

qNTQX® Fast Cell Direct SYBR Green RT-qPCR Kit

Cat. no. LM601013

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Product Specification

Product	qNTQX® Fast Cell Direct SYBR Green RT-qPCR Kit
Catalog No.	LM601013
Storage conditions	This product should be stored at –25 °C to –15 °C for 6 months.

Product Description

qNTQX® Fast Cell Direct SYBR Green RT-qPCR Kit is suitable for RNA extraction from various animal cells (such as cell-line adherent cells and suspension cells, primary culture cells, various stem cells, iPS cells, etc.), and without further RNA purification can be used directly for qPCR expression analysis. The workflow is short, simple to operate and has a low error rate — as little as 1.5 hours is needed to go efficiently from template preparation through reverse transcription to gene expression analysis. The kit contains both the reverse transcription reagent and the fluorescence detection reagent, allowing gene expression analysis to be performed easily.

Components

Cat. No.	Size
LM601013	40 T / 100 T

Instructions

A. Preparation of the Lysis Products

1. Allow the reagents to thaw at room temperature; invert to mix gently before use, and briefly centrifuge before use to avoid foaming. Do not vortex to mix — leaving the reagents on ice without adequate mixing will reduce reaction performance.
2. Transfer the cells to a centrifuge tube^{*} and collect the cells^{**} by centrifugation at 5,000 rpm for 2 min to fully remove the medium. If adherent cells are grown in 96-well plates, the medium can be removed directly.
3. 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 pipette to mix approximately 5 times to obtain the lysis product^{****}. When the number of cells exceeds 1×10^5 , lysis residue may commonly occur.

* The basic requirement for cell number in the 50 µL lysis system is 1×10^4 per well; the kit is validated for 1×10^3 – 1×10^5 cells. If the cell number is higher, the amount of FCD Lysis Buffer and DNase I solution can be increased proportionally.

** Different cell types require different centrifugation conditions; please centrifuge using the speed suitable for the cells used.

*** 5 µL of lysate requires 2.5 µL of FCD Stop Solution. When the amount of lysate is increased, the amount of FCD Stop Solution should be increased proportionally.

**** For long-term storage of the cell lysis product solution, store at –25 °C to –15 °C.

B. Reverse Transcription

1) After thawing 4× qNTQX® FCD RT Mix at room temperature, mix gently on ice and set up the reaction system according to the table below:

Components	Volume (μL)	Final concentration
4× qNTQX® FCD RT Mix	5	1×
Lysate	X	—
RNase-free H ₂ O	up to 20	—

2) Gently mix the prepared reaction solution by pipetting and perform the reverse transcription reaction according to the following programme:

Temp.	Time
55 °C	15 min
85 °C	5 min

C. Reaction Programme

Prepare the reaction solution using the following proportions (on ice):

Components	Volume (μL)	Final concentration
2× qNTQX® FCD qPCR SYBR Master Mix	10	1×
Forward primer (10 μM)	0.4	200 nM
Reverse primer (10 μM)	0.4	200 nM
Reverse-transcription product	X	—
RNase-free H ₂ O	up to 20	—

Run the quantitative fluorescence PCR using the following routine amplification programme:

Cycle step	Temp.	Time	Cycles
Pre-denaturation	95 °C	30 s	1
Denaturation	95 °C	10 s	35–40
Annealing	60 °C	30 s	
Extension	60 °C	30 s	
Melt curve phase	Instrument default settings	—	1

Notes

- Before use, ensure the frozen components are fully thawed and gently mixed.
- During the experiment, please try to use contamination-free consumables to avoid contamination.
- This product should avoid repeated freeze-thaw cycles, and strong light exposure should be avoided during preparation.
- For your safety and health, please wear a laboratory coat and disposable gloves during operation.
- This product is for research use only.