May 2023

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**.

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/µL).

Cell lysis and RT-qPCR

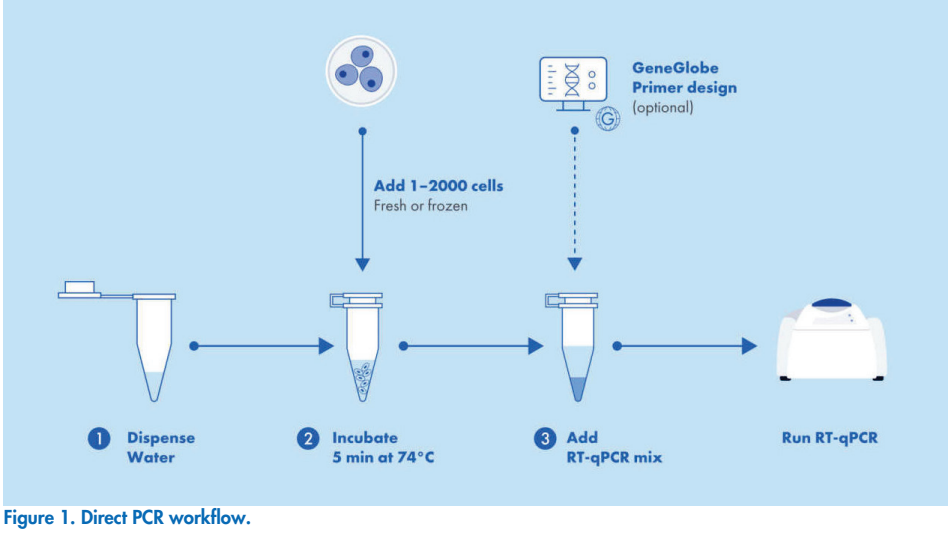

Figure 1. Direct PCR workflow.

1. Add 5.8 µL RNase-free water per well to a 96-well real-time PCR plate or PCR tube.
2. Transfer 2 µL of diluted cells to the 5.8 µL pre-aliquoted RNase free water, vortex the plate, and spin it down briefly. As control, we recommend using 2 µL cell culture medium and 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 or tube for 5 min at 74°C, either in the real-time PCR cycler or in any suitable end-point PCR cycler.
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 µL 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 µL (5.8 µL water plus 2 µL cell dilution).

Table 1. Reaction mix setup

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

* 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 cycler 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 cycler 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
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.

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.