Hyperactive Universal CUT&Tag Assay Kit for Illumina

TD903

Vazyme

Instruction for Use

Version 23.1

Vazyme

Contents

01/Product Description

Hyperactive Universal CUT&Tag Assay Kit for Illumina is a kit developed specifically for the Illumina high-throughput sequencing platform to study protein-DNA interaction. CUT&Tag (Cleavage Under Target & Tagmentation) technology is a new method for studying protein-DNA interaction. By fusing Protein A/G with transposase, under the guidance of the antibody, it allows accurate targeting of the target protein and cutting of the DNA sequence near the target site. This kit optimizes the experimental reaction system and library construction process. Compared with the traditional ChIP-Seq, it has the advantages of low cell input, short experimental cycle, high signal-to-noise ratio, and good repeatability. It is especially suitable for such fields as early embryonic development, stem cells, tumors, and epigenetics. All the reagents in the kit have undergone rigorous quality control and function testing, to ensure the optimal stability and repeatability of library preparation.

02/Components

	Component	TD903-01 (12 rxns)	TD903-02 (48 rxns)
BOX 1	ConA Beads	130 μl	520 μl
	DNA Extract Beads	250 μl	1 ml
	Buffer L/B	2 × 750 μl	6 ml
	Buffer WA	1.5 ml	6 ml
	Buffer WB	1.5 ml	6 ml
BOX 2	pA/G-Tnp (2 μM)	24 μl	96 μl
	5% Digitonin	150 μl	600 μl
	10 × Binding Buffer	400 μl	2 × 800 μl
	NE Buffer	1.2 ml	4 × 1.2 ml
	10 × Wash Buffer	2 × 1 ml	8 ml
	10 × Dig-300 Buffer	1.2 ml	4 × 1.2 ml
	Antibody Buffer (-)	1 ml	4 × 1 ml
	5 × TTBL	150 μl	600 μl
	Proteinase K	80 μl	320 μl
	2 × CAM	600 μl	2 × 1.2 ml

▲ The color marked in the components table represents the color of tube cap of each component;

▲ pA/G-Tnp = Hyperactive pA/G-Transposon; NE Buffer = Nuclear Extract Buffer; TTBL = Trueprep Tagment Buffer L; CAM = CUT&Tag Amplification Mix.

03/Storage

BOX 1: Store ConA Beads and DNA Extract Beads at 2 ~ 8°C and the other components at room temperature (15 ~ 25°C). Adjust transportation methods are according to different destinations.

BOX 2: Store 5% Digitonin at -30 ~ -15°C, and can be stored at room temperature (15 ~ 25°C) for 1 week; store the other components at -30 ~ -15°C and transport at ≤0°C.

04/Applications

This product is intended for the protein-DNA interaction study in mammalian cells, and the input number of cells indicated is 60 - 100,000. Plant tissues, cells and others can also be used in this kit for related experiments through special treatments.

05/Self-prepared Materials

Antibody: primary antibody, secondary antibody;

The recommended protease inhibitors include Roche Complete Protease Inhibitor EDTA-Free Tablets (Sigma-Aldrich, 5056489001);

Magnetic beads for purification: VAHTS DNA Clean Beads (Vazyme #N411);

DNA quality control: Agilent Technologies 2100 Bioanalyzer or equivalent;

DNA Adapter: TruePrep Index Kit V2 for Illumina (Vazyme #TD202);
TruePrep Index Kit V3 for Illumina (Vazyme #TD203);
TruePrep Index Kit V4 for Illumina (Vazyme #TD204-TD207);
Please select the corresponding adapter kit according to the sample quantity.

Other materials: anhydrous ethanol, sterilized ddH_2O, low adsorption EP tube, PCR tube, rotary mixer, magnetic stand, PCR instruments and others.

06/Notes

For research use only. Not for use in diagnostic procedures.

Due to a number of factors such as the experimental samples, expression abundance of target protein in samples, and operations, it may be necessary to adjust the library preparation according to the actual situation. In order to obtain a high-quality library, it is important to read the following notes carefully. In case of any questions during use, please contact Vazyme's technical support for help: support@vazyme.com.

06-1/Use of Magnetic Beads

◇The experimental process involves the use of three different magnetic beads. The kit contains ConA Beads (for binding cells/nuclei) and DNA Extract Beads (for DNA extraction). For the library purification process, VAHTS DNA Clean Beads (Vazyme #N411) is recommended, please distinguish different magnetic beads before use.

◇General notes for magnetic Beads operations:

- ▲ Store magnetic beads at 2 ~ 8°C, do not freeze them at -30 ~ -15°C.
- ▲ Magnetic beads should be equilibrated to room temperature (placed at room temperature) before use, and all magnetic bead operations should be performed at room temperature.
- ▲ Magnetic beads should be fully mixed by vortex shaking or pipetting up and down prior to each adsorption.

◇ Notes for ConA Beads operations:

- ▲ After ConA Beads are combined with cells, do not shake violently or use a pipette to blow and mix evenly to avoid cells falling off from the magnetic beads.
- ▲ Do not place magnetic beads or magnetic bead-cell complexes in the air for a long time to avoid dry binding of magnetic beads, which will affect subsequent experiments.
- ▲ In the process of treating magnetic beads or magnetic bead-cell complexes, do not centrifuge them at high speeds or place them on a magnetic stand for long periods of time, to avoid the aggregation of magnetic beads due to human factors.
- ▲ It is normal for some magnetic beads to adhere to the wall/aggregate during the incubation process. As long as the magnetic bead-cell complexes are within the range of solution infiltration, the subsequent experimental results will not be affected. For different types of cells, the degree of adherence to the wall/aggregation may vary after their binding with magnetic beads; for the same type of cells, the phenomenon of adherence to the wall/aggregation becomes more obvious as the sample input volume increases. If adherence to the wall/aggregation occurs, gently flick the bottom of the tube to fully mix the magnetic bead-cell complexes. Avoid opening the lid repeatedly and pipetting to fully mix the magnetic bead-cell complexes during the experiment.

06-2/Sample Preparation and Antibody Selection

◇ If living cells are used for experiments, for common suspending cell lines, discard the upper layer of culture medium after centrifugation and collect the cells for experiments. For most adherent cells, collect the cell suspension after trypsinization, then discard the upper layer of culture medium after centrifugation and collect the cells. For some cell lines, trypsinization may potentially affect the binding effect of cells and ConA Beads, and the judgment can be performed according to the actual condition.

◇ Cells used in the CUT&Tag experiment can be stained with trypan blue for cell viability assay. The cell viability is preferably >90%. Cell operations during the experiment should be gentle to maintain cell viability. For cells with poor growth or dead cells, the binding state of protein and DNA will change, and even the protein may detach and become naked DNA. Random cleavage of transposons may produce strong background noise, affecting the experimental results.

◇ If cell nuclei are used for experiments, this kit provides a lysis solution applicable for conventional cells. For embryos and other insoluble cells, it is necessary to choose appropriate cell lysis solution according to the sample type.

◇ If animal/plant tissues are used for experiments, it is recommended to extract tissue nuclei, and explore appropriate nuclei extraction conditions according to different tissue types.

◇ For proteins of interest with low expression abundance in the sample, as well as some special transcription factors, cells or nuclei can be lightly cross-linked to obtain better experimental results.

◇ It is recommended to set up positive control and negative control groups in the experiment. It is recommended to use histones with higher expression abundance in the sample for the positive control, and use the experimental group that adds transposons and secondary antibodies instead of primary antibodies for the negative control, to determine whether there is any abnormality during the entire experiment. It is not necessary to add non-specific IgG negative control, which cannot provide valuable information in sequencing analysis. The addition of such control is selected depending on the experimental needs.

◇ It is recommended to use ChIP-level antibodies for the primary antibody in the experiment. If no ChIP-level antibodies are commercially available for the protein of interest, antibodies applicable for Immune Fluorescence (IF) experiments are allowable.

◇ It is recommended to select a secondary antibody with high affinity to Protein A/G and without modification. Please refer to the table below:

Species	Antibody subtype	Protein A/G
Human	Total IgG	+++
	IgG1	+++
	IgG2	+++
	IgG3	+++
	IgG4	+++
	IgM	+
	IgD	-
	IgA1	+
	IgA2	+
	Fab	+
	scFv	+
Mouse	Total IgG	+++
	IgM	-
	IgG1	++
	IgG2a	+++
	IgG2b	+++
	IgG3	+++
Rat	Total IgG	++
	IgG1	++
	IgG2a	+++
	IgG2b	+
	IgG2c	+++
Rabbit	Total IgG	+++
Goat	Total IgG	+++
Sheep	Total IgG	+++
Guinea pig	Total IgG	+++
Hamster	Total IgG	++
Donkey	Total IgG	+++
Pig	Total IgG	+++
Dog	Total IgG	+++
Cat	Total IgG	+++
Chicken	Total IgY	-
Cow	Total IgG	+++
Horse	Total IgG	+++

+++ represents strong binding; ++ represents medium binding; + represents weak binding;
- represents no binding

06-3/Input of Cells and Number of Amplification Cycles

◇ The appropriate cell input volume for the kit is 60 - 100,000 cells. It is affected by the cell type, antibody selection, and the expression abundance of the protein of interest. The minimum input of cells compatible in the experiment is not constant. It is recommended to use 10,000 - 50,000 cells in the early experiments, to explore the relationship between the input of cells in the CUT&Tag experiment for the protein of interest and the number of amplification cycles.

▲ Taking K562 cells as an example, histones with medium and high expression abundance (such as H3K4me3 and H3K27me3) are used for the CUT&Tag experiment. The relationship between input of cells, number of amplification cycles and library yield is shown in the table below:

input of cells	Number of Amplification Cycles	Library Yield (Qubit Quantitation)
60	18 - 20	10 - 40 ng/μl
1,000	15 - 17	
10,000	12 - 14	
100,000	9 - 11	

◇ The CUT&Tag experiment requires a small number of cells, which provides the possibility for very low initial cell input and single-cell experiments. However, it is not recommended to use too low initial cell input for CUT&Tag experiments. For single-cell experiments, the process should be optimized to obtain more ideal experimental results.

◇ For PCR amplification, the library output is qualified when it meets the loading requirements, and an excessively high number of amplification cycles is not necessary to obtain a higher library yield. An excessive number of cycles will lead to various adverse effects such as over-amplification, amplification bias, and PCR duplicates.

06-4/Library Quality Control

Generally, the quality of a well-constructed library can be evaluated through concentration analysis and size distribution.

◇ **Library Concentration Assay:**

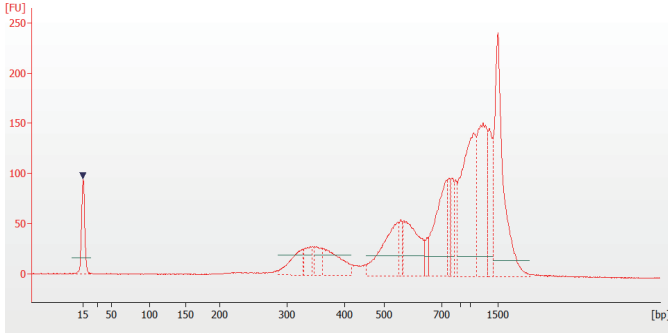
▲ There are two common library concentration assay methods: the method based on double-strand DNA fluorescent dye, such as the Equalbit 1 × dsDNA HS Assay Kit (Vazyme #EQ121) and PicoGreen; and the method based on qPCR absolute quantification, such as the VAHTS Library Quantification Kit for Illumina (Vazyme #NQ101-NQ106).

▲ Too many amplification cycles can cause non-specific annealing of the amplification product after it is unlinked, resulting in non-complementary strand cross-annealing products. These products migrate slower and are observed as secondary, higher molecular weight peaks in electrophoretic analysis. They are composed of single-stranded libraries with correct lengths, and can be normally bound with Flow Cell and sequenced after denaturation. Therefore, their presence has no significant

impact on library sequencing. However, because over-amplified libraries contain a large quantity of incomplete double-stranded structures, when library quantification is performed using fluorescent dye for double-stranded DNA identification, quantification results will be lower than actual values. However, the qPCR-based library quantification system involves a denaturation process in the process of quantification, and it is not affected by over-amplification.

◇ **Library Length Distribution Test:**

Library length distribution can be tested using devices based on the principle of electrophoretic separation, including LabChip GX, GXII, GX Touch (PerkinElmer), Bioanalyzer, Tapestation (Agilent Technologies), Fragment Analyzer (Advanced Analytical). For the library obtained using 10,000 K562 cells for histone H3K27me3 CUT&Tag experiment, the length distribution test is shown in the figure below:



06-5/Further Notes

◇ Thaw all the components in BOX 2 (except pA/G-Tnp and 2 × CAM) at room temperature before use, and all components should be thoroughly mixed before use.

◇ Pay attention to the storage conditions of different reagents to avoid invalidation.

◇ To avoid cross-contamination of samples, it is recommended to use tips with a filter and to replace the tip between different samples.

◇ PCR products are highly susceptible to aerosol contamination due to improper operation, which can accordingly affect the accuracy of experimental results. Therefore, we recommend enforcing physical isolation on the PCR reaction system preparation area and the PCR product purification and testing areas, using equipment such as dedicated pipettes, and periodically cleaning each experimental area (Vazyme #R504) using RNase and nucleic acid remover, to ensure the cleanliness of the lab environment.

◇ This kit is compatible with 8-strip tubes and 1.5 ml EP tubes for experiments. Compared with the 1.5 ml EP tubes, the 8-strip tubes have a smaller inner wall area, which can improve the adhesion of magnetic beads to the wall; when using the 8-strip tubes, gently open the lids to avoid liquid splashing and sample contamination from opening the lids at one side with too much force.

07/Library Structure

Library Structure

Index 2 (i5)

5'-AATGATACGGCGACCACCGAGATCTACACIIIIIIIITCGTCGGCAGCGTCAGATGTGTATAAGAG
ACAG-NNNNNN-CTGTCTCTTATACACATCTCCGAGCCCACGAGACIIIIIIIIATCTCGTATGCCGTC
TTCTGCTTG-3'

Index 1 (i7)

IIIIIIII: Index 2 (i5), 8 bases IIIIIIII: Index 1 (i7), 8 bases -NNNNNN-: Insert Sequence

08/Experiment Process

08-1/Buffer Preparation

▲ The calculation here is based on a single sample, please prepare in proportion to the actual sample quantity.

1. Binding buffer: Mix 30 μl of 10 × Binding Buffer with ddH_2O to 300 μl.
2. Wash buffer: Mix 150 μl of 10 × Wash Buffer with 30 μl of 50 × Protease Inhibitor and 1,320 μl of ddH_2O.
   ▲ Preparation of 50 × Protease Inhibitor: Dissolve a protease inhibitor cocktail tablet (Sigma-Aldrich, 5056489001) in 1 ml of ddH_2O, and store at -20°C.
3. Antibody Buffer: Mix 50 μl of Antibody Buffer (-) with 0.5 μl of 5% Digitonin and pre-cool on ice.
4. Dig-wash Buffer: Mix 792 μl of the Wash Buffer prepared in step 2 with 8 μl of 5% Digitonin.
   ▲ Digitonin has contact toxicity. Please take protective measures during the preparation of the solution and avoid direct contact. The buffer solution added with digitonin should be prepared and used straight away and should not be stored for long periods of time.
5. Dig-300 Buffer: Mix 100 μl of 10 × Dig-300 Buffer, 2 μl of 5% Digitonin and 20 μl of 50 × Protease Inhibitor with 878 μl of ddH_2O.
6. For the first use of Buffer WA and Buffer WB, please add a specified amount of anhydrous ethanol according to the bottle labeling and mark them.

	Volume of anhydrous ethanol to be added (ml)	
	TD903-01	TD903-02
Buffer WA	2	8
Buffer WB	6	24

08-2/ConA Beads Treatment

1. For an 8-strip tube, each sample is added with 100 μl of Binding Buffer.
2. Fully resuspend the ConA Beads using a pipette, fully mix 10 μl of ConA Beads with the Binding Buffer in step 1, then place the mixture on a magnetic stand, discard the supernatant after the solution becomes clear (about 2 min).
3. Remove the 8-strip tube from the magnetic stand, add 100 μl of Binding Buffer, then gently pipette to fully mix (do not mix by vortexing).
4. Place the 8-strip tube on the magnetic stand, discard the supernatant after the solution becomes clear (about 2 min), then add 10 μl of Binding Buffer to resuspend the ConA Beads.

08-3/Cell Collection

▲ All steps before the cell is permeable are performed at room temperature to minimize the stress inflicted on the cells. Please avoid vigorous vortexing and oscillation during the experimental operation.

1. The cells are collected and counted at room temperature.
2. The required number of cells for the experiment are collected and placed in a 1.5 ml EP tube, centrifuged at 2,500 rpm (600 × g) at room temperature for 5 min at low speed, then discard the supernatant.
3. Resuspend cells with 500 µl of Wash Buffer at room temperature and centrifuge at 2,500 rpm (600 × g) for 5 min at low speed, then discard the supernatant.
4. Add 100 µl of Wash Buffer to each sample to resuspend the cells.

▲ Optional step —— Obtain Cell Nuclei

1. The cells are collected and counted at room temperature.
2. Collect and place the required number of cells for the experiment in a 1.5 ml EP tube, centrifuge at 2,500 rpm (600 × g) at room temperature for 5 min at low speed, then discard the supernatant.
3. Add 100 µl of pre-cooled NE Buffer to each sample, gently pipette to resuspend cells, then incubate on ice for 10 min.
4. Centrifuge cells at 2,500 rpm (600 × g) for 5 min at low speed at room temperature, then discard the supernatant.
5. Add 100 µl Wash Buffer to each sample to resuspend nuclei.

08-4/Cell (Nuclei) and ConA Beads Incubation

1. Transfer 100 µl of cells (nuclei) to an 8-strip tube containing activated ConA Beads, invert to mix and incubate at room temperature for 10 min. Invert to mix for 2 - 3 times during this period.
2. Briefly centrifuge to collect the reaction solution (<100 × g) and place the 8-strip tube on the magnetic stand, then discard the supernatant after the solution becomes clear (about 2 min).

▲ Do not aggregate the magnetic beads at the bottom of the tube by prolonged centrifugal time.

08-5/Primary Antibody Incubation

1. Add 50 µl of pre-cooled Antibody Buffer to each sample to resuspend the cell (nuclei)-magnetic bead complexes.
2. Add the antibodies to the 8-strip tube according to the recommended immune concentration in the antibody manual, and invert to mix.
3. Collect at the bottom of the tube with short-spin centrifugation (Do not aggregate the magnetic beads at the bottom of the tube by prolonged centrifugal time), then place the 8-strip tube at 2 ~ 8°C overnight.

▲ It is recommended to set up positive control and negative control groups in the experiment. Please refer to **06-2/Sample Preparation and Antibody Selection**.

08-6/Secondary Antibody Incubation

1. Use Dig-wash buffer to dilute the secondary antibodies at a certain ratio (1:100 dilution is recommended), 50 μl per sample.
2. Use the 8-strip tube in 08-5/Primary Antibody Incubation to collect the reaction solution with short-spin centrifugation, place the 8-strip tube on the magnetic stand, then discard the supernatant after the solution becomes clear (30 sec - 2 min).
3. Add the secondary antibodies diluted in step 1 of 08-6/Secondary Antibody Incubation, invert several times to fully mix the antibodies and the cell (nuclei)-magnetic bead complexes, then rotate and incubate for 30 - 60 min at room temperature.
4. Collect the reaction solution with short-spin centrifugation, place the 8-strip tube on the magnetic stand, then discard the supernatant after the solution becomes clear (30 sec - 2 min).
5. Add 200 μl of Dig-wash buffer to the 8-strip tube, invert several times to ensure that the buffer and the cell (nuclei)-magnetic bead complexes are fully mixed.
6. Repeat steps 4 - 5 twice (3 times in total).

08-7/pA/G-Tnp Incubation

1. Mix 2 μl of pA/G-Tnp with 98 μl of Dig-300 Buffer to a final concentration of 0.04 μM, 100 μl per sample.
   ▲ Under different experimental environments, the cleavage activity of transposons may vary. Please accordingly adjust the concentration of transposons used based on the actual situation.
2. Use the 8-strip tube in 08-6/Secondary Antibody Incubation to collect the reaction solution with short-spin centrifugation, place the 8-strip tube on the magnetic stand, then discard the supernatant after the solution becomes clear (30 sec - 2 min).
3. Dilute 100 μl of the pA/G-Tnp transposons diluted in step 1 of 08-7/pA/G-Tnp Incubation to each sample, invert several times, so that the transposons and the cell (nuclei)-magnetic bead complexes are fully mixed.
4. Rotate and incubate for 1 h at room temperature.
5. After short-spin centrifugation, place the 8-strip tube on the magnetic stand, then discard the supernatant after the solution becomes clear (30 sec - 2 min).
6. Add 200 μl of Dig-300 Buffer to the 8-strip tube, invert several times, so that the buffer and the cell (nuclei)-magnetic bead complexes are fully mixed.
7. Repeat steps 5 - 6 twice (3 times in total).

08-8/Fragmentation

1. Add 10 μl of 5 × TTBL to 40 μl of Dig-300 Buffer and fully mix.
2. Use the 8-strip tube in 08-7/pA/G-Tnp Incubation to collect the reaction solution with short-spin centrifugation, place the 8-strip tube on the magnetic stand, then discard the supernatant after the solution becomes clear (30 sec - 2 min).
3. Add 50 μl of TTBL diluted in step1 in 08-8/Fragmentation to each sample and fully mix.
4. Place the 8-strip tube in a PCR machine and incubate at 37°C for 60 min.
   ▲ It is not necessary to set up a heated lid. The lid of the PCR instrument can be kept open.

08-9/DNA Extraction

1. After fragmentation, Add 5 µl of Proteinase K, 100 µl of Buffer L/B and 20 µl of DNA Extract Beads to each sample, fully mix by vortexing and incubate at 55°C for 10 min. Invert 2 - 3 times during this period.
   ▲ In order to avoid experimental errors, DNA Extract Beads should be fully mixed before they are added.
2. Following short-spin centrifugation, place the 8-strip tube is placed on the magnetic stand for 2 - 3 min, then discard the supernatant carefully.
   ▲ Do not remove magnetic beads, and please remove the supernatant as much as possible.
3. Remove the above samples from the magnetic stand, add 200 µl of Buffer WA (please make sure to add anhydrous ethanol before use), then fully mix by vortexing. Collect the reaction solution with short-spin centrifugation, place the 8-strip tube on the magnetic stand for 2 min, then discard the supernatant.
4. Remove the above samples from the magnetic stand, add 200 µl of Buffer WB (please make sure to add anhydrous ethanol before use), then fully mix by vortexing. Collect the reaction solution with short-spin centrifugation, place the 8-strip tube on the magnetic stand for 2 min, then discard the supernatant.
5. Report step 4 once.
6. Open the lid and air dry at room temperature for 5 - 10 min until there is no liquid remaining in the tube and the surface of the magnetic beads is non-reflective.
   ▲ To ensure DNA purity, Buffer WB should be fully dried, but not overdried (cracked magnetic beads), which will affect the final yield.
7. Remove the above samples from the magnetic stand, add 22 µl of ddH_2O, pipette to fully mix, elute at room temperature for 5 min, then gently shake 2 - 3 times during this period.
8. Place the 8-strip tube on the magnetic stand, pipette 20 µl of supernatant into a new 8-strip tube after the solution becomes clear (about 1 min). The sample can be stored at -30 ~ -15°C for long periods of time, and avoid repeated freezing and thawing.

08-10/Library Amplification

1. Prepare the following components in a sterile PCR tube:

Components	Volume
Fragmented DNA after purification	15 µl
2 × CAM	25 µl
N5XX	5 µl
N7XX	5 µl
Total volume	50 µl

2. Gently pipette up and down, then place in the PCR instrument for the following reactions:

Temperature	Time	Number of cycles[b]
72°C[a]	3 min	
95°C	3 min	
98°C 60°C	10 sec 5 sec	9 - 20 cycles
72°C	1 min	
4°C	Hold	

a. Incubation at 72°C is used for chain replacement reactions. Do not delete this step;

b. Select the number of amplification cycles depending on the actual situation. Refer to **06-3/Input of cells and Number of Amplification Cycles**.

08-11/PCR Product Purification

1. Fully mix VAHTS DNA Clean Beads (Vazyme #N411) through vortex shaking, and aspirate 100 µl into the above PCR reaction product. Mix the whole system well by vortexing or pipette up and down 10 times, then incubate for 5 min at room temperature.
   ▲ Since magnetic beads are sticky, pipette sufficient volume and release slowly.

2. Briefly centrifuge the reaction tube and place it onto a magnetic stand to separate the magnetic beads from the fluid. When the solution becomes clear (about 5 min), carefully remove the supernatant. Do not disturb the beads.

3. Keep the PCR tube always on the magnetic rack, and add 200 µl of freshly prepared 80% ethanol to rinse the magnetic beads. Perform incubation for 30 sec at room temperature, and carefully remove the supernatant.

4. Repeat step 3, rinse twice in total.

5. Keep the PCR tube always on the magnetic rack, and open the lid to air-dry the magnetic beads for 3 - 5 min.

6. After the magnetic beads are dried, remove the PCR tube from the magnetic stand, and elute with 22 µl of ddH_2O, fully mix the beads by vortex shaking or gently pipetting up and down 10 times, then incubate at room temperature for 5 min.

7. Briefly centrifuge the PCR tube and keep it still in a magnetic stand. When the solution becomes clear (about 5 min), carefully transfer 20 µl of supernatant into a new PCR tube and store at -30 ~ -15°C.

08-12/Library Quality Testing

Refer to **06-4/Library Quality Control.**

09/FAQ & Troubleshooting

◇ What species is CUT&Tag applicable to?

CUT&Tag is widely used in researches of conventional mammalian cell protein-DNA interaction. Plants and other cells can be subjected to special treatments (cell wall degradation or nuclei extraction) prior to experiments. Please refer to the front-end processing method of CUT&RUN technology for procedures.

◇ What's the main function of ConA Beads?

The concanavalin A-coated ConA Beads can bind to glycoproteins on the cell membrane/nuclei membrane, thereby adsorbing cells, visualizing cell processing operations, and reducing cell loss during the subsequent experiments.

◇ Is CUT&Tag only restricted to Illumina platform sequencing?

The transposons provided by Vazyme #TD903 are specifically designed for the Illumina platform. For other sequencing platforms, it is necessary to replace the amplification primers applicable for the corresponding platform.

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