

qNTQX® Fast Cell Direct Probe RT-qPCR Kit

Cat. no. LM602007

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1. Product Specification

Product	qNTQX® Fast Cell Direct Probe RT-qPCR Kit
Catalog No.	LM602007
Size	40 T / 100 T
Storage conditions	LM602007-A and LM602007-B: unopened, stored at –25 to –15 °C. After thawing, store at 4 °C and prevent contamination. Other components: stored at –25 to –15 °C for long-term storage. Shelf life: 1 year.

2. Product Description

qNTQX® Fast Cell Direct Probe RT-qPCR Kit is suitable for quantitative gene expression analysis of various animal cells (such as cell line adherent cells and suspended cells, primary culture cells, various stem cells, iPS cells), without RNA extraction, and can be directly used for qPCR expression analysis, with short time, simple operation, and low error rate — from template preparation to reverse-transcription reaction and gene expression analysis in as little as 1.5 h. The kit contains reverse transcription reagents as well as a quantitative detection reagent, enabling gene expression analysis to be performed easily.

3. Components

Comp. No.	Comp. Name	40 T	100 T
LM602007-A	FCD Lysis Buffer*	2 mL	5 mL
LM602007-B	FCD Washing Buffer*	8 mL	20 mL
LM602007-C	FCD Stop Solution*	100 µL	250 µL
LM602007-D	DNase I*	80 µL	200 µL
LM602007-E	4× qNTQX® FCD RT Mix*	200 µL	500 µL
LM602007-F	2× qNTQX® FCD Probe qPCR Mix*	2 mL	5 mL
LM602007-G	RNase-Free H ₂ O	2 mL	5 mL

* If the supplied reagents are insufficient, the Lysis set (Cat# LM606001S), RT set (Cat# LM606002), and qPCR set (Cat# LM606003) can be purchased separately.

4. Instructions of Using

4.1. Preparation of the Lysis Products

- Place the reagents to melt at room temperature, invert before use, mix gently and well, and use after brief centrifugation to avoid foaming. Do not mix using an oscillator. Leaving reagents on ice for a prolonged period will lead to a decrease in reaction performance.
- Transfer cells to a centrifuge tube* and collect cells** by centrifugation at 5,000 rpm for 2 min to fully remove the medium. Cells can be aspirated directly if cultured in 96-well plates.
- Add 150 µL FCD Washing Buffer to each well, wash the cells by aspiration and centrifuge at 5,000 rpm for 2 min to remove the FCD Washing Buffer.

4. Add 48 μL FCD Lysis Buffer and 2 μL DNase I solution to each well, mix and let stand at room temperature for 5 min. After incubation, add 2.5 μL FCD Stop Solution*** and mix approximately 5 times to obtain the lysis product****. When the number of cells exceeds 1×10^5 cells, lysis residue may occur.

* The basic requirement for the number of cells in a 50 μL lysis system is 1×10^2 – 1×10^5 cells per well. If the number of cells is large, the amount of FCD Lysis Buffer and DNase I solution can be increased proportionally.

** Different cells require different centrifugation conditions; please centrifuge at a speed suitable for the cells used.

*** 50 μL of lysate requires 2.5 μL of FCD Stop Solution; increase the amount of FCD Stop Solution proportionally if the amount of lysate increases.

**** If the cell lysis product is stored for a long time, please store at -25 to -15 $^{\circ}\text{C}$.

4.2. Reverse Transcription

After melting 4 $\times$ qNTQX® FCD RT Mix at room temperature, mix gently on ice and configure the reaction system according to the table below:

Components	Volume (μL)	Final concentration
4 $\times$ qNTQX® FCD RT Mix	5	1 $\times$
Obtained lysate*	x	—
RNase-free H_2O	up to 20	—

* To avoid aspiration of cell debris, the recommended input volume is 2 μL , and the maximum should not exceed 55% of the total reaction volume.

Gently mix the prepared reaction solution using a pipette and perform the reverse transcription reaction according to the table below**:

Temp.	Time
37 $^{\circ}\text{C}$	5 min
85 $^{\circ}\text{C}$	5 s

** The recommended reverse transcription temperature is 37 $^{\circ}\text{C}$. The RT product can be used directly for downstream RT-qPCR. To minimize inhibition of qPCR by the RT system and obtain appropriate Ct values (10–35), dilute the product 10–1,000 $\times$ before use. If downstream assays are not performed immediately, store at -25 to -15 $^{\circ}\text{C}$.

4.3. Fluorescence-Based qPCR Reaction Programme

4.3.1 Reaction System: the reaction solution is prepared using the following proportions (preparation on ice):

Components	Volume (μL)	Final concentration
2 $\times$ qNTQX® FCD Probe qPCR Mix	12.5	1 $\times$
Forward Primer (10 μM)	0.5	200 nM
Reverse Primer (10 μM)	0.5	200 nM
Probe (10 μM)	0.25–1	100–400 nM
Reverse transcription products	x	—
RNase-free H_2O	up to 25	—

* High template concentrations may cause non-specific amplification; dilute the template 5–50 $\times$ as appropriate. The recommended template input is 4 μL . If reaction performance is suboptimal, adjust the primer/probe concentration within the range of 0.2–1.0 μM .

4.3.2 Fast Fluorescence-Based qPCR Programme (recommended): this product recommends the use of a rapid programme for amplification, which can greatly reduce the reaction time. The annealing temperature can be adjusted when amplification is difficult.

Cycle step	Temp.	Time	Cycles
Predenaturation	95 °C	10 s	1
Denaturation	95 °C	5 s	45
Annealing/Extension**	60 °C	10 s	

** The annealing/extension temperature and final extension time can be adjusted as required. The fast cycling programme is suitable for most genes; for a small number of genes with complex secondary structures, a standard cycling programme may be used instead.

4.3.3 Regular Fluorescence-Based qPCR Programme: a regular programme can also be used for amplification when the experimental instrument does not meet the rapid reaction time.

Cycle step	Temp.	Time	Cycles
Predenaturation	95 °C	10 s	1
Denaturation	95 °C	15 s	40–45
Annealing/Extension	60 °C	30 s	

5. Notes

1. Before use, the frozen components are fully thawed and mixed gently before use.
2. During the experiment, please try to use pollution-free consumables to avoid contamination.
3. This product should avoid repeated freezing and thawing, and strong light exposure should be avoided during preparation.
4. For your safety and health, please wear a lab coat and disposable gloves during operation.
5. This product is for research use only.