For research use only Version Number: 1.0-2208

(96-well) QuickEasy™ Cell Direct RT-qPCR Kit – SYBR Green I

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

Kit composition (50 μL lysis system/2 0 μL RT reaction system /20 μL qPCR reaction system)		DRT-03011 96T	Remark
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-SYBR	1 mL × 2	qPCR
	50× ROX Reference Dye	400 μL	
	RNase - Free ddH $_2$O	1.7mL	
Manual		1 piece	1

***:** The lysis reagent DNA Eraser is included in Part II of the kit; Cell Lysis, RT, and qPCR components can be purchased separately.

Product introduction

This product provides 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-SYBR provided in the box can quickly and effectively obtain real-time quantitative PCR results.

5× Direct RT Mix and 2× Direct qPCR Mix-SYBR have strong inhibitor tolerance, and can use the lysate of the sample to be tested as a template for efficient reverse transcription and specific amplification. The reagent contains Foregene unique Reverse Transcriptase with high affinity for RNA , as well as Hot D- Taq DNA Polymerase , dNTPs, $MgCl_2$, reaction buffer, PCR optimizer and stabilizer , and can be used in conjunction with the lysis buffer to detect the sample quickly, easily and accurately, and it has the characteristics of high sensitivity, strong specificity and good stability.

The kit is aimed at micro-system lysis of 96-well cultured cells, and 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 96-well cells plate for one-time use, avoiding the pollution caused by repeated opening, freezing and thawing of reagents and the degradation of reagent performance.

Transport and storage conditions

1. Transportation conditions: The kits should be transported in a low-temperature ice box to ensure that the kit is in a state of <4 ℃ .
2. Storage conditions : Kit Part I should be stored at 2-8 ℃ ; Part II should be stored at -20± 5 ℃ .

Kit component information

- ❖ Buffer CL: Provide the environment required for the cell lysis reaction.
- ❖ Foregene Protease Plus II : Under the environment of lysis buffer, cells are lysed to release nucleic acids .
- ❖ DNA Eraser: A DNA remover that removes the influence of the genome on subsequent experiments .
- ❖ Buffer ST: Terminate the active substances in the lysate to avoid affecting the subsequent RT.
- ❖ 5 × Direct RT Mix: Contains Foregene Reverse Transcriptase with high RNA affinity specially developed by Foregene , as well as RNase Inhibitor, dNTPs, stabilizers, enhancers, optimizers and reverse transcription primers with optimized ratios (Random Primer, Oligo(dT) 18 Primer).
- ❖ 2× Direct qPCR Mix-SYBR: This reagent contains Foregene's Hot D- Taq DNA Polymerase , dNTPs, $MgCl_2$, reaction buffer, SYBR Green I, PCR optimizer and stabilizer.

Precautions: (Please 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, which will cause RNA degradation.
- ◆ The cell lysis system should be freshly prepared and ready to use .
- ◆ 2× Direct qPCR Mix-SYBR should avoid repeated freezing and thawing, otherwise the PCR efficiency will be affected.

Operation Guide

A: Sample's RNA release

1. Cell pretreatment: wash the cell culture plate with cold PBS, and lyse the cells.

1.1. Adherent cells: Tilt the 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** , and 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 : Take out Buffer CL , let it equilibrate to room temperature, add DNA Eraser and Foregene Protease Plus II, prepare the lysis system according to the following table 1 : (the lysate is freshly prepared, ready to use).

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

Components (Cell Lysis Master Mix) 50 μL / well	Reagent addition 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 , room temperature (20-25 ℃) Incubate for 5 min.

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 phenomenon.

4. Add 5μL Buffer ST to the above liquid, pipette repeatedly 5-10 times or mix with a plate mixing instrument for 10 seconds , room temperature (20-25 ℃) Incubate for 2 min.

Note : The pipette tip is placed 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 ℃ or -80 ℃ (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 mix gently for use; take out RNase-Free ddH_2O and put it on an ice bath for use after melting . Prepare the reaction system on ice according to Table 2-1 below.

Table 2-1 : RT reaction system preparation

Components to RT system	Reagent addition	Final concentration
5 × Direct RT Mix	4 μL	1 ×
Cell Lysate (RNA Template)	4 μL	Addition range adjustment (10-40%)
RNase - Free ddH_2O	12μL	
Total Volume	20μL	

2. After the system preparation is complete, mix gently and centrifuge briefly, then carry out RT reaction according to the reaction conditions in Table 2-2 below .

Table 2-2 : RT reaction condition settings

Step	Temperature	Time	Components
1	42 ℃	20 minutes	cDNA synthesis
2	95 ℃	5 minutes	Inactivated reverse transcriptase
3	4 ℃	N/A	After the reaction is complete, store at 4 ℃ for use or -20 ℃ for storage

3. After the reaction is complete, the reaction product is placed on ice and used directly for qPCR. For long-term storage, please store it at -20 ℃ or -80 ℃ .

Note: Due to the use of non-purified templates , white precipitates may appear in the reverse transcription product , which is a normal phenomenon , and the supernatant can be taken by transient centrifugation for subsequent experiments . The obtained RT reaction solution is added to the next qPCR reaction system, and the recommended addition range is 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 a reaction system according to the following table 3-1.

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

Table 3-1: PCR reaction system preparation

Components to RT system	Reagent addition	Final concentration
2 × Direct qPCR Mix-SYBR	10μL	1 ×

Forward Primer (10 μM)	0.5 μL	50-900 nM
Reverse Primer (10 μM)	0.5 μL	50-900 nM
cDNA template (obtained in step B)	4 μL	10-30%
RNase-Free ddH_2O	5 μL	
50× ROX Reference Dye *	-	-
Total Volume	20μL _	

*: Choose the appropriate final concentration of ROX Reference Dye according to the different quantitative PCR instruments. The optimum concentration of ROX Reference Dye for common quantitative PCR instruments is shown in the table below :

Fluorescent quantitative PCR instrument	Final concentration of ROX Reference Dye
ABI PRISM7000/7300/7700/ 7900HT/Step One etc.	5× (such as 20 μL system , add 2 μL 50×ROX Reference Dye)
ABI 7500/7500 Fast and Stratagene Mx3000P/Mx3005P/Mx4000 etc.	1× (such as 20 μ L system, add 0.4 μL 50×ROX Reference Dye)

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

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

Table 3-2: Two-step qPCR reaction condition settings

Step	Temperature	Time	Number of cycles	Content
1	95 °C	3 minutes	1	Pre-denatured
2	95 °C	10 seconds	40	Template denaturation in cycle
3	60-65 °C	30 seconds		Annealing/extension
4	Draw a melting curve			

Table 3-2: Three-step qPCR reaction condition settings

Step	Temperature	Time	Number of cycles	Content
1	95 °C	3 minutes	1	Pre-denatured
2	95 °C	10 seconds	40	Template denaturation in cycle
3	55-65 °C	10 seconds		Annealing
4	72 °C	30 seconds		Extension
5	Draw a melting curve			