt at –30 to –15°C in a constant-temperature freezer and protected from light. QuantiNova Pathogen Master Mix can also be stored at 2–8°C for up to 1 month, depending on the expiration date.

Further information

- *QuantiNova Pathogen +IC Kit Handbook*: www.qiagen.com/HB-2147
- Safety Data Sheets: www.qiagen.com/safety
- Technical assistance: support.qiagen.com

Notes before starting

- This protocol is optimized for simultaneous detection of DNA and RNA targets using TaqMan[®] probes in a singleplex or multiplex reaction (up to 4 targets) with any real-time cyclers and conditions for fluorescence normalization. ROX dye is required 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 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.

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- The QuantiNova Pathogen Master Mix contains both the inactivated HotStarRT-Script Reverse Transcriptase, and the inactivated form of the QuantiNova DNA Polymerase. The Master Mix can be used for amplifying DNA and/or RNA templates simultaneously.
 - The reference 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. It is provided as a 100x concentrate and should be diluted (using water) to obtain a 1x final concentration.
 - For the highest efficiency in real-time RT-PCR using TaqMan probes, amplicons should ideally be 60–150 bp in length.
 - Before performing multiplex analyses, choose suitable combinations of reporter dyes and quenchers that are compatible with the detection optics of your real-time cyclers.
 - **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. For multiplex reactions, you may prepare a primer-probe mix containing all primers and probes for the addressed targets.
 - The QuantiNova Internal Control is available as DNA and RNA template. The use is optional and not mandatory. They are detected as a 200 bp 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.
 - Before use as extraction control, dilute the QN IC template 1:10 using QuantiTect Nucleic Acid Dilution Buffer and mix thoroughly by vortexing. Add the resulting Internal Control solution to the sample lysate at a ratio of 0.1 µl per 1 µl elution volume.
 - Before use as reverse transcription/amplification control only, dilute the QN IC template 1:100 using QuantiTect Nucleic Acid Dilution Buffer or RNase-free water and mix

thoroughly by vortexing. Add 1 µl of the resulting QN IC solution directly to the reaction mixture.

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

Table 1. Reaction mix setup

Component	96-well block, Rotor-Gene	384-well block	Final concentration
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 (optional) ***	2 µl	1 µl	1x
QN IC DNA or RNA (optional) †	1 µl	1 µl	
RNase-free water	Variable	Variable	-
Template DNA or RNA (added at step 4)	Variable	Variable	Variable
Total reaction volume	20 µl	10 µl	-

* To be used as a 20x concentrate for high ROX cyclers (i.e., ABI PRISM 7000, Applied Biosystems 7300, 7900, and StepOne Real-Time PCR Systems) and as a 200x concentrate for low ROX dye cyclers (i.e., Applied Biosystems 7500, ViiA7 and QuantStudio 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. 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 DNA and RNA Internal Controls

† Only if using the QN IC as reverse transcription/amplification control only.

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 cyclers.

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

Note: Data acquisition should be performed during the 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 (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.

7. For more detailed protocol description and interpretation of the QuantiNova IC Probe Assay results, please refer to the handbook *QuantiNova Pathogen +IC Kit*.



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