AcuGenix™ First-Strand cDNA Synthesis Kit (for Single-Cell 3′ RNA-Seq) V01

Product Description

AcuGenix™ First-Strand cDNA Synthesis Kit (for Single-Cell 3′ RNA-Seq) performs reverse transcription of single-cell transcripts using Oligo $(dT)_{18}$ and TSO primers. cDNA is enriched from reverse transcription products via universal primer PCR, and sequencing library construction can be subsequently completed with a matched enzymatic library construction kit.The kit is applicable to high-throughput single-cell full-length cDNA synthesis in mammalian cells and eukaryotic cells without cell walls.The kit is compatible with 10 pg to 1 μg poly(A)-containing total RNA.The kit is not suitable for prokaryotic total RNA and degraded RNA, such as FFPE RNA.

Components

Components	BR3N801-01 (8T)	BR3N801-02 (20T)	BR3N801-07 (100T)
4×RT Buffer	151 μL	376 μL	0.94 mL×2
Reverse Transcriptase	36 μL	90 μL	450 μL

Storage

Store at −20±5℃.

Notes

1.This kit does not contain RNase inhibitor; please prepare it separately.
2.Other reagents required for library construction shall be prepared or purchased separately.
3.Please use RNase-free consumables and keep the experimental area clean.
4.Fully mix this kit before use, and avoid repeated freezing and thawing.
5.For research use only.

Ptotocol

Method 1: For high-throughput single-cell experiments, protocols shall be adjusted according to different single-cell instruments. Taking the 10×Genomics single-cell platform as an example:

1.Reaction System Preparation

1.1 Reaction Mixture Preparation:

Thaw 4×RT Buffer, Template Switch Oligo (prepared by user), Reducing Agent B (prepared by user) and RNase Inhibitor (40 U/μL, prepared by user) in advance. Invert to mix well, centrifuge, and place on ice for later use.

Table 1 Reaction System Composition

Component	Volume (μL)
4× RT Buffer	18.8
Template Switch Oligo	3.1
Reducing Agent B	2
RNase Inhibitor (40 U/μL)	1.6
Reverse Transcriptase	4.5
Total	30

1.2 Mix each component in a low-binding centrifuge tube according to Table 1 above. Gently pipette up and down to mix, perform brief centrifugation, place on ice, and use for subsequent experiments.

1.3 Please refer to the corresponding instrument manual for subsequent procedures.

Method 2: For reverse transcription reactions, please follow the steps below:

1. RNA Pre-denaturation

1.1 Thaw Oligo$(dT)_{18}$ (20~50 μM, prepared by user) at room temperature or on ice. Invert or flick gently to mix, perform brief centrifugation, and place on ice for later use. Prepare the reaction mixture according to Table 2.

Table 2 RNA Pre-denaturation Reaction System Composition

Component	Volume (μL)
Oligo(dT)18 (20~50 μM) (prepared by user)	1
Lysed cells or RNA	10
Total	11

1.2 Gently pipette to mix well, perform brief centrifugation to collect the reaction mixture at the bottom of the tube. Perform the following reaction on a PCR instrument (heated lid at 80℃):70℃ for 5 min; immediately place on ice for 3 min.

2. 2.First-strand cDNA Synthesis

2.1 Remove first-strand synthesis reagents from -20℃ storage. Thaw at room temperature or on ice, invert to mix well, perform brief centrifugation to collect liquid on tube walls, and place on ice for later use. Prepare the reaction mixture for first-strand cDNA synthesis according to Table 3:

Table 3 First-strand cDNA Synthesis Reaction System Composition

Component	Volume (μL)
Previous step	11
4× RT Buffer	5
TSO Primer (Prepared by user; concentration to be optimized by user.)	1
RNase Inhibitor (40 U/μL)	0.5
Reverse Transcriptase	1
ddH2O	1.5
Total	20

2.2 Gently pipette to mix well, and perform brief centrifugation to collect the reaction mixture at the bottom of the tube. Immediately place into a PCR instrument for the following reaction (heated lid at 105℃):42℃ for 60 min; 75℃ for 15 min; 4℃ hold.

2.3 The product can be directly used for second-strand cDNA synthesis, or stored temporarily at −20℃. For long-term storage, please store at −80℃.