*Trans*NGS® Whole Transcriptome Amplification Kit

Please read the manual carefully before use.
Version No. Version 2.0

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Cat. No. KC921

Storage: WTA TSO should be stored at -70°C or below for one year. The other components should be stored at -18°C or below for one year.

Description

*Trans*NGS® Whole Transcriptome Amplification Kit is capable of obtaining amplified full-length cDNA from single cell. It is suitable for amplification and library construction of full transcriptome from 1-10^5 cells or 10 pg-100 ng total RNA. The kit uses WTA 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 full transcriptome amplification products.

Features

- 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).
- The compatible sample volume is up to 6 μl, which can be adapted to different concentrations of RNA or different numbers of cells.

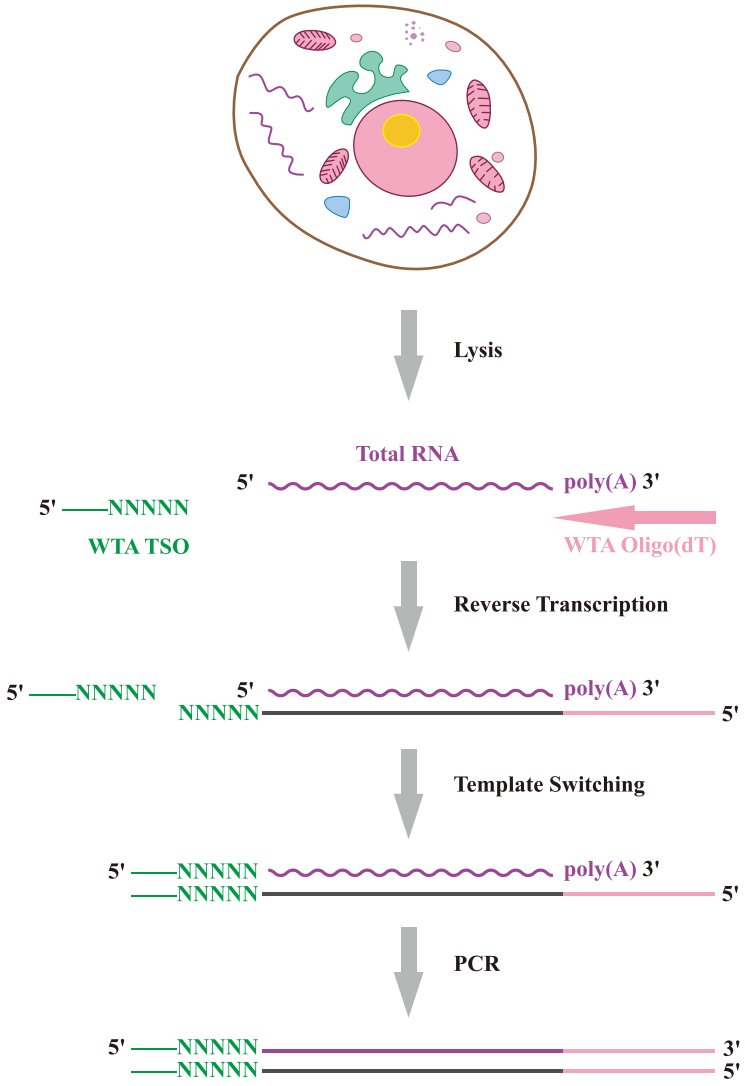
Suitable sample types

- 1-10^5 mammalian cells or eukaryotic cells without cell wall structures (such as protoplasts).
- 10 pg-100 ng total RNA (including mRNA with poly(A) sequences).

Kit contents

Component	KC921-01 (12 rxns)	KC921-02 (96 rxns)
WTA Lysis Buffer II	12 μl	96 μl
WTA Oligo(dT)	24 μl	192 μl
10 mM dNTPs	24 μl	192 μl
Ribonuclease Inhibitors	12 μl	96 μl
WTA RT Buffer	48 μl	384 μl
DTT	24 μl	192 μl
WTA TSO	12 μl	96 μl
WTA Reverse Transcriptase II	24 μl	192 μl
2×WTA Amplification SuperMix II	600 μl	4.8 ml
WTA PCR Primer	24 μl	192 μl
RNase-free Water	1 ml	2×4 ml

Experimental principle schematic diagram



Recommended self-prepared reagents

- *TransDetect*® Cell LIVE/DEAD Viability/Cytotoxicity Detection Kit (Cat. No. FC301).
- *MagicPure*® Size Selection DNA Beads (Cat. No. EC401).
- *Trans*NGS® Tn5 DNA Library Prep Kit for Illumina® (Cat. No. KP101/KP111/KP111).
- *Trans*NGS® Tn5 Index Kit for Illumina® (Cat. No. KI101).
- Pre-cooled 1×PBS, freshly prepared 80% ethanol, sterile ultrapure water, etc.

Sample Preparation

- **For Cell Sample**

After cell collection, it is recommended to resuspend the cells in 1× PBS to remove the culture medium, as it may interfere with subsequent reactions. Cell samples should be assessed for viability using methods such as trypan blue staining.

For cells to be sorted, please label them with a viability dye for flow cytometry (such as FC301 from the recommended reagents or

self-prepared calcein dye, etc.). After dye labeling, wash and resuspend the cells in PBS to avoid affecting sorting quality. Sort cells into reaction wells pre-loaded with lysis buffer using methods such as fluorescence-activated cell sorting (FACS). After sorting, centrifuge the plates as soon as possible to ensure the sorted single cells are fully immersed in the lysis buffer. Since the volumes of both the lysis buffer and the liquid carried by the single cells are very small, immediate centrifugation can prevent cells sorted near the edge of the well from drying on the wall, thereby improving the success rate of library preparation. The volume of the buffer carried by the sorted single cells is negligible. The sorted cell samples can be stored at low temperatures (<-70°C) and shipped on dry ice. It is recommended to store them for no more than half a month.

Note: Low cell viability or impure cellular environments leading to RNA degradation will reduce cDNA yield, result in smaller peak sizes, and severely impact library preparation efficiency.

• **For RNA Sample**

Please assess RNA integrity using agarose gel electrophoresis or the Agilent RNA 6000 Pico Kit before proceeding. Degraded RNA will result in reduced cDNA yield, smaller peak sizes, and significantly impact library construction efficiency.

Operational steps:

1.Cell lysis or RNA pretreatment (perform in a clean bench)

(1) For **cell samples**, prepare the following Preserving Buffer (**6 µl**) on ice:

Component	Volume
WTA Lysis Buffer II	1 µl
WTA Oligo(dT)	2 µl
10 mM dNTPs	2 µl
Ribonuclease Inhibitors	1 µl
Total volume	**6 µl**

Add the above reaction system to the cell sample, making a total volume of **11 µl**:

Component	Volume
Preserving Buffer	6 µl
Cell Sample	X µl
RNase-free Water	up to 11 µl
Total volume	**11 µl**

Gently mix the samples, incubate at 72°C for 3 minutes in a thermal cycler, after then immediately place the PCR tube on ice for 2 minutes. Then perform cDNA first-strand synthesis (step 2 in this protocol).

(2) For **RNA samples**, prepare the following Preserving Buffer (**5 µl**) on ice:

Component	Volume
WTA Oligo(dT)	2 µl
10 mM dNTPs	2 µl
Ribonuclease Inhibitors	1 µl
Total volume	**5 µl**

Add the above reaction system to the RNA sample, making a total volume of **11 µl**:

Component	Volume
Preserving Buffer	5 µl
RNA Sample	X µl
RNase-free Water	up to 11 µl
Total volume	**11 µl**

Gently mix the samples, incubate at 72°C for 3 minutes in a thermal cycler, after then immediately place the PCR tube on ice for 2 minutes. Then perform cDNA first-strand synthesis (step 2 in this protocol).

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

(1) During ice incubation, prepare the first-strand cDNA synthesis mix (total volume **9 µl**) and place it on ice.

Component	Volume
WTA RT Buffer*	4 µl
DTT	2 µl
WTA TSO	1 µl
WTA Reverse Transcriptase II	2 µl
Total volume	**9 µl**

*WTA RT Buffer should be completely thawed **at room temperature** and **thoroughly vortexed** before use.

(2) Add 9 µl of the first-strand cDNA synthesis mix to the step 1 samples that have completed the 2-minute ice incubation (operate on ice), gently mix with a pipette, and centrifuge briefly, reaching a total volume of **20 µl**.

(3) Run the following program in a thermal cycler (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) Set up the reactions listed below. Choose the amplification volume according to the sample capacity* of the thermal cycler. Below are the two reaction systems with different amplification volumes.

Reaction system with a total amplification volume of 100 µl:

Component	Volume
The Product of the Previous Step	20 µl
2×WTA Amplification SuperMix II	50 µl
WTA PCR Primer	2 µl
RNase-free Water	28 µl
Total volume	**100 µl**

Reaction system with a total amplification volume of 50 µl:

Component	Volume
The Product of the Previous Step	20 µl
2×WTA Amplification SuperMix II	25 µl
WTA PCR Primer	1 µl
RNase-free Water	4 µl
Total volume	**50 µl**

* Check the sample capacity of the thermal cycler. If using the 100 µl amplification system, 100 µl liquid level of sample in tube should not extend past the surface of the block, otherwise the library yield will be affected due to insufficient heating of the liquid in the tube. If the metal hole of the thermal cycler is shallow, it is recommended to use a 50 µl amplification system.

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

Temperature	Time	Cycle
98°C	3 min	1
98°C 67°C 72°C	15 s 20 s 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
10 cells	100 pg	13-14
100 cells	1 ng	10-11
1000 cells	10 ng	7-8
10000 cells	100 ng	6-7

4. Purification of full-Length cDNA products

It is recommended to use 0.6× *MagicPure*® Size Selection DNA Beads (Cat. 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 stay still for 30 minutes at room temperature before use.

(2) Vortex the beads thoroughly, pipet **60 µl** of beads (**0.6**×) to 100 µl of PCR products, or pipet **30 µl** of beads (**0.6**×) to 50 µl of PCR products.

(3) Pipet to mix well, stay still for 5 minutes at room temperature.
Note: Insufficient mixing will significantly affect experimental results.

(4) Place the PCR tube on the magnetic stand and stay still at room temperature until the solution is clear (about 5 minutes). Make sure the beads settle to the magnet completely. Discard the supernatant.
Note: Spin down briefly before put on magnetic stand if there is liquid on the wall. Make sure the beads settle to the magnet completely. Be careful not to disturb the beads, otherwise will affect the final yield

(5) Keep the PCR tube on the magnetic stand, add 200 µl of freshly prepared 80% ethanol to the tube, do not pipet the beads, stay still for 30 seconds at room temperature, and discard the supernatant.
Note: Be sure to use freshly prepared ethanol, otherwise it will affect the experimental results.

(6) Repeat step (5) once.

(7) Air dry the beads at room temperature while the tube is on the magnetic stand.
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 RNase-free Water. Mix by pipetting or vortexing, stay still for 3 minutes at room temperature.

(9) Place the PCR tube on the magnetic stand and stay still at room temperature until the solution is clear (about 2 minutes). Make sure the beads settle to the magnet completely.
Note: Spin down briefly before put on magnetic stand if there is liquid on the wall. Incubation time can be extended to 5 minutes at room temperature.

(10) Carefully pipet 20 µl of cDNA and transfer it to a clean centrifuge tube for yield and peak identification. The remaining cDNA product can be stored at -20°C.

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