YeaCell™ 1st Strand Synthesis kit for Single Cell 3' RNA-seq

Product description

YeaCell™ 1st Strand Synthesis kit for Single Cell 3' RNA-seq can use Oligo (dT)18 or TSO Primer to reverse transcription of single cell experiment, which was suitable for microfluidic microsystems. The cDNA of the product can be enriched by PCR reaction with universal primers, and then constructed a DNA library using Yeasen's enzymatic library construction kit. This product uses a new reverse transcriptase based on M-MLV(RNase H-)Reverse Transcriptase through multi-point mutation, which has the advantages of high reverse transcriptase efficiency, low mismatch rate and high fidelity.

Specifications

Cat.No.	13594ES04 / 13594ES08 / 13594ES95
Size	4 T / 8 T / 120 T

Components

Components No.	Name	13594ES04	13594ES08	13594ES95
13594-A	RT buffer	76 μL	152 μL	2×1130 μL
13594-B	RT Enzyme mix	36 μL	72 μL	1056 μL

Storage

This product should be stored at -25~-15℃ for 1 years.

Application

1. High-throughput single-cell full-length cDNA synthesis for mammalian or eukaryotic cells without cell walls.
2. 10 pg~1 μg total RNA with poly (A).
3. This product is not suitable for prokaryotic total RNA and degraded RNA, such as FFPE RNA.

Instructions

Plan A : If the experimental scheme is a high-throughput single-cell experiment, appropriate adjustments should be made according to different single-cell instruments.

1) To prepare the Reaction Buffer, thaw the RT buffer, Template Switch Oligo (customer)and Reducing Agent B (customer) at room temperature in advance, mix it upside-down, centrifuge it at the bottom of the tube and place it on ice for use.

Table 1 Reaction Buffer prepare

Name	Value（μL）
RT buffer	18.8
Template Switch Oligo	2.4
Reducing Agent B	2
RT Enzyme mix	8.8
Total	32

2) Mix thoroughly by gently pipetting up and down at least 10 times. Briefly spin down the tube in a microcentrifuge to collect the liquid from the side of the tube.

3) Refer to the instructions for Cat#12520ES (for 10x Genomics single cell instrument) for subsequent use.

Plan B : For reverse transcription experiments, refer to this procedure

Step 1 RNA denaturation

1. Prepare the reaction liquid according to Table 2:

Table 2 RNA denaturation reaction

Name	Value（μL）
Oligo (dT)18（20~50 μM）(customer)	1
Lysis cell or RNA	12
Total	13

2. Mix thoroughly by gently pipetting up and down at least 10 times. Briefly spin down the tube in a microcentrifuge to collect the liquid from the side of the tube.

3. Place tube in a thermocycler and run the following program: (heated lid 80°C on) 70°C，5 min,then place it on ice immediatly.

Step 2 1st Strand Synthesis

1. Remove the first chain of synthetic reagents from -20°C, thaw at room temperature, mix thoroughly and spin down. Prepared the 1st strand synthesis reagents according to the Table 3.

Table 3 1st Strand Synthesis reaction

Name	Value（μL）
Above step	13
5×RT Buffer	4
TSO Primer（20~50 μM）(customer)	1
RNase Inhibitor(40 U/μL)（customer）	1
Reverse Transcriptase for Single Cell Full Length cDNA	1
Total	20

2. Mix thoroughly by gently pipetting up and down at least 10 times. Briefly spin down the tube in a microcentrifuge to collect the liquid from the side of the tube.

3. Place tube in a thermocycler and run the following program:（heated lid 105°C on）42°C，90 min；70°C，15min；4°C，hold。

4. The product can be directly used for two-strand cDNA synthesis or temporary storage at -80°C.

Notes

1. This product is for research use only.

2. Please operate with lab coats and disposable gloves，for your safety.

3.Supplies free of RNase contamination and cleaning the experimental area regularly are necessary. ThermoFisher's RNAZap™ high-efficiency nucleic acid removal spray was recommended to remove RNase contamination.

4. Other materials should be asked if we want to construct a DNA library.