*Trans*NGS® CUT&Tag Library Prep Kit for Illumina®

Please read the datasheet carefully prior to use.

Version No. Version 2.0

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Cat. No. KP172

Storage: Box 1 is stored at 2℃-8℃ for 1 year and Box 2 is stored at -18℃ or below for 1 year.

Description

The kit is designed to prepare CUT&Tag libraries for mammalian cells for the Illumina high-throughput sequencing platform. The prepared libraries can be used for Single-end sequencing and Paired-end sequencing. Cleavage Under Target & Tagmentation (CUT&Tag) is a new method for studying protein interactions with DNA. Protein A/G-Tn5 transposase fusion protein and the target protein can form an immune complex through antibody bridging. Tn5 transposase is activated in the presence of Mg^{2+}, and so short sequences containing adapters can be inserted into both ends of the DNA fragment that binds to the target protein. Finally, library can be constructed by PCR amplification. The kit is suitable for DNA research on the interaction of DNA-binding proteins such as transcription factors and co-regulators. Compared with traditional ChIP-seq, it has the advantages of more accurate and stable protein-DNA interaction information in low input of cells.

Applications

10-10^5 mammalian cells, or processed cell nucleus, and eukaryotic cells without cell wall (such as plant cell protoplasts, etc.).

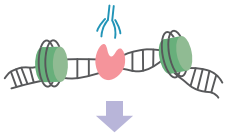
Reagents not included in the kit

- Primary antibody (ChIP Grade).
- Secondary antibody: Goat Anti-Rabbit lgG H&L (Cat. No. HS001) and Goat Anti-Mouse lgG H&L (Cat. No. HS002).
- *Trans*NGS® Tn5 Index Kit for Illumina® (Cat. No. KI101).
- *Trans*NGS® Spike-in DNA Controls for CUT&Tag (Cat. No. KS201).
- Pre-cooled 1×PBS solution, absolute ethanol, sterilized ultrapure water, etc.
- Low adsorption centrifuge tubes, including 1.5 ml centrifuge tubes and PCR tubes.
- If cell nucleus library preparation of animal or plant tissues is required, it is recommended to use cell nucleus isolation kit from SHBIO (human or animal samples, 52009-10; plant samples, 52305-10).

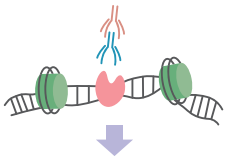
Principle Chart

Living cells collection and magnetic bead binding (30 min)

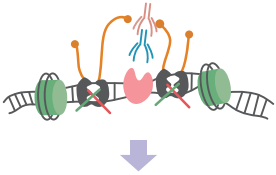
Primary antibody binding (1 h)



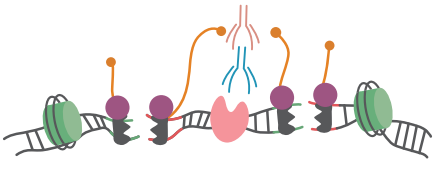
Secondary antibody binding (1 h)



Transposome binding (1 h)



DNA fragmentation and termination (70 min)



DNA extraction (20 min)

Amplification (30 min)

Kit Contents

	Component	KP172-01 (12 rxns)	KP172-02 (48 rxns)
Box 1	ConA Magnetic Beads II	60 µl	240 µl
	CUT&Tag DNA Clean Beads	2 ml	4 ml × 2
Box 2	ConA Beads Binding Buffer	660 µl × 2	2.7 ml × 2
	Cell Wash Buffer	10 ml × 3	40 ml × 3
	Nuclear Extraction Buffer	6 ml	6 ml × 4
	1×Pro-Wash Buffer	10 ml	40 ml
	pAG-Tn5	12 µl	48 µl
	Tagmentation Buffer	600 µl	2.4 ml
	Protease Inhibitor	420 µl	840 µl × 2
	Ab Enhancer	20 µl	80 µl
	Binding Enhancer	80 µl	320 µl
	500 mM EDTA	30 µl	120 µl
	Lysis Enhancer	20 µl	80 µl
	Proteinase K（20 mg/ml）	20 µl	80 µl
	2×CUT&Tag Library Amplification Mix	300 µl	600 µl × 2
	Nuclease-free Water	1 ml	4 ml

Starting Sample Preparation

It is recommended to use 10^4-10^5 cells for the first experiment. After the experiment is successful, the amount of cells can be adjusted according to the instructions. Ensure that the proportion of viable cells is greater than 90%, and dead cells can be identified by trypan blue staining. Adherent cells and tissue blocks should be digested into single cells as much as possible and resuspended in PBS. It is also possible to extract cell nucleus for cells that are difficult to digest into a single-cell state before constructing library. For details, see the instruction for routine cell nucleus extraction in the appendix. If the species type is special, the number of cells is not within the above range, or the expression abundance of the target protein in the sample is not high, etc., the experiment conditions need to be appropriately adjusted. Impurities such as trypsin and EDTA should be removed as much as possible from the cell suspension to avoid interfering with subsequent library construction.

Library Structure

5'-AATGATACGGCGACCACCGAGATCTACAC[i5]TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-XXXXXXXX-CTGTCTCTTATACACATCTCCGAGCCCACGAGAC[i7]ATCTCGTATGCCGTCTTCTGCTTG-3'

Protocol

1. Cell collection and magnetic bead binding

(1) Pipet 5 µl of ConA Magnetic Beads II * equilibrated at room temperature into a centrifuge tube. Place the tube on a magnetic stand. After the liquid is clear, discard the supernatant.

*Take out ConA Magnetic Beads II from the 4°C freezer in advance, equilibrate it at room temperature for 30 minutes, and pipet to mix before use. If there are multiple samples, this step can be performed in 8-tube strip.

(2) Add 50 µl of ConA Beads Binding Buffer. Flick to mix. Collect the beads and discard the supernatant on the magnetic stand. Repeat this step once.

(3) Add 10 µl of ConA Beads Binding Buffer to resuspend ConA Magnetic Beads II.

(4) Prepare the cell wash solution in the table below, 1.2 ml per sample.

Component	Volume
Cell Wash Buffer	1.2 ml
Protease Inhibitor	12 µl
Total volume	~1.2 ml

(5) Collect the required number of fresh cells in a 1.5 ml centrifuge tube. If the cell volume exceeds 100 µl, centrifuge at 600 g for 3 min at room temperature and carefully discard the supernatant; If the cell volume is less than 100 µl, proceed directly to step (6).

(6) Add 1 ml of the cell wash solution prepared in step (4) to each tube, gently pipet to resuspend the cells*, centrifuge at 600 g at room temperature for 3 min, and carefully discard the supernatant.

(7) Add 90 µl of the cell wash solution prepared in step (4) to each tube and gently pipet to resuspend the cells*.

(8) Transfer each tube of resuspended cells to the 10 µl ConA Magnetic Beads II prepared in step (3), mix 10 times with gentle pipetting*, place immediately on a rotary mixer, and incubate for 10 minutes at room temperature.
Note: Do not shake to mix. It is recommended to gently flick the tube to mix.

2. Incubate primary antibody, secondary antibody, pA/G-Tn5 and perform interruption reaction

Incubate primary antibody:

(1) Prepare the **primary antibody incubation solution**, **50 µl** for each sample. Vortex to mix and **pre-cool** in ice bath for later use. If there are multiple samples, prepare (N+1) × 50 µl.

Component	Volume
Cell Wash Buffer	47.8 µl
Protease Inhibitor	0.5 µl
Ab Enhancer	1 µl
Binding Enhancer	0.5 µl
500 mM EDTA	0.2 µl
Total Volume	50 µl

(2) Gently spin the incubated ConA Magnetic Beads-cell conjugate and place it on a magnetic stand for 2 minutes. Carefully discard the supernatant after the liquid is clear. Dilute the primary antibody* with 50 µl primary antibody incubation solution (the dilution ratio of the primary antibody should strictly follow the recommended ratio in the antibody manual, generally the dilution ratio is 1:50 or 1:100), resuspend the ConA Magnetic Beads-cell conjugate, gently flick the tube to mix, and **incubate at room temperature with rotation for 1 hour****.

*Antibodies should be verified and suitable for ChIP or CUT&Tag experiments. If there is no corresponding level of antibodies, IP level antibodies can be used, but the optimal dosage of the antibody needs to be further explored. **Do not freeze and thaw the primary antibody repeatedly**. Repeated freezing and thawing more than 10 times will seriously affect the results of library construction.

**This step can also be incubated on a rotating device at 4°C overnight.

Incubate secondary antibody:

(3) Prepare the secondary antibody incubation solution, 500 µl for each sample. Prepare this solution freshly before use.

Component	Volume
Cell Wash Buffer	490 µl
Protease Inhibitor	5 µl
Binding Enhancer	5 µl
Total Volume	500 µl

(4) For each sample, dilute the secondary antibody* with 100 µl of secondary antibody incubation solution. The final concentration of the secondary antibody is 10 µg/ml.

*Secondary antibodies should be unmodified, unlabeled, and have high affinity for Protein A/G.It is recommended to use Goat Anti-Rabbit lgG H&L (Cat. No. HS001) or Goat Anti-Mouse lgG H&L (Cat. No. HS002)

(5) After incubating the primary antibody, quick spin gently, place the tube on the magnetic stand for 2 minutes. Carefully discard the supernatant after the liquid is clear. Add 100 µl of the diluted secondary antibody solution to each tube to resuspend the ConA Magnetic Beads-cell conjugate, gently flick the tube to mix, and incubate at room temperature with rotation for 1 hour.

(6) After incubating the secondary antibody, quick spin gently, place the tube on the magnetic stand for 2 minutes. Carefully discard the supernatant after the liquid is clear. Add 150 µl of the secondary incubation solution, gently flick the tube to mix, and stay still at room temperature for 5 minutes. Quick spin gently. Place the tube on the magnetic stand for 2 minutes. Carefully discard the supernatant after the liquid is clear.

(7) Wash again: Add 150 µl of secondary antibody incubation solution, gently flick the tube to mix, and stay still at room temperature for 5 minutes.

Transposome binding:

(8) Prepare the following transposition buffer in advance, 500 µl for each sample.

Component	Volume
1×Pro-Wash Buffer	494 µl
Protease Inhibitor	5 µl
Binding Enhancer	1 µl
Total volume	500 µl

(9) Quick spin gently. Place the tubes on a magnetic stand to clear (about 2 minutes) and carefully remove the supernatant with a pipette. Add 99 µl transposition buffer and 1 µl pAG-Tn5 per tube. Carefully resuspend the ConA Magnetic Beads-cell cconjugate, gently flick the tube wall to mix, and incubate at room temperature with rotation for 1 hour.

(10) After a quick spin gently, place the tubes on a magnetic stand to clear (2 minutes) and remove the supernatant with a pipette. Add 150 µl transposition buffer, gently flick the tube wall to mix, and stay still for 5 minutes at room temperature. After a quick spin gently, place the tubes on a magnetic stand to clear (2 minutes) and carefully remove the supernatant.

(11) Wash again: Add 150 µl of transposition buffer, gently flick the tube to mix, and stay still at room temperature for 5 minutes.

DNA transposition and termination:

(12) After a quick spin gently, place the centrifuge tube on the magnetic stand for 2 minutes. Carefully discard the supernatant after the solution is clear. Add 50 µl Fragmentation Buffer to resuspend ConA Magnetic Beads-cell conjugate per tube and gently flick the tube to mix. Incubate at 37°C for 1 hour with shaking.

*This step can be performed on thermal cycler. Before place it on the machine, gently flick the tube to mix and quick spin gently.

(13) After a quick spin gently, mix the magnetic beads with a pipette. Add 1.5 µl 500 mM EDTA and 0.5 µl Lysis Enhancer per tube, and gently vortex to mix*. Add 0.5 µl Proteinase K, vortex to mix, and incubate at 70°C for 10 minutes to terminate the transposition reaction. Perform DNA extraction immediately after the reaction is completed.

*If quantitative analysis is required, add Spike-in DNA (Cat. No. KS201) after adding EDTA and Lysis Enhancer and mix well. The recommended amount of Spike-in DNA is 1 pg/100,000 cells. Add gradient dilution of spike-in DNA according to the actual cell numbers.

3. DNA extraction

Take out CUT&Tag DNA Clean Beads from the 4°C refrigerator in advance and equilibrate it at room temperature for 30 minutes.

(1) Add 120 µl (2.4×) CUT&Tag DNA Clean Beads to each centrifuge tube mentioned above, vortex thoroughly, and incubate for 5 minutes at room temperature.

(2) Place the centrifuge tube on a magnetic stand for 2 minutes. Carefully discard the supernatant when the solution is clear. Add 200 µl freshly prepared 80% ethanol, incubate for 30 seconds at room temperature, and carefully remove the supernatant.

(3) Keep the centrifuge tube on the magnetic stand, add another 200 µl of freshly prepared 80% ethanol, incubate for 30 seconds at room temperature, and carefully remove the supernatant. After quick spin, place it on the magnetic stand to separate the beads from the residual ethanol. Use a 10 µl pipette tip to carefully aspirate the remaining liquid without touching the beads.

(4) After air-drying the beads at room temperature for 2-3 minutes*, take out the centrifuge tube and add 22 µl Nuclease-free Water. Vortex thoroughly and incubate for 3 minutes at room temperature before placing it on the magnetic stand. Carefully pipet 20 µl of the supernatant into a new PCR tube. This is the Tag DNA**.

* Do not overdry the beads.

** Tag DNA samples can be stored at -20°C or used for the following amplification reaction, avoiding repeated freezing and thawing.

4. Library amplification and fragment selection

(1) Prepare the following amplification system on ice.

Component	Volume
Tag DNA	20 μl
N5XX Primer*	2.5 μl
N7XX Primer*	2.5 μl
2×CUT&Tag Library Amplification Mix	25 μl
Total volume	50 μl

*N5XX/N7XX Primers are components of the self-prepared reagent kit KI101.

(2) Mix by pipetting or vortexing, and quick spin.

(3) The PCR amplification procedure is as follows.

58°C	5 min	
72°C	5 min	
98°C	30 sec	
98°C	10 sec	N cycles*
60°C	10 sec	
72°C	1 min	
4°C	Hold	

*For different input cells, the number of amplification cycles is as follows:

Input Cell	PCR Cycles
100,000	9-11
50,000	10-12
10,000	12-14
1,000	15-17
500	16-18
100	17-19
50	19-21
10	23-25

(4)Take out CUT&Tag DNA Clean Beads from the 4°C refrigerator in advance and incubate for 30 minutes at room temperature before use.

(5) Add 65 μl (1.3×) CUT&Tag DNA Clean Beads to the PCR product, vortex to mix, and incubate for 5 minutes at room temperature. Place the PCR tube on the magnetic stand, carefully discard the supernatant after the liquid is clear.

(6) Keep the PCR tube on the magnetic stand, add 200 μl of freshly prepared 80% ethanol, incubate for 30 seconds at room temperature, and carefully discard the supernatant.

(7) Keep the PCR tube on the magnetic stand, add another 200 μl of freshly prepared 80% ethanol, incubate for 30 seconds at room temperature, and carefully discard the supernatant. After quick spin, place it on the magnetic stand

to separate the beads from the residual ethanol. Use a 10 µl pipette tip to carefully aspirate the remaining liquid without touching the beads.

(8) After air-drying the beads at room temperature for 2-3 minutes*, take out the PCR tube and add 22 µl Nuclease-free Water. Vortex to mix and incubate for 3 minutes at room temperature before placing the tube on the magnetic stand. Carefully pipet 20 µl of the supernatant into a new centrifuge tube. This is the Library DNA**.

* Do not overdry the beads.

** Library DNA samples can be stored at -20°C or sent directly for sequencing, avoiding repeated freezing and thawing.

Appendix

1. Cell Nucleus Extraction Steps:

1.1 Collect the required amount of fresh cells in a 1.5 ml centrifuge tube, centrifuge at 600 g for 3 minutes at room temperature, and carefully discard the supernatant.

1.2 Add 500 µl of PBS to resuspend the cells, centrifuge at 600 g for 3 minutes at room temperature, and carefully discard the supernatant.

1.3 Add 500 µl of pre-cooled Nuclear Extraction Buffer, mix gently, and keep on ice for 10 minutes.

1.4 Centrifuge at 1300 g for 4 minutes at 4 °C, discard the supernatant and invert the tube on a paper towel for a few seconds.

1.5 Add 500 µl of PBS to resuspend the cell nucleus, centrifuge at 1300 g for 4 minutes at 4 °C, discard the supernatant, and invert the tube on a paper towel for a few seconds.

1.6 Add 90 µl of prepared cell wash solution to resuspend the cell nucleus, and proceed to step (8) of "Cell collection and magnetic bead binding".

2. Library Peak Shape:

Using the H3K4me3 antibody with 293T cells as an example, the peak shape of the CUT&Tag library after 13 cycles of amplification is shown below.

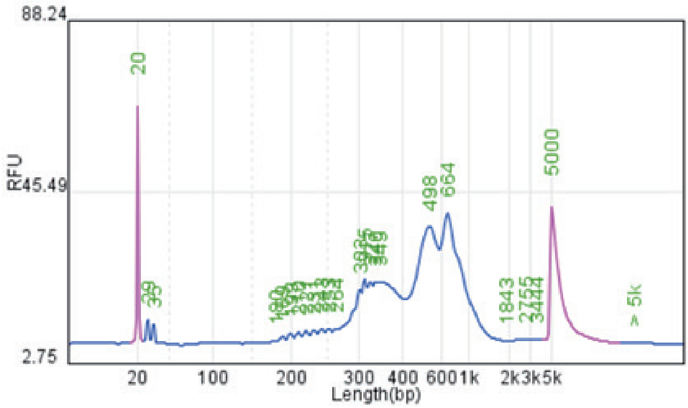


Figure 1: Library peak shape generated from 10,000 293T cells with Qsep

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