For research use only Version Number：1.0-2208

(96-well) QuickEasy™ Cell Direct RT-qPCR Kit–Taqman

For direct RT-qPCR using 10-10^5 cells cultured by 96-well plate

COMPONENT (50 μLlysis systerm/20 μL RTReaction System/20 μL qPCR Reaction System)		DRT-03021 96 T	Note
Part I	Buffer CL	5 mL	Cell Lysis
	Foregene Protease Plus II	100 μL	
	Buffer ST	500 μL	
Part II	DNA Eraser	100 μL	
	5× Direct RT Mix	400 μL	RT
	2× Direct qPCR Mix-Taqman	1 mL × 2	qPCR
	20× ROX Reference Dye	200 μL	
	RNase-Free ddH_2O	1.7 mL	
Instruction Manual		1 份	1 份

*: Part of lysis reagents and DNA Eraser are pack in Part II；Cell Lysis、RT、qPCR can be purchase separately.

Product Introduction

This product uses a unique lysis buffer system to quickly release RNA from cultured cell samples for RT-qPCR reactions, eliminating the time-consuming and laborious RNA purification process, and only takes 7 minutes to obtain the required RNA template. The 5× Direct RT Mix and 2× Direct qPCR Mix-Taqman provided in the kit can quickly obtain effective real-time quantitative PCR results. 5× Direct RT Mix and 2× Direct qPCR Mix-Taqman have strong inhibitor tolerance, and can use the lysate of the sample to be tested as a template for efficient reversal and specific amplification. The reagent contains Foregene Reverse Transcriptase, which with high affinity for RNA, as well as Hot D-Taq DNA Polymerase, dNTPs, MgCl2, reaction buffer, PCR optimizer and stabilizer, and can be used in conjunction with the lysis buffer to quickly and easily detected the sample, and has the characteristics of high sensitivity, strong specificity, and good stability.

The kit is aimed at micro-system lysis of 96-well cultured cells, which has good uniformity and consistency; the kit components provide 96 lysis reactions, 96 reverse transcription reactions and 96 × 2 qPCR reactions, which can meet the needs of the whole 96-well plate of cells is used for one-time use, avoiding the pollution caused by repeated opening, freezing and thawing of reagents and the degradation of reagent performance.

Storage Condition

1. Shipping condition：The whole process of low temperature ice pack box transportation to ensure that the kit is in the <4 °C state
2. Storage conditions： Store part I at 4°C and Part II at -20±5°C。

Kit component information

- ❖ Buffer CL： Provides the environment required for cell lysis reactions.
- ❖ Foregene Protease Plus II：In the context of lysis buffer, cells are lysed to release nucleic acids.
- ❖ DNA Eraser： DNA remover, the effect of removing the genome on subsequent experiments
- ❖ Buffer ST： Terminates the active substance in the lysate to avoid effects on subsequent RT.
- ❖ 5× Direct RT Mix：Contains high RNA affinity Foregene Reverse Transcriptase, RNase Inhibitor, dNTPs, stabilizers, enhancers, optimizers, and reverse transcription primers for optimal alignment (Random Primer, Oligo(dT) 18 Primer).
- ❖ 2× Direct qPCR Mix-Taqman： This reagent contains Hot D-Taq DNA Polymerase, dNTPs, MgCl2, reaction buffer, PCR optimizer, and stabilizer.
- ❖ 20× ROX Reference Dye: Generally used on Real Time PCR amplification instruments of ABI, Stratagene and other companies, it is used to adjust the difference between PCR tubes and tubes caused by PCR dosing errors. The 20× ROX Reference Dye concentration required for different instruments is different, and the user can add it according to the recommended concentration of the instrument.
- ❖ RNase-Free ddH2O: RNase-free sterilized ultra pure water for two-step RT-qPCR reactions.

Precautions: (Be sure to read the precautions carefully before using the kit)

- ◆ Pay attention to the operation method of the experiment to avoid cross-contamination between samples.
- ◆ Pay attention to the cleanliness of the experimental environment and utensils to avoid RNase contamination and RNA degradation.

- Please prepare the cell lysis system freshly, Set up right before use.
- 2× Direct qPCR Mix-Taqmanshould avoid repeated freeze-thaw, otherwise it will affect reverse transcription and PCR efficiency.

Operation Guide

A: Sample RNA Release

1. Cells were pretreated: Wash the cell culture plate with cold PBS, then lyse the cells.

1.1. Adherent cells: Adherent cell culture plate, and use a pipette to suck out the medium in the well (the medium should be aspirated as clean as possible to avoid affecting the subsequent lysis reaction). Add 50 μL of pre-cooled 1× PBS to each well. Do not pipette repeatedly to remove PBS from the wells. Tilt the plate to remove as much PBS as possible. Proceed to step 2.

Note: Make sure the cells are firmly attached to avoid massive cell loss during washing.

1.2. Suspension cells: After counting the number of cells, centrifuge at 1000 × g for 10 minutes, remove the supernatant, and collect the cell pellet; add 50 μL pre-cooled PBS to wash, centrifuge at 1000 × g for 10 minutes, remove the supernatant (remove as much PBS as possible, so as not to affect the subsequent cleavage reaction). Collect the cell pellet and proceed to step 2.

Note: The centrifugation conditions are different for different cells, please use the centrifugation speed suitable for the cells used for centrifugation.

2. Cell lysis: Remove Buffer CL, its temperature equilibrated to room temperature, DNA Eraser and Foregene Protease Plus II, according to the following table 1 prepared lysis system: (Lysis solution is ready for use).

Table 1: Preparation of Lysis System (Note: Please operate on ice when preparing)

Component (Cell Lysis Master Mix 50 μL/well	Reagent dosage for 1 well	96-well reagent volume
Buffer CL	48 μL	4608 μL
DNA Eraser	1 μL	96 μL
Foregene Protease Plus II	1 μL	96 μL

3. Pipette 50 μL of cell lysis master mix into each well, pipette repeatedly 5-10 times or mix with a plate mixing instrument for 10 seconds, and incubate at room temperature (20-25°C) for 5 minutes.

Note: To avoid the formation of air bubbles, keep the volume of the pipette below 50 μL when pipetting. Cells may appear slightly cloudy after lysis, which is normal.

4. Add 5 μL Buffer ST to the above liquid, pipette repeatedly 5-10 times or mix with a plate mixing instrument for 10 seconds, and incubate at room temperature (20-25°C) for 2 minutes.

Note: Place the tip of the pipette below the liquid level to ensure that the stop solution is added to the lysate. To avoid the formation of air bubbles, please adjust the scale of the pipette to below 50 μL when pipetting.

5. The lysate is used for subsequent RT-qPCR experiments. If subsequent experiments cannot be performed in time, please place it on an ice bath (not exceeding 2 hr), or store it at -20°C or -80°C (not exceeding three months) .。

B: RT system preparation

1. Take out 5 × Direct RT Mix and place it on an ice bath, let it melt naturally, and gently mix it for later use; take out RNase-Free ddH2O and melt it and place it on an ice bath for later use. Prepare the reaction system on ice according to Table 2-1 below.

Table 2-1: Preparation of RT reaction system

RT system add content	With the amount	Final concentration
5× Direct RT Mix	4 μL	1×
Cell Lysates (RNA template)	4 μL	Add range adjustment (10 -40%)
RNase-Free ddH_2O	12 μL	
Total Volume	20 μL	

2. After completion of system formulation, gently mixed and centrifuged briefly in the following table 2 -2 reaction conditions RT reaction.

Table 2-2: RT Reaction condition setting

Step	Temperature	Time	Content
1	42°C	20 min	cDNA synthesis
2	95°C	5 min	Inactivated reverse transcriptase
3	4°C	N/A	After the reaction is complete, store at 4°C for use or store at -20°C

3. After completion of the reaction, the reaction product was placed directly on ice for qPCR , please put the long-term preservation -20℃ or -80 ℃ .

Note: Due to the use of non-purified template, white precipitates may appear in the reverse

transcription product. This is a normal phenomenon. Centrifuge the supernatant immediately for subsequent experiments.

The resulting RT reaction solution is added to the next Step qPCR reaction systems, it is recommended to add amounts ranging from 10-30% of the reaction system.

C: qPCR reaction system preparation

1. Take an appropriate amount of the cDNA template prepared in step B to prepare the reaction system according to the following table 3-1.

Note: When used for subsequent qPCR detection, the amount of template accounts for 10-30% of the qPCR system. For example, in a 20 μL qPCR system, it is enough to add 2-6 μL of lysate, but not more than 6 μL.

表 3-1: Preparation of PCR reaction system

RT system add content	With the amount	Final concentration
2× Direct qPCR Mix-Taqman	10 μL	
Forward Primer (10 μM)	0.8 μL	50-900 nM
Reverse Primer (10 μM)	0.8 μL	50-900 nM
Probe Primer (10 μM)	0.4 μL	200 nM
cDNA template (obtained in step B)	4 μL	10-30%
RNase-Free ddH_2O	4 μL	
20× ROX Reference Dye *	-	-
Total Volume	20 μL	

*: Choose the appropriate final concentration of ROX Reference Dye according to the different quantitative PCR instruments. The most suitable for common quantitative PCR instrument Refer to the table below for the concentration of Reference Dye:

Real Time PCR Instrument	ROX Reference Dye Final Concentration
ABI PRISM7000/7300/7700/ 7900HT/Step One,etc.	1×(eg. 20 μl system, add 1 μl 20×ROX Reference Dye)
ABI 7500/7500 Fast 和 Stratagene Mx3000P/Mx3005P/Mx4000,etc.	0.5×(eg. 20 μl system,add 0.5 μl 20×ROX Reference Dye)

2. The qPCR reaction was performed according to the optimized qPCR conditions (annealing temperature, etc.) (reaction conditions are shown in Table 3-2 below).

Note: Try to use the optimized conditions for qPCR reaction to get better results.

Table 3-2: qPCR reaction condition setting

Step	Temperature	Time	Cycle	Content
1	95°C	3 min	1	Pre-denatured
2	95°C	10 sec	40	Template denaturation in cycle
3	60-65°C	30 sec		Annealing/extension