Single Cell Sequence Specific Amplification Kit

P621

Version 22.1

Product Description

The Single Cell Sequence Specific Amplification Kit is a one-step RT-PCR-based amplification method. It is used to achieve transcriptome amplification in single cells or trace amounts of total RNA, which is convenient to reveal the expression levels of different genes between single cells. This kit incluedes RNA extraction and purification, reverse transcription and PCR in one tube, and no additional operations are required. In this case, the kit has the advantages of saving time, reducing experimental errors, reducing pollution, and improving sensitivity. This kit is also suitable for one-step amplification of 2 - 1,000 cells, and the number of cycles of one-step amplification should be adjusted according to the number of cells.

Components

	Components	P621-01 (200 rxns)
■	2 × Reaction Mix[a]	500 µl
■	RT/Taq enzyme[b]	20 µl
□	Nuclease-free ddH_2O	2 × 1.25 ml

a. It contains dNTP Mix, Mg^{2+} specific enhancer.

b. It contains Hiscript II Reverse Transcriptase, RNase inhibitor, Champagne Taq DNA Polymerase.

Storage

Store at -30 ~ -15℃ and transport at ≤0℃.

Applications

It is applicable for amplification of transcriptomes in single cells and trace amounts of total RNA.

Notes

For research use only. Not for use in diagnostic procedures.

1. RT/Taq enzyme contains high concentration of glycerol. Please centrifuge briefly and mix gently before use. Collect to the bottom of the tube, mix thoroughly and pipet accurately.
2. Please use Nuclease-free pipette tips, EP tubes and other Nuclease-free materials to avoid contamination during the experiment.

Experiment Process

1. Preparation of Assay Pool

Mix the amplification primers of different target genes to make an Assay Pool (the final concentration of each primer is 0.1 µM). For example, take 20 µl of Actb gene primers (10 µl of upstream primer and 10 µl of downstream primer with a concentration of 10 µM), add 980 µl of Nuclease-free ddH_2O to a final volume of 1 ml (the final concentration of each primer is 0.1 µM). 500 pairs of amplification primers for different genes can be mixed.

2. Prepare the following reaction system in an Nuclease-free centrifuge tube:

2 × Reaction Mix	2.5 µl	■
0.1 µM Assay Pool	0.5 µl	
RT/Taq enzyme	0.1 µl	■
Cell sample*	0.5 - 1.0 µl	
Nuclease-free ddH_2O	Up to 5.0 µl	□

*Cells can be stored in PBS buffer.

Put it on ice for later use, add cells, seal the lid, and immediately place the mixture in a -70℃ refrigerator for 2 min; centrifuge at 3,000 rpm (1,000 × g) for 2 min, and immediately put the tube into the PCR machine for the following reaction:

Steps	Temperature	Time	Cycles
Reverse Transcription	50℃	60 min	1
Initial Denaturation	95℃	3 min	1
Denaturation	95℃	15 sec	20*
Annealing, Extension	60℃	15 min	
Hold	4℃		

*We recommended the number of cycles could be adjusted by the number of input cells: 20 cycles for 1 cell, 17 cycles for 10 cells, 14 cycles for 100 cells, and 11 cycles for 1,000 cells.

After the reaction, add 20 μl Nuclease-free ddH_2O (1:5 dilution) to each tube and mix thoroughly by vortexing. Centrifuge at 3,000 rpm (1,000 × g) for 2 min and proceed to subsequent qPCR reactions immediately or store at -20℃. Applicative qPCR systems include the Fluidigm BioMark high-throughput qPCR system, and many other 96-well or 384-well qPCR systems.

3. qPCR reaction system (Using ABI StepOnePlus)

Prepare the following reaction mix:

Ace qPCR SYBR Green Master Mix	10.0 μl	
Primer 1 (10 μM)	0.5 μl	
Primer 2 (10 μM)	0.5 μl	
50 × ROX Reference Dye 1	0.4 μl	
Template DNA*	1.0 μl	
Nuclease-free ddH_2O	7.6 μl	☐

*Template DNA needs to be further diluted (10 times dilution) to reduce errors caused by operations.

Centrifuge at 3,000 rpm (1,000 × g) for 2 min, then immediately place in a qPCR instrument to perform the following reactions:

Stage 1	Initial Denaturation	Rep：1	95℃	5 min
Stage 2	Cycling Reaction	Reps：40	95℃	10 sec
			60℃	30 sec
Stage 3	Melting Curve	Rep：1	95℃	15 sec
			60℃	60 sec
			95℃	15 sec