

## Supplementary Protocol

# QuantiNova® SYBR® Green 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 SYBR Green RT-PCR Kit (cat. no. 208152, 208154, and 208156). 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).

## 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.
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/ $\mu$ L).

### Cell lysis and RT-qPCR

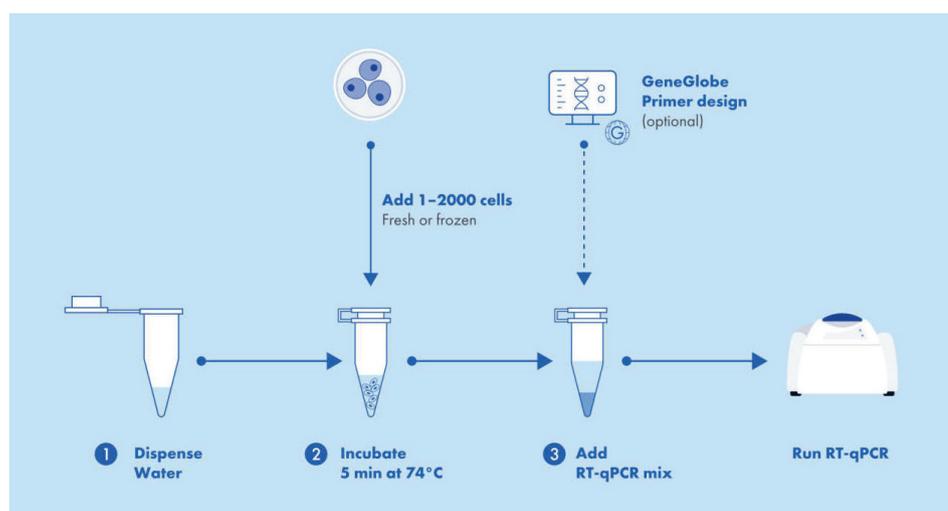


Figure 1. Direct PCR workflow.

1. Add 5.8  $\mu\text{L}$  RNase-free water per well to a 96-well real-time PCR plate or PCR tube.
2. Transfer 2  $\mu\text{L}$  of diluted cells to the 5.8  $\mu\text{L}$  pre-aliquoted RNase free water, vortex the plate, and spin it down briefly. As control, we recommend using 2  $\mu\text{L}$  cell culture medium and 5.8  $\mu\text{L}$  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 or tube 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 or tube briefly, proceed to step 5.
5. Set up the real-time, one-step RT-PCR in a final volume of 20  $\mu\text{L}$  using the set up protocol from the *QuantiNova SYBR Green RT-PCR Kit Handbook* or *Quick-Start Protocol* provided in the kit or refer to Table 1.

The template volume is 7.8  $\mu\text{L}$  (5.8  $\mu\text{L}$  water plus 2  $\mu\text{L}$  cell dilution).

**Table 1. Reaction mix setup**

Component	96-well block, Rotor-Gene	Final concentration
2x SYBR Green RT-PCR Master Mix	10 $\mu\text{L}$	1x
QN ROX Reference Dye (AB instruments only)	1 $\mu\text{L}$ /0.1 $\mu\text{L}$ *	1x
QN SYBR Green RT-Mix	0.2 $\mu\text{L}$	1x
20x primer	1 $\mu\text{L}$ †	0.5 $\mu\text{M}$ forward primer 0.5 $\mu\text{M}$ reverse primer
RNase-free water	5.8 $\mu\text{L}$ added in step 1	—
Template RNA	2 $\mu\text{L}$ added in step 2	—
Total reaction volume	20 $\mu\text{L}$	—

\* 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.

6. Program the real-time cyclers according to protocol from the *QuantiNova SYBR Green RT-PCR Kit Handbook* or *Quick-Start Protocol* provided in the kit or refer to Table 2.
7. Place the PCR tubes or plates in the real-time cyclers and start the cycling program according to protocol from the *QuantiNova SYBR Green 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	50°C	Maximal/fast mode
PCR initial heat activation	2 min	95°C	Maximal/fast mode
<b>2-step cycling</b>			
Denaturation	5 s	95°C	Maximal/fast mode
Combined annealing/extension	10 s*	60°C	Maximal/fast mode
Number of cycles	40†		

\* If your cyclers 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.

## Document Revision History

Date	Changes
05/2023	Initial release.

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**For products comprising ROX dye**

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