ABScript Full Length Reverse Transcriptase for Single Cell

RK20414

Product Components

Components	Catalog	Size-1 5,000 U	Size-2 20,000 U
Reverse Transcriptase	RM21003	25 μL	100 μL
5X RT Buffer	RM21005	100 μL	400 μL

Product Description

ABScript Full Length Reverse Transcriptase for Single Cell is a genetically engineered M-MuLV reverse transcriptase with reduced RNase H activity and Template-switching activity. It adds adapter sequences to the 3' end of cDNA and, using Oligo (dT) VN Primer and Template-switching oligo (TSO) Primer, can initiate reverse transcription reactions from as little as 10 pg of total RNA, yielding higher full-length cDNA production. It is suitable for reverse transcription of small amounts of template and low-copy genes.

Product Source

The gene encoding the mutated M-MuLV Reverse Transcriptase (RNase H-) was expressed in Escherichia coli and purified through multiple steps.

Unit Definition

One unit (U) of activity is defined as the amount of enzyme required to incorporate 1 nmol of dTTP into acid-insoluble material in 10 minutes at 37℃, using Poly(rA)•Oligo d(T)18 as a template in a 50 μL reaction.

Storage Temperature

-20℃

Application

- Suitable for single-cell full-length cDNA synthesis from eukaryotic mRNA or Total RNA samples containing a poly(A) tail.
- Input Sample Amount: 10 pg ~ 10 ng Total RNA (from 1~1000 lysed cells) as the starting material.

Precautions

- All buffers and enzymes should be kept on ice at all times. - Exercise caution when using various reagents to avoid cross-contamination between reagents and samples.
- Maintain a clean experimental area; wear clean gloves and masks during operations. All consumables used in the experiment, such as centrifuge tubes and pipette tips, must be RNase-free.

Instructions

1. RNA Pre-denaturation

1.1. Prepare the RNA denaturation reaction system according to the table below:

Reagent	Volumn
Total RNA/cell*	10 μL
Oligo (dT) RT Primer（10 μM） （Self-prepared）	1 μL
dNTP（5 mM） （Self-prepared）	1 μL
Total volum	**12 μL**

1.2. Gently mix by pipetting, briefly centrifuge, and place on ice. Run the following program in a PCR machine (heated lid at 105℃):

Temperature	Time
72℃	5 min
Immediately place on ice after reaction completion*	2 min

Note: Immediately place on ice for at least 2 minutes after the reaction has concluded.

2. First Strand cDNA Systhesis

2.1. Remove the 5X RT Buffer and Reverse Transcriptase components from the -20°C freezer and place them on ice to thaw. Once the components are fully dissolved, vortex to mix thoroughly and briefly centrifuge. Prepare the first-strand cDNA synthesis reaction mixture according to the table below.

Reagent	Volumn
Products from step 1.2	12 μL
5X RT Buffer	4 μL
TSO Primer（20 μM）（Self-prepared）	2 μL
RNase Inhibitor（20 U/μL）（Self-prepared）	1 μL
Reverse Transcriptase	1 μL
Total volum	**20 μL**

2.2. Gently mix by pipetting up and down 15-20 times, and centrifuge briefly. Place the tube in a thermocycler，and run the following program (Lid preheated to 105℃).

Temperature	Time
42℃	90 min
70℃	10 min
12℃	Hold

2.3. After the program has completed, retrieve the first strand cDNA product. It can be directly used for dscDNA synthesis or stored at -80℃ for later use.