

Supplementary Protocol

QuantiNova® Probe RT-PCR Kit for Direct RT-qPCR from Single or Multiple Cells

The following protocol was successfully used for quantitative real-time RT-PCR directly from cultured cells without prior RNA extraction, using the QuantiNova Probe RT-PCR Kit (cat. no. 208352, 208354, and 208356). For product details, reagent preparation, and cycling conditions, please refer to the respective kit handbook, available on [qiagen.com](https://www.qiagen.com).

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 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).

Important point before starting

Please refer to the QuantiNova Probe RT-PCR Kit Handbook or Quick-Start Protocol provided in the kit.

Procedure

Cell wash

1. Cultivate cells under the standard culture conditions for the cell line being used.
2. Wash the cells with cell-culture medium or PBS buffer.
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 microliter (appropriate number of cells: 0.5–1000 cell/ μ L).

Cell lysis and RT-qPCR

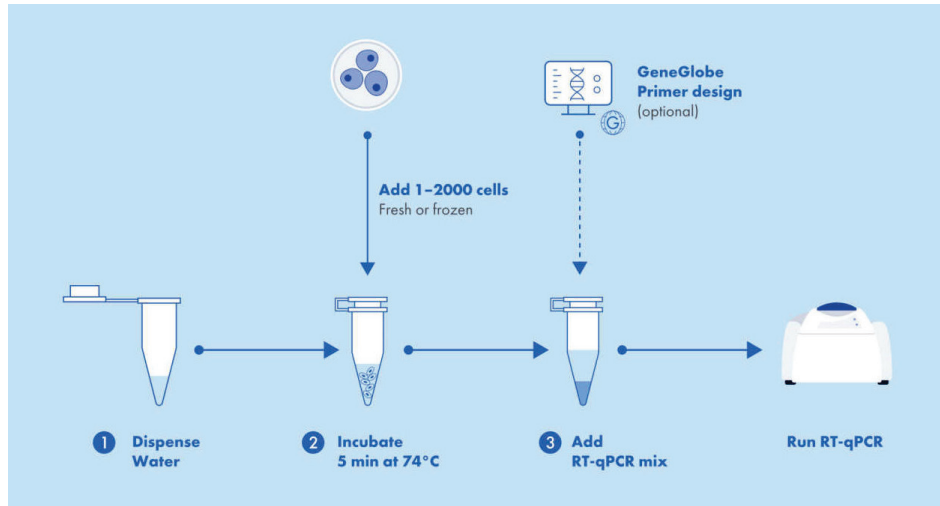


Figure 1. Direct PCR workflow.

1. Add 4.8 μL RNase-free water per well to a 96-well real-time PCR plate.
2. Transfer 2 μL of diluted cells to the 4.8 μL pre-aliquoted RNase free water, vortex the plate, and spin it down briefly. As control, we recommend using 2 μL cell culture medium, added to the 5.8 μL RNase free water in one well of the plate to check that no contaminating template is introduced by the cell culture medium.
3. Incubate the plate for 5 min at 74°C, either in the real-time PCR cyclers or in any suitable end-point PCR cyclers.
4. Centrifuge the plate briefly, proceed to step 5.
5. Set up the real-time, one-step RT-PCR in a final volume of 20 μL using the set up protocol from the *QuantiNova Probe RT-PCR Kit Handbook*.

The template volume is 6.8 μL (4.8 μL water plus 2 μL cell dilution).

6. To monitor successful reverse transcription and amplification, we 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 Probe 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.

Table 1. Reaction mix setup

Component	96-well block, Rotor-Gene	Final concentration
2x Probe RT-PCR Master Mix	10 µL	1x
QN ROX Reference Dye (AB instruments only)	1 µL /0.1 µL*	1x
QN Probe RT-Mix	0.2 µL	1x
20x primer–probe mix 1	1 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	0.8 µM forward primer 2 0.8 µM reverse primer 2 0.2 µM TaqMan probe 2
QN IC RNA (optional)	1 µL	1x
RNase-free water	4.8µl added in step 1	–
Template RNA	2 µL cells added in step 2	

* Results in a 1:20 dilution for high ROX dye cyclers (i.e., ABI PRISM® 7000; Applied Biosystems® 7300 and 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 of the 10x QuantiNova IC Probe Assay.

7. Program the real-time cyclers according to the protocol from the *QuantiNova Probe RT-PCR Kit Handbook* or *Quick-Start Protocol* provided in the kit or refer to Table 2.
8. Place the PCR tubes or plates in the real-time cyclers and start the cycling program according to protocol from the *QuantiNova Probe RT-PCR Kit Handbook* or *Quick-Start Protocol* provided in the kit or refer to Table 2.

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.

Document Revision History

Date	Changes
05/2023	Initial release.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

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