Single Cell Full Length mRNA-Amplification Kit

N712

Vazyme Biotech Co., Ltd.
Web: www.vazyme.com
Tel: 400-600-9335
Sales: global@vazyme.com
Support: global@vazyme.com
Service: global@vazyme.com

Instruction for Use

Version 21.1

Vazyme

Contents

01/Product Description

Single Cell Full Length mRNA Amplification Kit can achieve enough amount of full-length cDNA through using 1 - 500 of cells or 10 pg - 10 ng of total RNA as templates. In this kit, Oligo (dT) VN Primer is used as reverse transcription primer and template-switching activity of Sc Reverse Transcriptase is utilized to add adapter sequences at the 3' of cDNA. Subsequently, PCR amplification is performed through the adapter sequences to obtain full-length cDNA amplification products, which effectively avoids 3' preference and rRNA contamination in the cDNA synthesis process. The obtained full-length cDNA amplification products can be used for genetic regulation information analysis such as differential gene expression, alternative splicing, and fusion genes. Single Cell Full Length mRNA Amplification Kit is compatible with sample volumes within 2.5 μl. Generally, 2 - 20 ng of cDNA amplification products can be obtained in one reaction.

02/Components

	Components	N712-01 (12 rxns)	N712-02 (24 rxns)	N712-03 (96 rxns)
Box 1	5' TS Oligo Primer	6 μl	12 μl	48 μl
	Control Total RNA (1 μg/μl)	5 μl	5 μl	10 μl
Box 2	Lysis Buffer	230 μl	460 μl	2 × 920 μl
	RNase Inhibitor	30 μl	60 μl	320 μl
	Oligo (dT) VN Primer	12 μl	24 μl	96 μl
	dNTP Mix	12 μl	24 μl	96 μl
	DTT	6 μl	12 μl	48 μl
	1st Strand Buffer	24 μl	48 μl	192 μl
	Sc Reverse Transcriptase	12 μl	24 μl	96 μl
	2 × Amplification Mix	150 μl	300 μl	2 × 600 μl
	PCR Primer	6 μl	12 μl	48 μl
	Elution Buffer	1 ml	1 ml	2 × 1 ml
	Nuclease-free ddH_2O	1 ml	1 ml	2 × 1 ml

▲The color marked in the above table represents the color of the lid of each component.

03/Storage

Box 1, store at -85 ~ -65℃ and shipped on dry ice;
Box 2, store at -30 ~ -15℃ and shipped on dry ice.

04/Applications

In this kit, Oligo (dT) VN Primer is used as primer to amplify RNA with poly A sequence. It is applicable to the following sample types:

◇1 - 500 of mammalian cells, or other eukaryotic cells without cell walls;

◇10 pg - 10 ng of purified total RNA with poly A sequence;

▲It is not suitable for prokaryotic cells or fixed cells.

05/Self-prepared Materials

Purification Beads: VAHTS DNA Clean Beads (Vazyme #N411) or other equivalents;
Library Preparation Kit: TruePrep DNA Library Prep Kit V2 for Illumina (Vazyme #TD503) or other equivalents;
Other Materials Required: RNase-free PCR tube, low-adsorption EP tube, magnetic stand, Agilent Technologies 2,100 Bioanalyzer, etc.

06/Notes

1. Oligo dT Primer is used to perform amplification from RNA with poly A sequence. Please make sure there are no DNA with poly A sequence.
2. Cell walls can't be lysed effectively with this kit. Eukaryotic cells with cell walls must be lysed after removing the cell walls, or use purified RNA for reaction. Mammalian cells can be lysed directly using this product.
3. This product is not suitable for fixed cells.
4. When using RNA as template, make sure that the RNA is intact and free from contamination.
5. This product has high detection sensitivity, and the experimental operation should be completed in a positive pressure ultra-clean workbench. Please do not operate in ordinary PCR operating platforms.
6. All components of this product should be stored in nucleic acids and nuclease-free environment to avoid experimental failure.
7. All components in the kit have been optimized, please do not change the reaction system during use.
8. For the first time, it is recommended to set up positive and negative control at the same time to verify whether the system is working properly.

07/Sample Preparation

Some of the components in the cell culture medium or the sample may inhibit the reaction, please try to reduce unnecessary sample volume in order to reduce the possible influence on the reaction system.

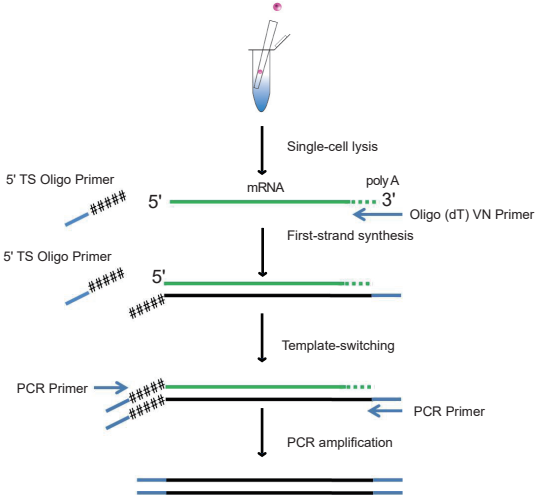
07-1/Cell Sample Preparation

◇ Cell viability should be detected by trypan blue staining or other methods. In dead cells, RNA can be seriously degraded, which will cause the failure of the experiment;
◇ Please confirm if the cell culture medium has an inhibitory effect on the test before experiment. It is recommended to resuspend the cells in 1 × PBS before proceeding to the subsequent steps;
◇ The starting template can be 1 - 500 cells. Excessive cells may inhibit the reaction.
◇ The cells obtained by flow cytometry sorting should be visualized under a microscope to check the cell numbers and cell state.
◇ If subsequent procedures are not performed, please refer to 09-1/step 2 and step 3. Store the prepared samples at -70℃ or lower temperature.

07-2/RNA Sample Preparation

For purified RNA samples, it is recommended to evaluate the integrality of the RNA using Agilent RNA 6000 Pico Kit before reaction. Taking degraded RNA as starting template will affect the cDNA yield and product size distribution, and may lead to the failure of the experiment.

08/Mechanism & Workflow



cDNA Synthesis Mechanism Based on Template-switching

09/Experiment Process

09-1/1st Strand cDNA Synthesis (Please operate in an ultra-clean workbench)

1. Take out all of the components for 1st strand cDNA synthesis and dissolve them on ice. Mix by vortex and briefly centrifuge to collect the solution at the bottom of the tube, and then place on ice.

▲There may be precipitates in 1st Strand Buffer, please mix by vortex and dissolve thoroughly before use.

▲Gently flick the tube to mix 5' TS Oligo Primer and Sc Reverse Transcriptase before use. Please do not vortex.

2. Prepare Sample Buffer as follows:

Components	Volume
Lysis Buffer	18 μl
RNase Inhibitor	2 μl
Total	20 μl

▲Mix thoroughly by gently pipetting, and briefly centrifuge to collect the solution at the bottom of the tube. Avoid bubbles during this procedure.

3. Sample preparation:

Set up control and test sample reactions as follows:

Components	Negative Control	Positive Control	Sample
Sample Buffer	1 μl	1 μl	1 μl
Diluted Control Total RNA	–	0.5 - 2.5 μl	–
RNA/Cell Sample	–	–	0.5 - 2.5 μl
Nuclease-free ddH_2O	up to 3.5 μl	up to 3.5 μl	up to 3.5 μl

▲The concentration of the Control Total RNA in the kit is 1 μg/μl, please carry out 10-fold serial dilution with Nuclease-free ddH_2O before use.

▲Cell viability should be detected by trypan blue staining or other methods.

▲If the starting templates are small amount of cells obtained by flow cytometry sorting, please check the cell numbers and cell state through a microscope.

▲Some of the components in the cell culture medium may inhibit the reaction. It is recommended to resuspend the cells in 1 × PBS and try to reduce the unnecessary sample volume.

▲If the subsequent amplification is not performed immediately, the cell samples prepared in this step can be stored at -70˚C or lower temperature and shipped on dry ice.

4. Prepare the reaction system as follows:

Components	Volume
RNA/Cell Sample from step 3	3.5 μl
Oligo (dT) VN Primer	1 μl
dNTP Mix	1 μl
Total	5.5 μl

5. Mix thoroughly by gently pipetting, briefly centrifuge, and place on ice.

6. Run the program as follows in a PCR instrument:

Temperature	Time
72˚C	3 min
Chill on ice immediately	2 min

7. Prepare the reverse transcription system as follows:

Components	Volume
Products from step 6	5.5 μl
1st Strand Buffer	2 μl
DTT	0.5 μl
RNase Inhibitor	0.5 μl
5' TS Oligo Primer	0.5 μl
Sc Reverse Transcriptase	1 μl
Total	10 μl

▲Gently flick the tube to mix Sc Reverse Transcriptase and 5' TS Oligo Primer before use. Please do not vortex.

8. Mix thoroughly by gently pipetting, briefly centrifuge, and place on ice.

9. Run the following program in a PCR instrument:

Temperature	Time
42˚C	90 min
70˚C	15 min
4˚C	Hold

The reaction products here can be stored at 4˚C overnight, and the storage time should not exceed 12 h.

09-2/Full-length cDNA Amplification (Please operate in an ultra-clean workbench)

1. Prepare PCR amplification system as follows:

Components	Volume	
1st strand cDNA synthesis products	10 μl	
Nuclease-free ddH_2O	2 μl	□
PCR Primer	0.5 μl	
2 × Amplification Mix	12.5 μl	
Total	25 μl	

2. Mix thoroughly by gently pipetting, briefly centrifuge and place on ice.
3. Run the following program in a PCR instrument:

Temperature	Time	Cycles
98℃	1 min	
98℃	10 sec	
65℃	15 sec	x
72℃	6 min	
72℃	5 min	
4℃	Hold	

Cycle numbers for different starting templates for reference:

Total RNA	Cells	Cycles
10 ng	500 cells	8 - 9
1 ng	100 cells	11 - 12
100 pg	10 cells	14 - 15
10 pg	1 cell	17 - 18

▲ The cycle numbers in the above table are obtained using 293T cells as test samples. Due to the large difference in RNA amounts of different cells, please adjust the cycles according to the different cell types. When testing a cell type for the first time, please set the cycle number gradient to determine the optimal number of cycles.

4. Place the products on ice.

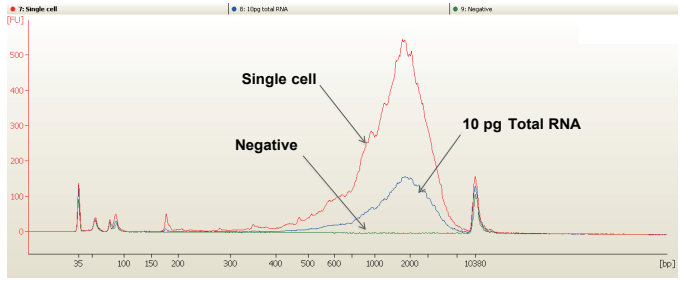
The products of this step can be store at 4℃ overnight, but do not exceed 12 h.

09-3/Amplified Products Purification and Detection

1. Equilibrate VAHTS DNA Clean Beads to room temperature and mix the beads thoroughly by vortex. Add 25 μl of VAHTS DNA Clean Beads to the cDNA amplification products, pipette 10 times to mix the reaction system thoroughly.
2. Incubate at room temperature for 8 min .
3. Briefly centrifuge the tube and place it on a magnetic stand to separate the beads from the solution. After the solution is clear (about 5 min), remove the supernatant carefully.
4. Keep the PCR tube on the magnetic stand. Add 200 μl of freshly prepared 80% ethanol without disturbing the beads. Incubate at room temperature for 30 sec, then carefully remove the supernatant.
5. Repeat step 4 (rinse the beads twice in total).
6. Always keep the PCR tube on the magnetic stand. Open the lid and air-dry the beads for 5 - 10 min until there is no ethanol residue.
7. After air-drying the beads, take the PCR tube out of the magnetic stand. Add 17 μl of Elution Buffer and mix them by pipetting. Incubate at room temperature for 2 min. If the beads are over-dried and cracking, please extend the incubation time.
8. Briefly centrifuge the PCR tube and then place on the magnetic stand to separate the beads and solution until the solution is clear (about 5 min).
9. Transfer 15 μl of the supernatant to a new low-adsorption PCR tube. Store at -20℃.

cDNA Amplification Products Detection

Analyze 1 μl of amplified cDNA using Agilent 2,100 Bioanalyzer and High Sensitivity DNA Chip. A single 293T cell and 10 pg of total RNA were amplified for 18 cycles. The amplified products were distributed between 400 bp and 10,000 bp. The peak of the libraries were around 2,000 bp. No template negative control yielded no products.



09-4/Library Preparation for Illumina Platform

1 ng of cDNA is recommend to prepare the library through TruePrep DNA Library Prep Kit V2 for Illumina (Vazyme #TD503) or Nextera XT DNA Library Preparation Kit (Illumina #FC-131-1024).