Version Number：1.0

# QuickEasy™ Cell Direct RT-qPCR Kit–SYBR Green I

For cell direct RT-qPCR using 10-1,000,000 cells

| Kit composition (20 μl qPCR reaction system) | | DRT-01011 200 T | DRT-01012 1000 T | comment |
|---|---|---|---|---|
| **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-SYBR | 1 ml × 2 | 1.7 ml × 6 | qPCR |
| | 50× ROX Reference Dye | 40 μl | 200 μl | |
| | RNase-Free dd$H_2O$ | 1.7 ml | 10 ml | - |
| Manual | | 1 serving | 1 serving | 1 serving |

***:** Cell Lysis ， RT and qPCR components can be purchased separately. For details， see Appendix 1 of the full version of the manual (PAGE 13).

## Product Introduction

This product uses a unique lysis buffer system to quickly release RNA from cultured cell samples for use in RT-qPCR reactions, eliminating RNA purification process. The required RNA template can be obtained in just 7 minutes, with reagents the 5× Direct RT Mix and 2× Direct qPCR Mix -SYBR can quickly and effectively obtain real-time quantitative PCR results.

## Transport and storage conditions

1. Transportation conditions: The whole process is transported in a low-temperature ice box to ensure that the kit is in a state of <4 °C.
2. Storage conditions: Part I of the kit is stored at 4 °C; Part II is stored at -20°C.

## Precautions:

- ◆ Attention to the cleanliness of the experimental environment and utensils to avoid RNase pollution and RNA degradation.
- ◆ Do not use cell samples that have been repeatedly frozen and thawed.
- ◆ Cell lysis system should be prepared fresh and ready to use.

## 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 $1 \times 10^5$, 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 | $1\times 10^6$ | 1000 μl |
| 12-well | $2\times 10^5$ | 400 μl |
| 24-well | $10^5$ | 200 μl |
| 96-well | $10^4$ | 50 μl |
| 384-well | $5\times 10^3$ | 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 $1\times10^5$ 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 1000 μl/well | 12-well plate 400 μl/well | 24-well plate 200 μl /well | 96-well plate 50 μl/well | 384-well plate 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 dd$H_2O$ | 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.

## 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 / 3-3).
   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 -SYBR | 10 μl | 1 × |
| Forward Primer (10μM) | 0.5 μl | 50-900 nM |
| Reverse Primer (10μM) | 0.5 μl | 50-900 nM |
| cDNA template (obtained from step B) | 4 μl | 10-30% |
| RNase-Free dd$H_2O$ | 5 μl | |
| 50× ROX Reference Dye 3* | - | - |
| Total Volume | 20 μl | |

**Table 3-2: Two-step qPCR reaction conditions are provided**

| Step | Temperature | Time | Cycles | Content |
|---|---|---|---|---|
| 1 | 95 °C | 3 min | 1 | Predenaturation |
| 2 | 95 °C | 5-10 sec | 40 | Template denaturation |
| 3 | 60-65 C | 20-30 sec | | Annealing / Extension |
| 4 | Melting curve | | | |

**Table 3-3: Three-step qPCR reaction conditions are provided**

| Step | Temperature | Time | Cycles | Content |
|---|---|---|---|---|
| 1 | 95 °C | 3 min | 1 | Predenaturation |
| 2 | 95 °C | 5-10 sec | 40 | Template denaturation |
| 3 | 55-65 °C | 10 sec | | Annealing |
| 4 | 72 °C | 20-30 sec | | Extension |
| 5 | Melting curve | | | |