*Trans*NGS® Single Cell Full Length cDNA Synthesis&Amplification Kit

Please read the datasheet carefully prior to use.

Version No. Version 1.0

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Cat. No. KC901

Storage: Single cell TSO should be stored at -70℃ for one year. The other components should be stored at -20℃ for one year.

Description

*Trans*NGS® Single Cell Full Length cDNA Synthesis&Amplification Kit is a reagent kit capable of obtaining amplified full-length cDNA from single cell. It is suitable for amplifying and constructing cDNA libraries from 1-500 cells or 10 pg-10 ng total RNA. The kit uses Single Cell Oligo (dT) as the reverse transcription primer and employs a reverse transcriptase with high synthesis efficiency and template-switching activity to add a special sequence to the 3' end of the cDNA, thereby obtaining full-length cDNA products. The kit is compatible with various cell types and tissue types, suitable for cell materials with varying RNA content. Typically, a single-cell library can yield 10-60 ng of amplified cDNA products.

Feature

- Strong compatibility with different cell types, suitable for cells with low RNA content (such as immune cells).
- Strong compatibility with different tissue types, suitable for tissues that are difficult to dissociate (such as brain tissue).
- High yield in constructing single-cell libraries, excellent peak shapes, high efficiency in gene detection (FPKM > 1).

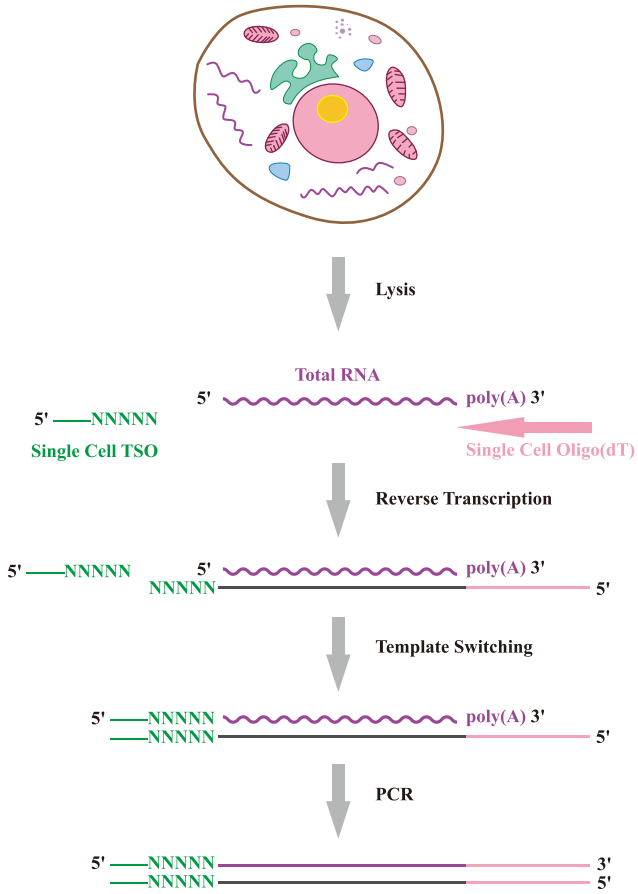
Suitable sample types

- 1-500 mammalian cells or eukaryotic cells without cell wall structures (such as protoplasts).
- 10 pg-10 ng total RNA (including mRNA with poly(A) sequences).

Kit content

Component	KC901-01 (12 rxns)	KC901-02 (24 rxns)	KC901-03 (96 rxns)
Single Cell Lysis Buffer	12 μl	24 μl	96 μl
Single Cell Oligo(dT)	12 μl	24 μl	96 μl
10 mM dNTPs	12 μl	24 μl	96 μl
Ribonuclease Inhibitors	5 μl	10 μl	40 μl
Single Cell RT Buffer	24 μl	48 μl	192 μl
DTT	12 μl	24 μl	96 μl
Single Cell TSO	6 μl	12 μl	48 μl
Single Cell RT Enhancer	6 μl	12 μl	48 μl
Single Cell Reverse Transcriptase	12 μl	24 μl	96 μl
2×Single Cell Amplification SuperMix	300 μl	600 μl	2.4 ml
Single Cell PCR Primer	12 μl	24 μl	96 μl
RNase-free Water	1 ml	2 ml	5 ml
Library Elution Buffer	300 μl	600 μl	2.4 ml

Experimental principle schematic diagram



Recommended self-prepared reagents

- *TransDetect*® Cell LIVE/DEAD Viability/Cytotoxicity Detection Kit (Catalog No: FC301).
- *MagicPure*® Size Selection DNA Beads (Catalog No: EC401).
- *Trans*NGS® Tn5 DNA Library Prep Kit for Illumina® (for 1 ng DNA) (Catalog No: KP111).
- *Trans*NGS® Tn5 Index Kit for Illumina® (Catalog No: KI101).
- Pre-chilled 1×PBS solution, freshly prepared 80% ethanol, sterile ultrapure water, etc.

Initial sample preparation

• **Cell sample preparation:** After collecting cells, it is recommended to resuspend them in 1×PBS to remove substances from the culture medium, to avoid interference with subsequent reactions. Cell samples should be identified for viability using methods such as trypan blue. For cells intended for sorting, label them with fluorescence-activated dyes (recommended reagents such as FC301 or self-prepared calcium yellow-green dye). After labeling, wash the cells with PBS and resuspend them to avoid affecting sorting quality. RNA degradation due to low cell viability or impure cell environments can result in reduced cDNA yield, smaller peak sizes, and significantly impact library construction.

• **RNA sample preparation:** For total RNA samples extracted, evaluate RNA integrity using techniques such as agarose gel electrophoresis or Agilent RNA 6000 Pico Kit before proceeding. RNA degradation can lead to decreased cDNA yield, smaller peak sizes, and a severe impact on library construction.

Operational steps:

1. Cell collection and Lysis/RNA sample pretreatment (perform under aseptic conditions)

(1) Prepare the reverse transcription pretreatment system for cell/RNA samples.

Reaction system for cell sample (4 μl in total).

Component	Volume
Single Cell Lysis Buffer	1 μl
Ribonuclease Inhibitors	0.2 μl
Single Cell Oligo(dT)	1 μl
10 mM dNTPs	1 μl
RNase-free Water	0.8 μl

Reaction system for RNA sample (4 μl in total).

Component	Volume
Total RNA (10 ng/ μl-10 pg/ μl)	1 μl
Single Cell Oligo(dT)	1 μl
10 mM dNTPs	1 μl
RNase-free Water	1 μl

(2) For cell samples, sort the cells into wells filled with the lysis system. After sorting, centrifuge as soon as possible*. The prepared cell samples can be subjected to the reaction in step (3) directly on ice, or stored at -70°C or lower temperatures**, utilizing dry ice for transportation . For RNA samples, step (2) can be skipped and directly proceed to step (3).

* Centrifuging immediately after cell sorting ensures the smooth entry of the individually sorted single cells into the lysis solution. Since both the lysis solution and the liquid volume of the single cell itself are minimal, immediate centrifugation prevents single cells, especially those positioned off-center, from drying on the walls of the well, thereby improving the success rate of library construction.

** Cell samples that are not used immediately for library construction can be stored in the lysis solution and kept at low temperatures (<-70°C). It is recommended to store them for no more than one month.

(3) Gently mix the samples, incubate in a PCR machine at 72°C for 3 minutes, and immediately place on ice for 2 minutes.

2. cDNA first-strand synthesis (perform in a clean bench)

(1) During ice bath, prepare the first-strand cDNA synthesis mix (total volume 6 µl) and place it on ice.

Component	Volume
Single Cell RT Buffer*	2 µl
DTT	1 µl
Single Cell TSO	0.5 µl
Single Cell RT Enhancer	0.5 µl
Single Cell Reverse Transcriptase	1 µl
RNase-free Water	1 µl

*Single cell RT buffer should be completely thawed **at room temperature** and **thoroughly vortexed** before use.

(2) Add 6 µl of the first-strand cDNA synthesis mix to the samples already on ice, gently mix with a pipette, and promptly centrifuge briefly, reaching a total volume of 10 µl.

(3) Run the following program in a PCR machine (with a heated lid set at 85°C).

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

3. Full-length cDNA amplification (perform in a clean bench)

(1) Add the following components to the samples that have completed the reaction on ice, with a total volume of 50 µl.

Component	Volume
2×Single Cell Amplification SuperMix	25 µl
Single Cell PCR Primer	1 µl
RNase-free Water	14 µl

(2) Vortex the sample thoroughly and centrifuge briefly. Run the following program in a PCR machine (with a heated lid set at 105°C).

Temperature	Time	Cycle
98°C	3 min	1
98°C 67°C 72°C	15 sec 20 sec 3 min	X*
72°C	5 min	1
4°C	Hold	1

*The recommended amplification number of cycles are as follows, and they can be adjusted accordingly based on the specific cell type.

Cell count	Total RNA	Cycles
1 cell	10 pg	17-18
10 cells	100 pg	13-14
100 cells	1 ng	10-11
500 cells	10 ng	7-8

4. Purification of full-Length cDNA products

It is recommended to use 0.6× *MagicPure*® Size Selection DNA Beads (Catalog No: EC401) for the purification of full-length cDNA products. The specific steps are as follows:

(1) Remove the magnetic beads from 2-8°C and let them stand at room temperature for 30 minutes before use.

(2) Vortex the beads thoroughly, pipette 30μl of beads (0.6×) to 50 μl of PCR products.

(3) Pipette up and down to mix thoroughly, let it stand at room temperature for 5 minutes.
Note: Insufficient mixing will significantly affect experimental results.

(4) Place the PCR tube on the magnetic stand and let it stand at room temperature until the solution is clear (approximately 5 minutes). This ensures that the beads are fully attached to the tube wall near the magnet. Discard the supernatant.
Note: If there is liquid on the tube wall, spin it down and place it back on the magnetic stand to ensure all beads are attached to the tube wall. Avoid pipette up the beads, as it may affect the final yield.

(5) Keep the PCR tube on the magnetic stand, add 200 μl of freshly prepared 80% ethanol to the tube without pipetting up the beads, let it stand at room temperature for 30 seconds, and discard the supernatant.
Note: Always use freshly prepared ethanol, as using old ethanol can affect experimental results.

(6) Repeat step (5) once.

(7) Keep the PCR tube on the magnetic stand and let the beads air dry at room temperature.
Note: Do not heat dry the beads, as it may affect the final yield.

(8) Remove the PCR tube from the magnetic stand, add 22 μl of Library Elution Buffer. Pipette up and down or vortex to mix thoroughly, let it stand at room temperature for 3 minutes.

(9) Place the PCR tube on the magnetic stand and let it stand at room temperature until the solution is clear (approximately 2 minutes), ensuring that the beads are fully attached to the tube wall near the magnet.
Note: If there is liquid on the tube wall, spin it down and place it back on the magnetic stand; let it stand at room temperature for up to 5 minutes.

(10) Carefully pipette up 20 μl of elution buffer and transfer it to a clean EP tube for yield and peak identification.

(11) It is recommended to use 1 ng of purified cDNA for Tn5 sequencing library preparation, and the remaining samples can be stored long-term at -20°C. It is recommended to use *Trans*NGS® Tn5 DNA Library Prep Kit for Illumina® (for 1ng DNA) (Catalog No: KP111) and *Trans*NGS® Tn5 Index Kit for Illumina® (Catalog No: KI101).

Appendix

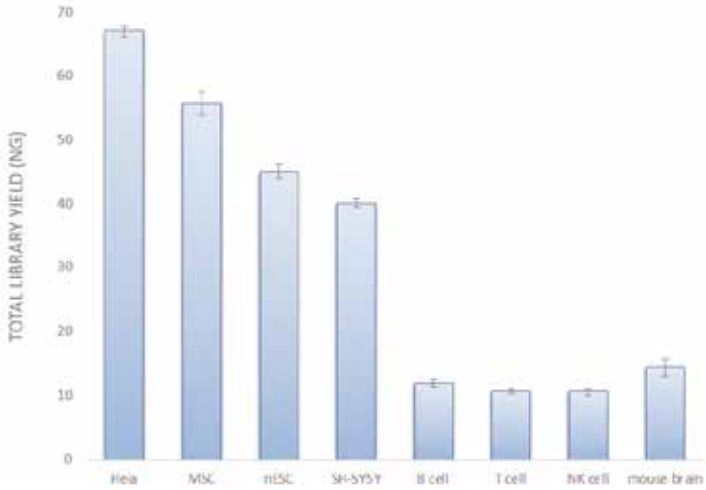


Figure 1: Full-length cDNA library across various cell types.

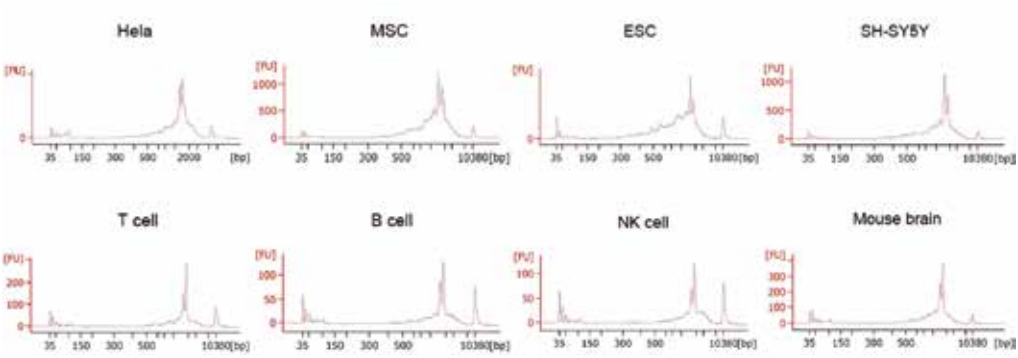


Figure 2: Peak shape of full-length cDNA across different cell types.

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