Single Cell Sequence Specific Amplification Kit

P621-01/02

Version 8.1

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Introduction

The Single Cell Sequence Specific Amplification Kit is designed for transcriptome amplification with single or micro total RNA as template, the technology based on one-step RT-PCR, applicable to uncover the expression levels of different genes between individual cells. Each kit contains reagents for RNA extraction and purification, reverse transcription, and PCR amplification that have been optimized together in a simple workflow in one tube, which can save time, reduce experimental error, reduce pollution, and improve sensitivity. The kit can also be applied to one-step amplification of 2-1000 cells, and the cycle number should be reduced according to the number of cells.

Package Information

Components	P621-01 (200 rxn)
2 x Reaction Mix[a]	500 µl
RT/Taq enzyme[b]	20 µl
Nuclease-free H_2O	1.25 ml x 2

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

b. Contains Hiscript® II Reverse Transcriptase, RNase inhibitor, Champagne Taq™ DNA Polymerase.

Storage Conditions

Store at -20˚C.

Quality Control

All components were tested without exonuclease, endonuclease and RNase residues.

Functional test: Take a single mouse hybridoma cell as template to amplify the Actb1 gene, and the Ct value of qPCR was less than 15.

Protocol

1. Preparation of Assay Pool

Mix the amplification primers of the different genes to be tested to prepare an Assay Pool (the final concentration of each primer was 0.1 µM). For example, mix 10 µl of Actb with 10 µl of Gapdh gene primer (10 µM, upstream and downstream primers), add 980 µl of Nuclease free water to a final volume of 1 ml (ensure the final concentration of each primer is 0.1 µM), and it can amplify up to 500 of different genes in one reaction.

2. Prepare RT–PreAmp Master Mix as follows in a Nuclease free tube:

2 x Reaction Mix	2.5 µl
0.1 µM Assay Pool	0.5 µl
RT/Taq enzyme	0.1 µl
Nuclease free H_2O	1.9 µl
Total	5.0 µl

Place on ice, add a single cell, and cover the tube cap, immediately place it in a refrigerator at -80˚C for 2 min, centrifuge at 3000 rpm for 2 min, and immediately put it into the PCR instrument to perform the following reaction:

Procedures	Temperature	Time	Cycle
Reverse Transcription	50˚C	60 min	1
Pre-denaturation	95˚C	3 min	1
Denaturation	95˚C	15 sec	20
Annealing & Extension	60˚C	15 min	
Hold	4˚C		

After the reaction, add 20 μl of Nuclease free water (1:5 dilution) to each tube, mix by vortexing, centrifuged at 3000 rpm for 2 min, and immediately subjected to subsequent qPCR reaction or storage at -20℃. It is applicable to Fluidigm BioMark high throughput qPCR system and other 96-well or 384-well qPCR systems.

2. Prepare RT–PreAmp Master Mix as follows in a Nuclease free tube:

Prepare the reaction system as follows:

Ace®qPCR SYBR Green Master Mix	10 μl
Primer 1 (10 μM)	0.5 μl
Primer 2 (10 μM)	0.5 μl
ROX Reference Dye1	0.4 μl
Tempate DNA[a]	1 μl
Nuclease free H_2O	7.6 μl

a.The template DNA needs to be further diluted (diluted 10 times) to reduce pipetting errors.

Centrifuged at 3000 rpm for 2 min, and immediately put it into the PCR instrument to perform the following reaction:

Stage1 Pre-denaturation	Reps: 1	95℃	5 min
Stage2 Cycles	Reps:40	95℃	10 sec
		60℃	30 sec
Stage 3 Melting Curve	Reps:40	95℃	15 sec
		60℃	60 sec
		95℃	15 sec

Notes

1. RT/Taq enzyme contains high concentration of glycerol, please centrifuge briefly before use to collect to the bottom of the reaction tube, mix thoroughly by gently pipetting, and absorb accurately.
2. Please use the Nuclease free Pipette tip, EP tube, etc. to prepare the reaction solution, in order to avoid contamination maximally.

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