Product Description

The AcuGenix™ CUT&Tag Assay Kit for Illumina is a library preparation kit developed for Illumina high-throughput sequencing platforms to study protein-DNA interactions. CUT&Tag (Cleavage Under Targets and Tagmentation) is a novel technique for investigating protein-DNA interactions: after specific antibodies bind to chromatin proteins in situ, a Protein A-Tn5 fusion protein targets and precisely cleaves the bound regions, efficiently generating fragment libraries with high resolution and extremely low background.

Compared to traditional ChIP-Seq, the CUT&Tag method provided by this kit offers advantages such as lower cell input requirements, simpler operation, shorter turnaround time, and higher signal-to-noise ratio. It is suitable for detecting specific transcription factor binding sites or sites of specific histone modifications, and can be applied in research areas including epigenetics, early embryonic development, stem cells, and oncology.

Components

Product Name	Cat	Specs
AcuGenix™ CUT&Tag Assay Kit for Illumina	BR3E101-51	8 T
	BR3E101-54	48 T

Component No.	Composition	BR3E101-51	BR3E101-54
BOX 1	ConA Beads	80 μL	480 μL
	DNA Clean Beads	1,650 μL	9.9 mL
	Stop Buffer	40 μL	240 μL
	Proteinase K	40 μL	240 μL
	Tnp Activator	40 μL	240 μL
BOX 2	pA-Tnp	8 μL	48 μL
	5% Digitonin	80 μL	480 μL
	10×Binding Buffer	176 μL	1,056 μL
	10×Wash Buffer	1,150 μL	1,150 μL×6
	25×Antibody Buffer	20 μL	120 μL
	10×Dig-300 Buffer	800 μL	1.2 mL×4
	Tag Amplification Mix	200 μL	1.2 mL

Note: Upon receipt of the kit, store BOX 1 at 2-8℃ and BOX 2 at −20±5℃.

Transportation and Storage Conditions

BOX 1: Transported with ice packs; store at 2-8℃.

BOX 2: Transported at −30 to −15℃; store at −20±5℃.

Application

Suitable for studying protein-DNA interactions in 100 to 100,000 intact mammalian cells or nuclei. Mammalian tissues, plant tissues, and plant cells can also be used with this kit after appropriate processing.

Notes

The library construction process is influenced by factors such as sample type, instrument model, operation, and target abundance, so some parameters may need to be adjusted. If you encounter any problems during use, please contact technical support for assistance.

1. Magnetic Bead Usage

1.1 This kit provides two types of magnetic beads: ConA beads (for cell binding) and DNA purification beads (for genomic

extraction and library purification). Please distinguish between them before use.
1.2 Do not store magnetic beads at temperatures below 0℃. Freezing will cause bead aggregation and sedimentation, leading to experiment failure.
1.3 Before use, equilibrate magnetic beads to room temperature (place at room temperature for approximately 30 min). Maintain room temperature throughout the operation.
1.4 ConA beads are coated with concanavalin A on the surface. Do not vortex vigorously before use. It is recommended to mix by gentle pipetting or inverting, followed by brief centrifugation.
1.5 After ConA beads have bound to cells, do not vortex vigorously or pipette harshly to avoid cell detachment or damage.
1.6 Avoid prolonged exposure of ConA beads or their complexes with cells to air, as this may cause the beads to dry and crack.
1.7 Avoid prolonged high-speed centrifugation or prolonged placement on a magnetic stand of ConA beads or their complexes with cells, as this may cause bead aggregation.
1.8 Slight aggregation of ConA beads may occur during operation, and the degree of aggregation varies with cell type and cell number. If aggregation occurs, gently flick the tube wall and invert to disperse the clumps; this will not affect the experimental results. Avoid vigorous vortexing or harsh pipetting.

2. Sample Preparation

2.1 If using intact cells for the experiment, note that the affinity for ConA beads may vary significantly due to differences in cell surface modifications across cell types. It is recommended to assess this before the experiment to avoid using low-affinity cell lines for low-input assays.

2.2 For common suspension cell lines, cells can be directly collected by centrifugation. For most adherent cell lines, after trypsinization into a single-cell suspension, the supernatant is removed by centrifugation, and the cells can be used normally. However, trypsin treatment may affect the binding efficiency of some cell lines to ConA beads.
2.3 For cells to be used in CUT&Tag experiments, it is recommended to determine the percentage of viable cells using trypan blue staining. Starting material with >90% viable cells is recommended. All operations should be performed as gently as possible to avoid cell damage. When cells die, surface modifications of some proteins may change, and some proteins may even detach from DNA, leading to increased background signal and possible false-negative results.

2.4 If using mammalian or plant tissues for the experiment, it is recommended to first isolate nuclei from the tissue. Appropriate nuclear extraction conditions should be used for cells of different origins and types to obtain high-quality nuclei.
2.5 For target proteins with low expression levels or transcription factors that interact transiently, mild UV crosslinking or formaldehyde crosslinking can be performed before the experiment to obtain better results.

3. Antibody Selection

3.1 For the primary antibody, it is recommended to use antibodies validated for CUT&Tag or CUT&RUN. If no CUT&Tag-validated antibody is available, the recommended order of preference is: ChIP-grade antibodies, followed by IP-grade, IF-grade, and then WB-grade antibodies.

3.2 For the secondary antibody, because this kit provides a Protein A-Tn5 fusion protein, a secondary antibody that has high affinity for Protein A and is unmodified should be selected.
3.3 Since the CUT&Tag procedure is relatively complex and it is difficult to determine success or failure, it is recommended to include positive and negative controls to monitor the experimental process. For the positive control, a highly abundant histone is recommended. For the negative control, it is recommended to omit the primary antibody and proceed with the addition of the secondary antibody and the transposon.

4. Cell Input Amount and Library Amplification

4.1 To minimize the impact of large DNA fragments and excess primers, it is recommended to perform at least 12

thermal cycles.

4.2 When selecting the number of amplification cycles, the library concentration only needs to meet the requirement for sequencing; excessively high library yield should not be pursued. Too many cycles can lead to amplification bias, mutation accumulation, large fragment contamination, increased duplication rates, and chimeric products.

4.3 CUT&Tag libraries constructed with this kit have a main peak distribution between 200 and 1,500 bp. No size selection is required before sequencing; the library can be directly loaded onto the sequencer.

4.4 This kit is suitable for studying protein-DNA interactions in 100 to 100,000 intact cells or nuclei. Due to variations in cell type, antibody selection, and target protein abundance, the actual compatible cell number may vary slightly. In general, 5,000~50,000 cells is an appropriate range. For subsequent experiments, the relationship between cell input amount and the number of library amplification cycles can be adjusted according to experience and data requirements.

4.5 Using 293T cells as an example, with H3K4me3 as the primary antibody, the relationships between cell input amount, number of amplification cycles, and library yield are as follows:

Cell Input	Amplification Cycles	Library Concentration (Qubit)
100	18-20	10-40 ng/μL
1,000	15-17	
10,000	12-14	
100,000	10-12	

5. Library Quality Control

5.1 The constructed library should undergo quality control in two aspects: library concentration and fragment size distribution.

5.2 For library concentration determination, double-stranded DNA fluorescent dye-based methods (e.g., Qubit, PicoGreen) or qPCR (using a library quantification kit) may be used. The dye-based method is simple and easy to perform, but it cannot accurately quantify over-amplified libraries. The qPCR method is more time-consuming but enables absolute quantification for all library types. The qPCR method is recommended.

5.3 For fragment size distribution analysis, 2% agarose gel electrophoresis or a fragment analyzer (e.g., Agilent Technologies 2100 Bioanalyzer) may be used. 2% agarose gel is inexpensive but cannot accurately determine the main peak position of the library and can only be used as a qualitative tool. The bioanalyzer is expensive but can precisely determine the main peak position. For quantitative library analysis prior to sequencing, a bioanalyzer must be used.

6. Other Precautions

6.1 The kit consists of two parts, BOX 1 and BOX 2. Please note the storage temperature for each part.

6.2 Digitonin is toxic upon contact. Please take appropriate personal protective measures during solution preparation and avoid direct contact. After the addition of Digitonin, the buffer can be temporarily stored at 2~8℃for one day, but it is recommended to prepare fresh immediately before use. 5% Digitonin can be stored at 2~8℃ for one week.

6.3 To ensure experimental reproducibility, it is recommended to use a thermal cycler with a heated lid and preheat the instrument to the reaction temperature before starting the reaction.

6.4 To avoid cross-contamination and aerosol contamination, it is recommended to use filter tips during experimental operations. After the experiment, clean the work area with a chlorine-containing disinfectant or DNA decontamination solution.

Materials

Reagents: Absolute ethanol, sterile ultrapure water, Qubit dsDNA quantification reagent.

Instruments: Fragment analyzer, Qubit fluorometer, thermal cycler, rotator mixer, etc.

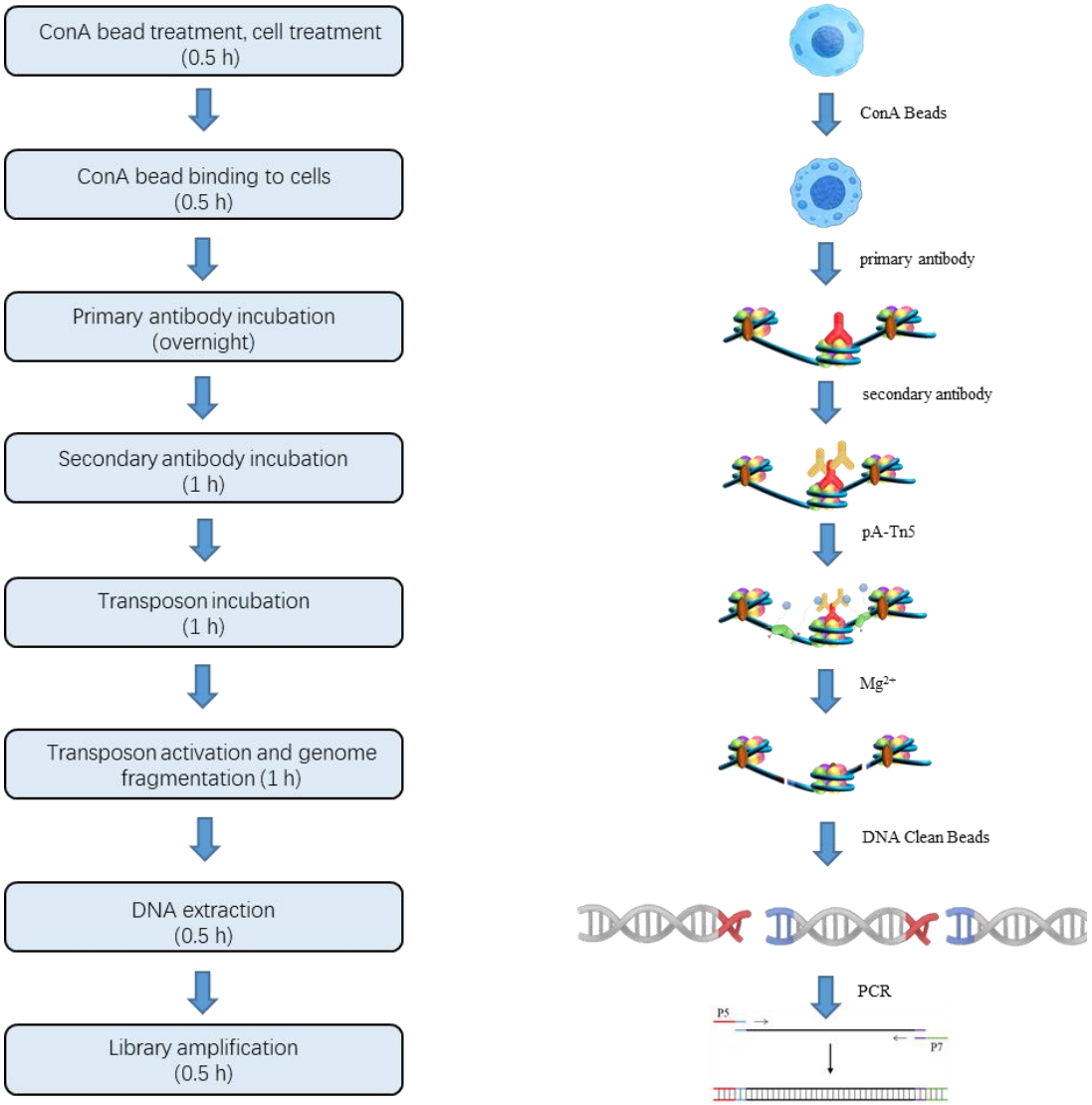
Consumables: Low-retention centrifuge tubes, 0.2 mL thin-walled tubes, 0.5 mL thin-walled tubes, magnetic stand, etc.

Antibodies: Primary antibody, secondary antibody.

Protease inhibitor: EDTA-free protease inhibitor must be used.

Library amplification primers: The NGS Tagment Index Kit for Illumina (Cat. No. BR3N108) is recommended.

Workflow



CUT&Tag principle and workflow

Cells are immobilized using concanavalin A-coated magnetic beads and permeabilized with digitonin, allowing the primary antibody, secondary antibody, and pA-Tn5 to enter the nuclei. Guided precisely by the antibodies, pA-Tn5 specifically cleaves the genomic DNA adjacent to the target protein and ligates sequencing adapters. After PCR amplification, DNA fragments interacting with the target protein are enriched.

Library structure:
5'-AATGATACGGCGACCACCGAGATCTACAC [Barcode 2] TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGNNNNNNNN NNCTGTCTCTTATACACATCTCCGAGCCCACGAGAC [Barcode 1] ATCTCGTATGCCGTCTTCTGCTTG- 3'

Note: N represents the insert sequence, and Barcode represents an 8-base tag sequence.

Protocol

1. Reagent Preparation

The volumes listed here are for a single reaction. Scale up proportionally according to experimental needs.

1.1 Binding Buffer：Take 22 μL of 10× Binding Buffer, add 198 μL of ddH_2O. Total volume: 220 μL.

1.2 Wash Buffer：Take 140 μL of 10× Wash Buffer, add 28 μL of 50× Protease Inhibitor, add 1,232 μL of ddH_2O. Total volume: 1,400 μL.

1.3 Dig-wash Buffer：Take 792 μL of the Wash Buffer prepared in 1.2, add 8 μL of 5% Digitonin. Total volume: 800 μL.

1.4 Antibody Buffer：Take 48 μL of the Dig-wash Buffer prepared in 1.3, add 2 μL of 25× Antibody Buffer. Total volume: 50 μL.

1.5 Dig-300 Buffer：Take 100 μL of 10× Binding Buffer, add 20 μL of 50× Protease Inhibitor, add 2 μL of 5% Digitonin, add 878 μL of ddH_2O. Total volume: 1,000 μL.

1.6 Tagment Buffer：Take 45 μL of the Dig-300 Buffer prepared in 1.5, add 5 μL of Tnp Activator. Total volume: 50 μL.

1.7 80%乙醇：Take 800 μL of absolute ethanol, add 200 μL of ddH_2O. Total volume: 1,000 μL.

2. ConA Beads Activation

2.1 Gently pipette to resuspend the ConA Beads. Transfer 10 μL of ConA Beads to a 0.2 mL tube, add 100 μL of Binding Buffer, and mix by pipetting. Place the 0.2 mL tube on a magnetic stand for 2 minutes.
2.2 Remove the liquid completely and take the tube off the magnetic stand. Add 100 μL of Binding Buffer, mix by pipetting, and briefly centrifuge to collect the liquid.
2.3 Place the tube on a magnetic stand for 2 minutes, remove the liquid completely, and resuspend the beads in 10 μL of Binding Buffer. Set aside for later use.

3. Cell Collection and Binding

3.1 Collect cells at room temperature and count them.

3.2 Take the desired number of cells, centrifuge at 600 × g for 3 minutes at room temperature, and discard the supernatant completely.

3.3 Resuspend the cells in 500 μL of Wash Buffer, centrifuge at 600×g for 3 minutes at room temperature, and discard the supernatant completely.

3.4 Resuspend the cells in 100 μL of Wash Buffer, transfer the suspension to the activated ConA Beads, mix by inverting, and incubate at room temperature for 10 minutes.
3.5 Briefly centrifuge to collect the liquid, place the 0.2 mL tube on a magnetic stand for 2 minutes, and discard the supernatant completely.

4. Primary Antibody Incubation

4.1 Resuspend the cell-ConA bead complex in 50 μL of pre-chilled Antibody Buffer. Add 1 μL of primary antibody, mix by inverting, and briefly centrifuge to collect the liquid.
4.2 Incubate overnight to several days at 2~8℃. Alternatively, incubate for 2 hours at room temperature.

5. Secondary Antibody Incubation

5.1 Dilute the secondary antibody at a ratio of 1:100 with Dig-wash Buffer. Prepare 100 μL per sample.

5.2 Take the primary antibody-cell-ConA bead complex from step 4.2, briefly centrifuge to collect the liquid, place the 0.2 mL tube on a magnetic stand for 2 minutes, and discard the supernatant completely.

5.3 Add 100 μL of the diluted secondary antibody, mix by inverting to resuspend, place on a rotator mixer, and incubate at room temperature with rotation for 1 hour.

5.4 Briefly centrifuge to collect the liquid, place the 0.2 mL tube on a magnetic stand for 2 minutes, and discard the supernatant completely.

5.5 Add 200 μL of Dig-wash Buffer, mix by inverting to resuspend.

5.6 Repeat steps 5.4~5.5 twice (after secondary antibody incubation, wash three times with 200 μL of Dig-wash Buffer).

6. pA-Tn5 Transposome Complex Incubation

6.1 Take 1 μL of pA-Tn5 transposome complex and add it to 99 μL of Dig-300 Buffer. Prepare 100 μL per sample.

6.2 Take the secondary antibody-primary antibody-cell-ConA bead complex from step 5.6, briefly centrifuge to collect the liquid, place the 0.2 mL tube on a magnetic stand for 2 minutes, and discard the supernatant completely.

6.3 Add 100 μL of the diluted pA-Tn5 transposome complex, mix by inverting to resuspend, place on a rotator mixer, and incubate at room temperature with rotation for 1 hour.

6.4 Briefly centrifuge to collect the liquid, place the 0.2 mL tube on a magnetic stand for 2 minutes, and discard the supernatant completely.

6.5 Add 200 μL of Dig-300 Buffer, mix by inverting to resuspend.

6.6 Repeat steps 6.4~6.5 twice (after pA-Tn5 transposome complex incubation, wash three times with 200 μL of Dig-300 Buffer).

7. Fragmentation

7.1 Take the 0.2 mL tube from step 6.6, briefly centrifuge to collect the liquid, place on a magnetic stand for 2 minutes, and discard the supernatant completely.

7.2 Add 50 μL of Tagment Buffer, mix by inverting to resuspend, and incubate at 37℃ for 1 hour.

7.3 Briefly centrifuge to collect the liquid, add 5 μL of Stop Buffer and 5 μL of Proteinase K, vortex to mix, and incubate at 55℃ for 10 minutes.

8. Nucleic Acid Extraction

8.1 Equilibrate the DNA Clean Beads to room temperature in advance. Add 120 μL of DNA Clean Beads (2.0× ratio) to the cell lysate from step 7.3, vortex to mix, and incubate at room temperature for 5 minutes.

8.2 Briefly centrifuge to collect the liquid, place on a magnetic stand for 2 minutes, and discard the supernatant completely.

8.3 Add 200 μL of 80% ethanol, let stand at room temperature for 30 seconds, and discard the supernatant completely.

8.4 Add 200 μL of 80% ethanol, let stand at room temperature for 30 seconds, and discard the supernatant completely.

8.5 Open the lid and let stand at room temperature for 2-5 minutes until the bead surface appears dull.

8.6 Add 25 μL of ddH_2O to resuspend the beads, incubate at room temperature for 5 minutes, briefly centrifuge to collect the liquid, place on a magnetic stand to capture the beads, and collect the nucleic acid eluate.

9. Library Amplification

9.1 Preheat the thermal cycler according to the table below:

Temperature	Time	Cycles
105℃	/	/
72℃	3 min	1
98℃	1 min	1
98℃	10 sec	12
60℃	10 sec	

72℃	1 min	1
4℃	∞	1

Note: The number of amplification cycles should be adjusted according to cell input amount. Refer to Precautions 4. Cell input amount and library amplification.

9.2 Take out the Tag Amplification Mix and the amplification primers matching the adapters. Thaw on ice, mix by inverting, briefly centrifuge, and keep on ice until use.

9.3 Prepare the library amplification reaction mix according to the table below:

Component	Component
Nucleic acid eluate from step 8.6	20
N5XX	2.5
N7XX	2.5
Tag Amplification Mix	25
Total	50

9.4 Gently pipette up and down or vortex to mix thoroughly, then briefly centrifuge to collect the reaction mixture at the bottom of the tube.

9.5 Place the tube into the thermal cycler preheated in step 9.1 and immediately start the library amplification reaction.

10. Product Purification

10.1 Take out the DNA Clean Beads that have been equilibrated to room temperature. Mix thoroughly by inverting and vortexing. Use a pipette to transfer 85 μL of beads (1.7× ratio) into the amplification product. Mix thoroughly by inverting and vortexing, then incubate at room temperature for 5 min.

10.2 Briefly centrifuge to collect the beads at the bottom of the tube. Place the tube on a magnetic stand to separate the beads from the liquid. After the liquid becomes clear (approximately 3 min), carefully discard the liquid. A residual volume of about 5~10 μL may be left at the bottom to avoid losing beads.

10.3 Add 200 μL of freshly prepared 80% ethanol, incubate at room temperature for 30 s, then completely discard the ethanol.

10.4 Add 200 μL of freshly prepared 80% ethanol, incubate at room temperature for 30 s, then completely discard the ethanol.

10.5 Keep the thin-walled tube on the magnetic stand with the lid open to allow the ethanol to evaporate fully. The bead surface will gradually lose its sheen; this process takes approximately 2 min. (If the bead surface cracks, the beads are over-dried and recovery efficiency will decrease.)

10.6 Remove the tube from the magnetic stand. Add 21 μL of ddH_2O, vortex thoroughly to mix, and let stand at room temperature for 2 min to elute the amplified products.

10.7 Briefly centrifuge, place the tube on the magnetic stand, and separate the beads from the liquid. After the liquid becomes clear (approximately 30 s), carefully transfer 20 μL of the supernatant to a new EP tube. Do not touch the beads.

11. Library Quality Control

See Precautions 5. Library Quality Control.