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

Please read the datasheet carefully prior to use.

Version No. Version 1.0

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Cat. No. KP172

Storage: Box 1 is stored at 4°C for 1 year and Box 2 is stored at -20°C 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

10^2-10^5 mammalian cells, or processed cell nucleus, and eukaryotic cells without cell wall (such as plant cell protoplasts, etc.).

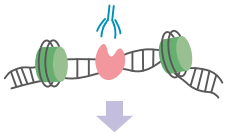
Reagents not included in the kit

- Primary antibody (ChIP Grade), secondary antibody (high affinity to Protein A/G and not modified).
- *Trans*NGS® Tn5 Index Kit for Illumina® (Cat. No. KI101).
- Pre-cooled 1×PBS solution, absolute ethanol, sterilized ultrapure water, etc.
- Low adsorption centrifuge tubes, including 1.5 ml centrifuge tubes and PCR tubes.

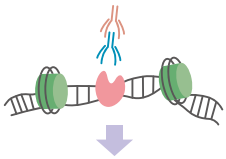
Principle Chart

Living cells collection (30 min)

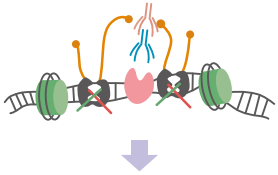
Primary antibody binding (2 h)



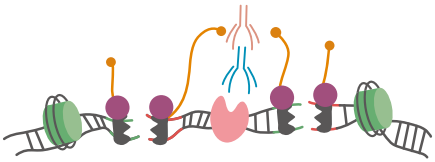
Secondary antibody binding (1 h)



Transposome binding (1 h)



DNA fragmentation (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	120 µl	480 µl
	CUT&Tag DNA Clean Beads	1.05 ml×2	4.2 ml×2
Box 2	ConA Beads Binding Buffer	800 µl×3	3.2 ml×3
	Cell Wash Buffer	10 ml×3	40 ml×3
	Nuclear Extraction Buffer	6 ml	6 ml×4
	1×Pro-Wash Buffer	7 ml×2	28 ml×2
	pAG-Tn5	12 µl	48 µl
	Ab Enhancer	20 µl	80 µl
	Tagmentation Enhancer	20 µl	80 µl
	Binding Enhancer	180 µl	720 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	1.2 ml
	Protease Inhibitor	420 µl	840 µl×2
	TE Buffer (1×)	1 ml	4 ml
	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 instructions 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 10 µl of ConA Magnetic Beads* 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 from the 4°C freezer in advance, equilibrate it at room temperature for 30 minutes, and pipet to mix before use.

(2) Add 100 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.

(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 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.

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

(1) Prepare the **secondary antibody incubation solution** in advance, **700 µl** for each sample. Vortex gently to mix. If there are multiple samples, prepare (N+1) × 700 µl.

Component	Volume
Cell Wash Buffer	686 µl
Protease Inhibitor	7 µl
Binding Enhancer	7 µl
Total volume	700 µl

(2) Prepare the **primary antibody incubation solution** based on the secondary 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
Secondary antibody incubation solution	49.3 µl
500 mM EDTA	0.2 µl
Ab Enhancer	0.5 µl
Total volume	50 µl

Incubate primary antibody:

(3) 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. Resuspend the ConA Magnetic Beads-cell conjugate with 50 μl of primary antibody incubation solution, add 0.5 μg of primary antibody*, mix by gentle pipetting, and **incubate at room temperature with rotation for 2 hours****.

*It is best to use antibodies that have been 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:

(4) For each sample, dilute the secondary antibody* with 100 μl of secondary antibody incubation solution in advance. 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.

(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, pipet gently to resuspend the ConA Magnetic Beads-cell conjugate, 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 200 μl of the secondary incubation solution, mix gently by pipetting 10 times, and stay still at room temperature for 5 minutes. Place the tube on the magnetic stand for 2 minutes. Carefully discard the supernatant after the liquid is clear.

(7) Wash again: Place the centrifuge tube on the magnetic stand for 2 minutes. Carefully discard the supernatant after the liquid is clear. Add 200 μl of secondary antibody incubation solution. Mix gently by pipetting 10 times and stay still at room temperature for 5 minutes.

Transposome binding:

(8) Prepare the following transposition buffer in advance, 700 μl for each sample.

Component	Volume
1×Pro-Wash Buffer	691.6 μl
Protease Inhibitor	7 μl
Binding Enhancer	1.4 μl
Total volume	700 μl

(9) 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 conjugate by pipetting and incubate at room temperature on an orbital rotator 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 200 μl transposition buffer, resuspend by gentle pipetting, and stay still for 5 minutes at room temperature.

(11) Wash again: place the tubes on a magnetic stand to clear and remove the supernatant with a pipette. Add 200 µl transposition buffer, resuspend by gentle pipetting, and stay still for 5 minutes at room temperature.

DNA transposition and termination:

(12) Prepare the following **Fragmentation Buffer** in advance, **50 µl** for each sample. Vortex to mix. If there are multiple samples, prepare (N+1) × 50 µl.

Component	Volume
Transposition buffer	49.5 µl
Tagmentation Enhancer	0.5 µl
Total volume	50 µl

(13) Place the centrifuge tube from step (11) on the magnetic stand for 2 minutes. Carefully discard the supernatant after the solution is clear. Add 50 µl Fragmentation Buffer to the washed ConA Magnetic Beads-cell conjugate per tube and mix by pipetting. Incubate at 37°C for 1 hour with shaking.

(14) 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.

3. DNA extraction

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

(1) Add 110µl (2.2×) 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 200µl 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 200µl 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 10µl 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 22µl 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℃ 5 min
72℃ 5 min
98℃ 45 sec
98℃ 15 sec
63℃ 10 sec
N cycles*
72℃ 1 min
4℃ Hold

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

Input Cell	PCR Cycles
100,000	12-13
10,000	14-15
1,000	17-18
100	19-20

(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 200µl 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 200µl 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 TE Buffer. 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.

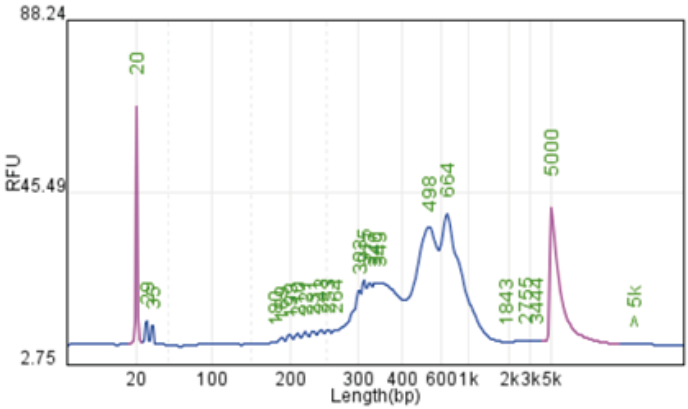


Figure 1: Library peak shape generated from 10,000 293T cells with Qsep

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