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FOREGENE

QuickEasy™ Cell Direct RT-qPCR Kit -Taqman

Cat.No.DRT-01021/01022

For cell direct RT-qPCR using ≤ 1000,000 cells

For performing RT-qPCR directly from cells without prior RNA purification

For research use only

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 7 minutes to obtain the required RNA template, with the 5× Direct RT Mix, 2× Direct qPCR Mix-Taqman provided by the kit can quickly and efficiently obtain real-time quantitative PCR results.

5× Direct RT Mix and 2× Direct qPCR Mix-Taqman have strong inhibitor tolerance and can perform efficient reversal and specific amplification using the lysate of the sample to be measured as a template. The reagent contains Foregene Reverse Transcriptase, Hot D-Taq DNA Polymerase, dNTPs, $MgCl_2$, Reaction Buffer, PCR Optimizer and Stabilizer, which can be used with lysis buffer to quickly and easily detect samples, and has the characteristics of high sensitivity, specificity and stability.

Product features

◆ Simple, effective Cell Direct RT technology that takes as little as 7 minutes to get RNA samples.

◆ Sample requirements are small, and a minimum of 10 cultured cells can be used for experimentation.

◆ High throughput for rapid RNA acquisition of cultured cells such as 384, 96, 24, 12, and 6-well plates.

◆ DNA Eraser is able to quickly remove released genomes, greatly reducing the impact on subsequent experimental results.

Optimized RT and qPCR systems enable two-step RT-PCR with more efficient reverse transcription, specificity, and stronger RT-qPCR reaction inhibitor tolerance.

Kit application

◆ Scope of application: Cultured cells.

◆ Sample lysis interpreted RNA: used only as a two-step RT-qPCR template.

◆ Kits can be used for the following purposes: gene regulatory expression analysis, allele testing, drug screening, etc.

Kit limitations

- Amplified fragments ≤ 300 bp.
- Kits are used to freshly culture cells.

Product quality control

According to the FOREGENE's Total Quality Management System, each batch of Cell Direct RT-qPCR series kits is rigorously tested multiple times to ensure the reliability and stability of the quality of each batch of kits.

Kit contents

QuickEasy™ Cell Direct RT-qPCR Kit-Taqman				
Kit components 20μl qPCR Reaction System		DRT-01021 200 T	DRT-01022 1000 T	Note
Part I	Buffer CL	4 ml	20 ml	Cell Lysis
	Foregene Protease Plus II	80 μl	400 μl	
	Buffer ST	400 μl	1 ml × 2	
Part II	DNA Eraser	80 μl	400 μl	
	5×Direct RT Mix *	160 μl	800 μl	RT
	2× Direct qPCR Mix-Taqman *	1 ml × 2	1.7 ml × 6	qPCR
	20×ROX Reference Dye	40 μl	200 μl	
	RNase-Free ddH_2O	1.7 ml	10 ml	
Instruction Manual		1 piece	1 piece	

***:**Cell Lysis, 5×Direct RT Mix, 2× Direct qPCR Mix-Taqman can be purchased separately,details are provided in Appendix 1 (PAGE 13).

Storage conditions

1. Shipping Conditions

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°C.

❖ The Foregene Protease Plus II should be stored at 4°C,not frozen at -20°C.

❖ Reagent 2× Direct qPCR Mix-Taqman is stored at -20°C, or at 4°C for short-term use if used frequently (within 10 days).

Kit component information

◆ Buffer CL: Provides the environment required for cell lysis reactions.

◆ Buffer ST: Terminates the active substance in the lysate to avoid effects on subsequent RT.

◆ DNA Eraser: DNA remover, the effect of removing the genome on subsequent experiments.

◆ 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).

◆ Foregene Protease Plus II: In the context of lysis buffer, cells are lysed to release nucleic acids.

◆ 2× Direct qPCR Mix-Taqman: This reagent contains Hot D-Taq DNA Polymerase, dNTPs, $MgCl_2$, 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 ddH_2O: 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.

◆Take fresh or well-preserved cell samples and never use repeated freeze-thawed cell samples.

◆ 5× Direct qPCR Mix,2× Direct qPCR Mix-Taqman should avoid repeated freeze-thaw, otherwise it will affect reverse transcription and PCR efficiency.

Preparations before operation

Be sure to read the instructions carefully before using this kit. The Cell Direct RT-qPCR Kit is simple, convenient, and fast to operate, and the instructions provide complete information about the entire kit and how to use it correctly. Please prepare the necessary experimental materials and equipment before use.

Experimental materials and equipment

- Culture cells.
- 1.5 ml or 2 ml, RNase-/DNase-Free centrifuge tube, RNase-/DNase-Free tip, 0.2 ml sterile qPCR tube.
- qPCR machine, pipette, tabletop centrifuge (≥13,400×g) (depending on experimental needs), etc.

Safety

- This product is for scientific research purposes only, please do not use it for pharmaceutical, clinical, food and cosmetic purposes.
- When using chemicals, wear appropriate lab clothes, gloves, protective glasses, etc.

Operation guides

Cell Lysis systems, RT systems, and qPCR reaction solution supplement packs can be purchased separately, for details in Appendix 1 (PAGE 13).

Operation guide

A: Sample RNA release

1. Cells were pretreated: Wash the cell culture plate with cold PBS, then lyse the cells (10-10^6), 10^6 than the amount of cells, is recommended Foregene the Cell an RNA Isolation Kit the Total (DE-03111) or Animal Total RNA Isolation Kit (DE-03011) for RNA extraction and purification.

1.1. Adherent cells (24- well plate as an example)

1.1.1. Determine the number of cells in each well, determine that the number of cells is 1 × 10^5, and use a pipette to remove the culture medium from the culture dish.

1.1.2. Add 200 µl of pre-chilled 1 × PBS to each well. Do not pipet repeatedly and remove PBS from the wells. Tilt the plate and remove as much PBS as possible. Proceed to step 2.

1.1.3. Different cell culture dish or a reference number table 1-1 in a cell culture dish was added precooled 1 × PBS for cell washing.

Table1-1: PBS dosage for different numbers of cells

Culture plate type	Number of cells / well	1 × PBS/ well
6-well	1× 10^6	1000 µl
12-well	2× 10^5	400 µl
24-well	10^5	200 µl
96-well	10^4	50 µl
384-well	5× 10^3	25 µl

Note：To ensure a firm adherent cells，a large number of cell loss avoided when washing .

1.2. Suspension cells or adherent cells cultured in non-porous plates

1.2.1. Adherent cells cultured in non-multi well plates (suspension cells start from the next step 1.2.2), collect and separate the cells according to the normal cell collection method, and place them in a culture plate or centrifuge tube; if trypsinization is used, require centrifugation to collect the cells and to remove residual trypsin, added PBS resuspended cells into individual cells to disperse the cells.

1.2.2. After the number of cells counted, aliquoted cells 1×10^5 one to centrifuge tubes, collect cells by centrifugation at 1000 × g for 10 min.

1.2.3. Add 200 µl PBS to the centrifuge tube, do not pipet repeatedly, and directly aspirate the PBS. proceed to step 2. (If difficult to precipitate and the cells were resuspended again, may be performed 1000×g centrifuged 10 min after discarding the supernatant, the cell pellet proceed to step 2)

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

Table1-2: cleavage system preparation (Note: in the preparation on ice)

Component (Cell Lysis Master Mix)	6-well plate	12-well plate	24-well plate	96-well plate	384-well plate
	1000 µl/well	400 µl/well	200 µl /well	50 µl/well	25 µl/well
Buffer CL	960µl	384µl	192µl	48µl	24µl
DNA Eraser	20µl	8µl	4µl	1µl	0.5 µl
Foregene Protease Plus II	20µl	8µl	4µl	1 µl	0.5 µl

3. (24 –well plate as an example) Pipette 200 µl of cell lysis master mix into each well, Repeatedly blow 5-10 times, Incubate at room temperature (20-25 ℃) for 5 min .

Note：To avoid the formation of bubbles, please when pipetting pipette scale was adjusted to 200µl or less. The cells may appear cloudy after lysis, which is normal.

4. (24 –well plate as an example) is added in the liquid 20 μl Buffer ST (different lysis systems Buffer ST added in an amount shown in Table 1-3) , repeated pipetting 5-10 times, at room temperature(20-25 ℃) were incubated for 2 min .

   Note： The pipette tip disposed below the surface, ensuring that the lysate was added， to avoid the formation of bubbles, please when pipetting pipette scale was adjusted to 200μl or less.

Table 1-3： Add Buffer ST

Buffer ST	6- well plate	12- well plate	24- well plate	96- well plate	384- well plate
	100 μl/well	40 μl/well	20 μl/well	5 μl/well	2.5 μl /well

5. The lysate is used for subsequent RT-qPCR experiments. If the subsequent experiments cannot be performed in time, please keep it on ice for no more than 2hr, and store at -20℃ or -80 ℃ (no more than 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	4μl	8 μl	1 ×
Cell Lysates (RNA template)	4 μl	8 μl	Add range adjustment (10 -40%)
RNase-Free ddH_2O	12 μl	24 μl	-
Total Volume	20 μl	40 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	15-30 min	cDNA synthesis
2	95 °C	5 min	Inactivated reverse transcriptase
3	4 °C	N/A	

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 Real Time PCR reaction systems, it is recommended to add amounts ranging from 10-30% of the reaction system.

C: qPCR reaction system preparation

1. Appropriate amount of B prepared in step cDNA template according to the following table 3-1 to prepare a reaction system.
   Note: The amount of cDNA template accounts for 10-30% of the qPCR system. For example, in a 20μl qPCR system, add 2-6 μl of lysis buffer, but not more than 6 μl.
2. The optimization good qPCR conditions (Annealing temperature， etc.) for qPCR reaction (The reaction conditions given at Table 3-2).
   Note: Try to use optimized conditions for qPCR reactions to get better results.

Table 3-1: Preparation of PCR reaction system

RT system add content	With the amount	Final concentration
2× Direct qPCR Mix-Taqman	10 μl	1×
Forward Primer (10μM)	0.4 μl	50-900 nM 1*
Reverse Primer (10μM)	0.4 μl	50-900 nM 1*
Probe(10μM)	0.2 μl	200nM
cDNA template (obtained in step B)	4 μl	10-30%
RNase-Free ddH_2O	—	
20×ROX Reference Dye 3*	—	—
Total Volume	20 μl	

1*: Primer concentration can be adjusted in the range of 50-900 nM when primer reaction performance is poor.
Note: The qPCR system can be adjusted according to experimental needs and fluorescence cycler model. For qPCR in a 50 μl system, adjust the reagent dosage proportionally according to the 20 μl system.
2*: Select the appropriate final concentration of ROX Reference Dye according to the fluorescence quantitative thermal cycler. The most appropriate ROX Reference Dye concentrations for common fluorescence quantitative cyclers are shown in the table below:

Real time PCR machine	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 and Stratagene Mx3000P/Mx3005P/Mx4000,etc.	0.5×(eg. 20 μl system,add 0.5 μl 20×ROX Reference Dye)

Table 3-2: qPCR reaction conditions are provided

Two-Step	Temperature	Time	Cycles	Content
1	95℃	3 min	1	Predenaturation
2	95℃	5-10 sec	40	Template denaturation
3	60-65℃	20-30 sec		Annealing / Extension

Note: In order to obtain the best qPCR effect, gradient PCR can be used to optimize the reaction conditions for different templates and different primers. PCR reaction conditions vary depending on the fluorescence analyzer, template, primer, etc. In the specific operation, the optimal reaction conditions need to be designed according to the specific conditions of the fluorescence quantitative thermal cycler, template type, size of the fragment of interest, base sequence of the amplified fragment and GC content and length of primers, including annealing temperature, reaction time, etc.

Real Time PCR primer design principles

Forward Primer and Reverse Primer

For Real Time PCR, primer design is very important. Primers are related to the specificity and efficiency of PCR amplification, and can be designed with reference to the following principles:

- Primer length: 18-30bp.
- GC content: 40-60%.
- Tm value: Primer design software, such as Primer 5, can give the Tm value of the primer. The Tm values of the upstream and downstream primers should be as close as possible. The Tm calculation formula can also be used: Tm = 4 °C (G + C) + 2 °C (A + T). When performing PCR, a temperature below the primer Tm value of 5 °C is generally selected as the annealing temperature (the corresponding increase in the annealing temperature can increase the specificity of the PCR reaction).
- Primers and PCR products:
  - Design primer PCR amplification product length is preferably 100-150bp.
  - Design primers in the secondary structural area of the template should be avoided as much as possible.
  - Avoid the formation of 2 or more complementary bases between the 3' ends of upstream and downstream primers.
  - Primer 3' terminal base can not be present with 3 additional consecutive G or C.
  - The primers themselves cannot have complementary structures, otherwise a hairpin structure will be formed, affecting PCR amplification.
  - ATCG should be distributed as evenly as possible in the primer sequence, and the 3' terminal base should be avoided as T.

Appendix 1：Cell Direct RT-qPCR Kit component supplement pack

1. Cell Lysis Solution

Kit components (24-well lysis system / well)		DRT-01011-A1 100 T	DRT-01011-A2 500 T
Part I	Buffer CL	20 ml	100 ml
	Foregene Protease Plus II	400 μl	1 ml × 2
	Buffer ST	1 ml × 2	10 ml
Part II	DNA Eraser	400 μl	1 ml × 2

2. RT Mix

Kit components (20 μl reaction system)	DRT-01011-B1 200 T
5× Direct RT Mix	800 μl
RNase-Free ddH_2O	1.7 ml × 2

3. qPCR Mix

Kit components (20 μl reaction system)	DRT-01021-C1 200 T	DRT-01021-C2 1000 T
2× Direct qPCR Mix-Taqman	1 ml × 2	1.7 ml × 6
20× ROX Reference Dye	40 μl	200 μl
RNase-Free ddH_2O	1.7 ml	10 ml

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