Hyperactive In-Situ ChIP Library Prep Kit for Illumina

Catalog# TD901/TD902

Version 9.1

Vazyme biotech co., ltd.

01/ Introduction

Hyperactive In-Situ ChIP Library Prep Kit for Illumina is specifically designed for Cleavage Under Targets and Tagmentation (CUT&Tag) technology. CUT&Tag technology is a new method for research on protein-genomic interaction by fusion of Protein G or Protein A with engineered ultra - active Tn5 transposase to form a novel dual-function fusion enzyme (Hyperactive pG- Tn5 / pA-Tn5 Transposase). It precisely binds the DNA sequence near the target protein under the antibody guidance and results in factor-targeted tagmentation, generating fragments used for PCR enrichment or DNA sequencing.

Compared with the traditional protein-genomic interaction research method of ChIP-Seq, CUT&Tag has significant advantages of low cell input, short operation time, high signal-to-noise ratio, good repeatability and is especially suitable for research on early embryo development, stem cells, tumors, and epigenetics.

02/ Components

	Components	TD901-01/02 12/48 rxn	TD902-01/02 12/48 rxn
BOX 1	ConA beads	130/520 µl	130/520 µl
	0.5 M EDTA	200/800 µl	200/800 µl
	10% SDS	50/200 µl	50/200 µl
BOX 2	Hyperactive pG-Tn5 Transposon(6.88 µM)	8/32 µl	-----
	Hyperactive pA-Tn5 Transposon(6.88 µM)	-----	8/32 µl
	5% Digitonin	0.5/2 ml	0.5/2 ml
	10 × Binding Buffer	1/4 ml	1/4 ml
	10 × Wash Buffer(-)	5/20 ml	5/20 ml
	10 × Dig-300 Buffer(-)	5/20 ml	5/20 ml
	30% BSA	20/80 µl	20/80 µl
	1 M $MgCl_2$	50/200 µl	50/200 µl
	Proteinase K (20 mg/ml)	40/160 µl	40/160 µl
	1 × TE(pH 8.0)	0.5/2 ml	0.5/2 ml
	5 × TAB	120/480 µl	120/480 µl
	TAE	12/48 µl	12/48 µl

▲ ConA beads = Concanavalin A-coated magnetic beads.

▲ TAE = TruePrep Amplify Enzyme; TAB = TruePrep Amplify Buffer.

03/ Storage

BOX 1: ConA beads should be stored at 2°C ~ 8°C, and the remaining components (EDTA / SDS) should be stored at room temperature. All reagents should be transported at 2 °C ~ 8 °C.

BOX 2: 5% Digitonin is stored at -30°C ~ -15°C and can be held at room temperature for up to 1 week.

10 × Binding Buffer is stored at -30°C ~ -15°C and can be held at 2°C ~ 8°C for 6 months.

The remaining components should be stored at -30°C ~ -15°C.

All reagents should be transported at ≤0°C.

04/ Applications

This product is suitable for protein-DNA interaction research of mammalian cells, and the cell input is 60 - 100,000. This kit is also applicable to specially treated yeast and plant cells.

05/ Additional Materials Required

Antibody (primary antibody/ secondary antibody);

Protease Inhibitor Tablets, Roche Complete Protease Inhibitor EDTA-Free Tablets (Sigma-Aldrich, 5056489001) is recommended;

PCI (phenol: chloroform: isoamyl alcohol = 25:24:1);

Chloroform;

Absolute ethanol;

Nuclease-free Water;

Rotary Mixer;

VAHTS DNA Clean Beads (Vazyme #N411);

Magnetic Stand;

Thermocycler (PCR instrument);

Low-absorption Nuclease-free PCR tubes and EP tubes;

Single Index: TruePrep Index Kit V4 for Illumina (Vazyme, #TD204/TD205/TD206/TD207);

Dual Index: TruePrep Index Kit V2/V3for Illumina (Vazyme, #TD202/TD203)

Note: Please select the appropriate kit according to the number of samples.

06/ Notes

◇ Tips for DNA Purification with Magnetic Beads

1. Equilibrate the beads to room temperature before use to assure capture efficacy.

2. Do not store the Magnetic Beads below 0 °C.

3. All operations with Magnetic Beads should be carried out at room temperature.

4. Mix the beads thoroughly every time before pipetting.

5. It is important not to discard or transfer any beads with the removal or transfer of supernatant. Please transfer the supernatant after the solution is completely clarified, avoid disturbing the beads, otherwise the subsequent enzymatic reaction will be affected.

6. Always use 80% ethanol freshly prepared. Do not leave any 80% ethanol supernatant behind in the washing step to reduce the residual impurities.

7. It is important to remove all the ethanol and dry the beads (the surface changes from bright brown to matte brown) before proceeding with subsequent reactions. However, over-drying of beads may make them difficult to resuspend, resulting in a dramatic loss of DNA.

◇ Avoid sample cross-contamination

1. It is recommended to use filter pipette tips;

2. Change tips when varying samples.

◇ Cell manipulation

Operate cells as gently as possible to maintain cell viability.

◇ Reagent usage

Different buffer reagents should be stored at the corresponding condition to avoid failure.

◇ Avoid PCR product contamination

It is recommended to separate preparation area and clean-up area physically, use dedicated pipettor, and clean experimental region by 0.5% sodium hypochlorite or 10% decolorizer timely.

07/ Sample preparation

◇Buffer Preparation

▲This operation is applicable to 12 samples. It can change according to the actual amounts of samples.

1. Binding Buffer: Take 1 ml of 10 × Binding Buffer, and add ddH2O to 10 ml. It can be stored at 2°C ~ 8°C for 6 months.

2. Wash Buffer: Take 5 ml of 10 × Wash Buffer (-), add ddH2O to 50 ml, then add a piece of Protease Inhibitor Tablet. It can be stored at 2°C ~ 8°C for 1 week.

3. Dig-wash Buffer: Take 40 ml of the Wash Buffer prepared in **Step 2**, add 400 μl of 5% Digitonin and mix thoroughly. It can be stored at 2°C ~ 8°C for 2 days.

▲Digitonin is toxic and DMSO can penetrate through the skin. Please do personal protection during solution preparation. If there is a small amount of precipitate after adding Digitonin, it will not affect the usage.

07/ Sample preparation

4. Antibody Buffer: Mix 8 μl of 0.5 M EDTA, 6.7 μl of 30% BSA and 2 ml of Dig-wash Buffer, then keep the tube on ice before use. The mixture should be prepared freshly.

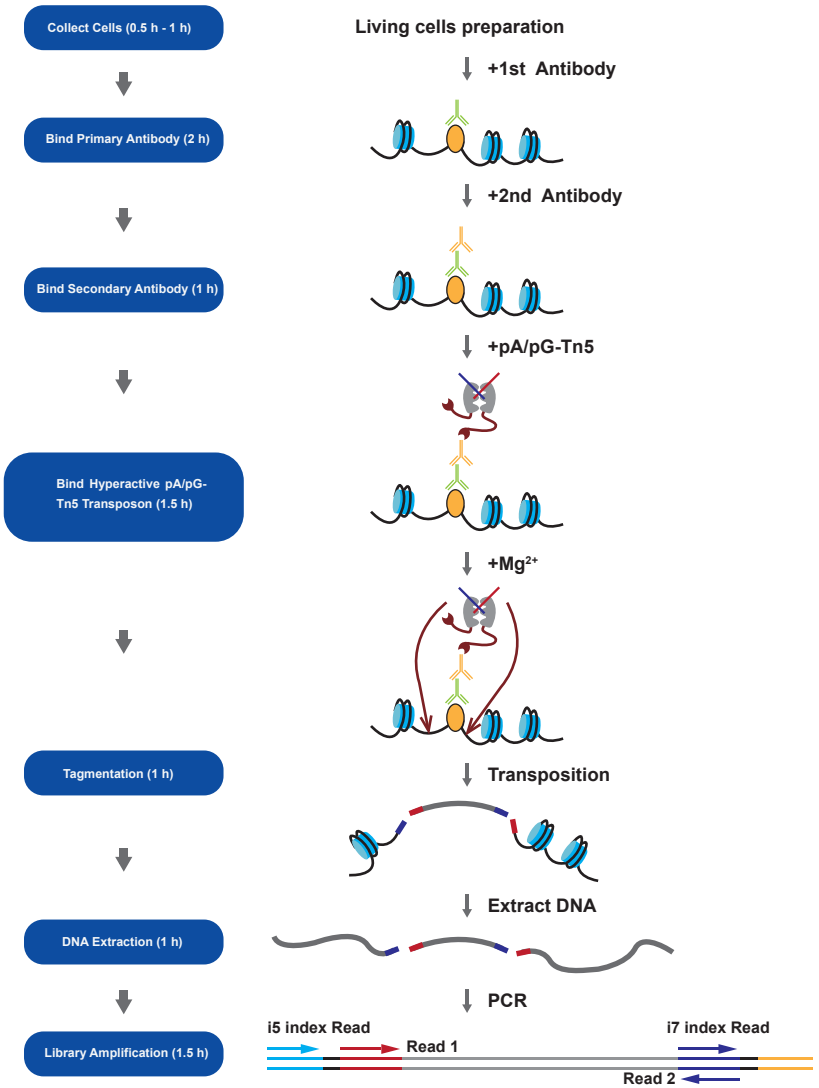
5. Dig-300 Buffer: Take 5 ml of 10 × Dig-300 Buffer (-), add ddH2O to 50 ml, then add 100 μl of 5% Digitonin and a piece of Protease Inhibitor Tablet. Digitonin (5%) should be added on the same day to reduce the formation of precipitates. The buffer without added Digitonin can be stored at 2°C ~ 8°C for one week.

6. Tagmentation Buffer: Take 5 ml of Dig-300 Buffer prepared in **Step 5**, add 50 μl of 1 M MgCl2 and mix thoroughly. The mixture should be prepared freshly.

◇ Cell Preparation

Harvest fresh culture at room temperature and count cells. Histones (or proteins of interest) of dead cells may detach from nucleosomes and become naked DNA. Random cleavage of the transposon results in strong background noise. It is recommended that the activity of the sample cells be at least >90%. Cell viability can be identified by trypan blue staining.

08/ Mechanism & Workflow



CUT&Tag basic Mechanism

The cells are bound to Concanavalin A-coated magnetic beads (ConA beads), and the cell membrane is permeabilized by Digitonin (Non-ionic detergent). The enzyme (Hyperactive pG- Tn5 / pA-Tn5 Transposase) precisely binds the DNA sequence near the target protein under the antibody (primary / secondary) guidance and results in factor-targeted tagmentation. DNA sequence is tagmented, with adapters added at the same time at both ends, which can be enriched by PCR to form the sequencing-ready libraries.

08/ Mechanism & Workflow

Library Structure

Index 2 (i5)

5'-AATGATACGGCGACCACCGAGATCTACACIIIIIIIITCGTCGGCAGCGTCAGATGTGTATAAG
AGACAG-NNNNNN-CTGTCTCTTATACACATCTCCGAGCCCACGAGACIIIIIIIIATCTCGTATGC
CGTCTTCTGCTTG-3'

Index 1 (i7)

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

09/ Protocol

09-1/ ConA beads treatment

1.Add Binding Buffer (100 µl / sample) to a 1.5 ml EP tube.

2.Gently resuspend ConA beads by pipetting. Transfer 10 µL (per sample) of ConA beads into Binding buffer from **Step 1** and then mix gently by pipetting. Place the tube on a magnet stand to clear (about 2 min), then discard the supernatant.

3.Remove the EP tube from the magnetic stand, and add 100 µl (per sample) of Binding Buffer, then mix gently with a pipette (do not shake vigorously or vortex), and briefly centrifuge to collect the reaction solution at the bottom of the tube.

4.Place the tube on a magnet stand to clear (about 2 min), then discard the supernatant. Add 10 µl (per sample) of Binding Buffer to resuspend the beads.

09-2/ Cell collection

All steps prior to the cell permeabilization are performed at room temperature to minimize stress on the cells. We recommend that vigorous vortexing should be avoided.

1.Harvest fresh culture at room temperature and count cells.

▲The protocol is targeted at a cell input of 60-100,000.

2.Centrifuge at room temperature 600 x g for 3 min and discard the supernatant.

3.Add 500 µl of Wash Buffer to resuspend the cells at room temperature, and centrifuge at room temperature 600 x g for 3 min, discard the supernatant.

09-3/ Incubate cells with ConA beads

1.Add 100 µl (per sample) of Wash Buffer to resuspend the cells, then transfer resuspended cell to a new 1.5 ml EP tube. While vortexing gently (1100 rpm), add treated bead slurry (from **Step 09-1**) dropwise. Place on end-over-end rotator for 5-10 min.

2.Briefly centrifuge to collect the reaction solution at the bottom of the tube. Place the tube on a magnet stand to clear (about 2 min), then discard the supernatant.

09-4/ Primary antibody incubation

1.Add 50 µl (per sample) of ice-cold Antibody buffer to resuspend the cells, gently vortex and place on ice.

2.Add corresponding volume (according to the recommended immunization concentration of the antibody instructions) of primary antibody to each sample with gentle vortexing.

3.Place on nutator and incubate for 2 h at room temperature (or overnight at 2 °C -8 °C).

▲ Liquid should remain in the bottom and side of the tube while rocking.

▲ It should include both control antibodies of positive and negative in parallel.

09-5/ Secondary antibody incubation

1.Briefly centrifuge to collect the reaction solution at the bottom of the tube. Place the tube on a magnet stand to clear (about 30 s to 2 min), then discard the supernatant.

2.Dilute secondary antibody in Dig-wash buffer (regularly recommended to use 1:100 dilution). Add 50 µL (per sample) of the diluted antibody to each sample, while gently vortexing to mix throughly the antibody with ConA beads.

3.Place the tubes on a nutator at room temperature for 30 - 60 min.

09/ Protocol

09-5/ Secondary antibody incubation

4.Briefly centrifuge to collect the reaction solution at the bottom of the tube. Place the tube on a magnet stand to clear (about 30 s to 2 min), then discard the supernatant.

5.Add 800 µL of Dig-wash buffer. Invert 10 times or gently vortex to mix throughly the Buffer with ConA beads.

6.Repeat **Steps 4** and **Steps 5** twice.

09-6/ Hyperactive pG-Tn5/pA-Tn5 Transposon Incubation

1.Mix Hyperactive pG-Tn5/pA-Tn5 Transposon with Dig-300 Buffer to a final concentration of 0.04 µM (according to the references). For example, Add 0.58 µl of Transposon to 100 µl of Dig-300 Buffer (the concentration of the transposon provided in the kit is 6.88 µM).

▲In different experimental environments, the cleavage activity of transposase may be different. Please adjust the final concentration of Transposon based on the actual situation. The transposon concentration is 4 µM based on the reaction system recommended by the Vazyme #S602 or #S603 instructions.

2.Briefly centrifuge to collect the reaction solution at the bottom of the tube. Place the tube on a magnet stand to clear (about 30 s to 2 min), then discard the supernatant.

3.Add 100 µl (per sample) of the mixture (Hyperactive pG-Tn5/pA-Tn5 Transposon and Dig-300 buffer) from **Step1** to each sample, while gently vortexing to mix throughly the Transposon with ConA beads.

▲ Increased NaCl slows down the binding of Hyperactive pG-Tn5 Transposon to chromatin-openable regions.

▲White precipitate may appear after Dig-300 Buffer is added, which does not affect incubation and cleaning.

4.Place the tubes on a nutator at room temperature for 1 h.

5.Briefly centrifuge to collect the reaction solution at the bottom of the tube. Place the tube on a magnet stand to clear (about 30 s to 2 min), then discard the supernatant.

6.Add 800 µL of Dig-300 Buffer. Invert 10 times or gently vortex to mix the Buffer with ConA beads thoroughly.

7.Repeat **Steps 5** and **Steps 6** twice.

09-7/ Tagmentation

1.Briefly centrifuge to collect the reaction solution at the bottom of the tube. Place the tube on a magnet stand to clear (about 30 s to 2 min), then discard the supernatant.

2.Add 300 µL of Tagmentation buffer, and then vortex gently.

3.Incubate at 37°C for 1 h.

09-8/ DNA extraction

1.To terminate the tagmentation reaction, please add 10 µL of 0.5M EDTA, 3 µL of 10% SDS and 2.5 µLof 20 mg/mL Proteinase K to each sample at room temperature.

2.Mix by vortexing gently, and briefly centrifuge to collect the reaction solution at the bottom of the tube. Then incubate at 50°C for 1 h or at 37°C overnight.

3.Add 300 µL of PCI (Phenol: Chloroform: Isoamyl alcohol = 25:24:1) and mix by high-speed vortexing ~2 s.

▲Do not separate the liquid from the beads, add the PCI directly for DNA fragment extraction.

4.Centrifuge at room temperature 16,000 x g for 5 min.

5.Transfer the upper aqueous layer into the new EP tube, add 300 µL of Chloroform and invert 10 times to mix (do not vortex). Centrifuge at room temperature16,000 x g for 3 min.

6.Remove aqueous layer by pipetting to a fresh 1.5 mL tube containing 750 µL of 100% ethanol, pipetting up and down to mix. Place the tubes on ice.

7.Chill on ice and centrifuge at 4°C 16,000 x g for 15 min.

8.Carefully discard the supernatant and drain on a paper towel.

▲White plaques are usually not visible in this step. When discarding the supernatant, please try to be gentle to reduce DNA fragment loss.

9. Add 1 mL of 100% ethanol to rinse, and centrifuge at 4 °C 16,000 x g for 1 min.

10. Carefully pour off the liquid and drain on a paper towel. Air dry.

11. After the EP tube is dry, add 25 µl - 30 µl of 1 × TE (10 mM Tris-HCl pH 8.0, 1 mM EDTA). Store the sample at -30°C ~ -15°C or directly perform PCR amplification.

09/ Protocol

09-9/ Library Amplification

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

Components	Volume
Purified DNA product	24 µl
ddH_2O	5 µl
5×TAB	10 µl
P5 Primer X[a]	5 µl
P7 Primer X[b]	5 µl
TAE	1 µl
In total	50 µl

a. If using the Primer of TruePrep Index Kit V4 for Illumina (TD204/TD205/TD206/TD207), then P5 Primer X is N101, P7 Primer X is N9XX;

If using the Primer of TruePrep Index Kit V3 for Illumina (TD203), the corresponding P5 Primer X is N6XX, and P7 Primer X is N8XX;

If using the Primer for TruePrep Index Kit V2 for Illumina (Vazyme #TD202), the corresponding P5 Primer X is N5XX, and the P7 Primer X corresponds to N7XX.

Please select according to the number of samples and the index selection strategy.

b. If using a self-matching adapter, add 2 µl of P5 Primer X (10 µM) and P7 Primer X (10 µM).

2.Use a pipette to mix gently, and perform the following reaction in the PCR instrument.

Temperature	Time	Cycles
Hot lid of 105℃	On	
72℃[a]	3 min	
98℃	30 sec	
98℃	15 sec	15 - 20 cycles[b]
60℃	30 sec	
72℃	30 sec	
72℃	5 min	
4℃	Hold	

a. The 72 °C incubation step is used for strand displacement reaction. Do not delete this step.

b. Please choose the appropriate cycle numbers according to the actual situation. If the cell input is high (10,000 - 100,000), 15 cycles - 17 cycles is recommended. If the cell input is low (60 - 10,000), 17 cycles - 20 cycles is recommended.

09-10/ Purification of PCR Products

1.Equilibrate the VAHTS DNA Clean Beads to room temperature. Suspend the beads thoroughly by vortexing. Pipet 60 µl (1.2x) of beads into the PCR products. Mix thoroughly by pipetting up and down for 10 times. Incubate at room temperature for 5 min.

▲The beads are thick, use a pipette to make sure you get enough volume and squirt slowly.

2.After a quick spin, place the tube on a magnet stand. Wait until the solution clarifies (about 5 min). Keep it on the magnet stand and carefully discard the supernatant without disturbing the beads.

3.Keeping the sample on the magnet stand, add 200 µl of freshly prepared 80% ethanol to rinse the beads. DO NOT re-suspend the beads! Incubate for 30 sec at room temperature and carefully discard the supernatant without disturbing the beads.

4.Repeat the Step 3 for a total of two rinses.

5.Open the tube and air-dry the beads for 3 - 5 min.

▲The environmental humidity varies in different areas. The time for drying is different. The magnetic beads just dry and the surface is dull. Excessive drying can cause difficulty in elution. If not completely dried, alcohol residue will affect subsequent experimental reactions.

6.Take the tube out of the magnet stand. Add 22 µl ddH2O. Mix thoroughly by pipetting. Incubate at room temperature for 5 min. If the beads are dry and cracked, extend the incubation time appropriately

7.After a quick spin, place the tube back on the magnet stand and wait until the solution clarifies (about 5 min). Carefully transfer 20 µl of the supernatant to a new tube, stored at -30℃ ~ -15℃.

09/ Protocol

09-11/ Library Quality Control

Library concentrations detection

Detect library yield based on a fluorescent dye method (e.g. Qubit or fluorescent dye PicoGreen) that specifically recognizes double-stranded DNA.

Library distribution detection

Use Agilent Bioanalyzer 2100 (or 2% agarose gel electrophoresis) to detect library fragment distribution.

10/ FAQs and Solutions

◇ What species does CUT&Tag apply to?

CUT&Tag Protocol is suitable for protein-DNA interaction research of conventional mammalian cells. For cells such as yeast and plants, please make adjustments according to the needs (e.g. breaking down cell walls or extracting the nucleus).The operation can refer to the sample treatment methods in CUT & RUN technology.

◇ What is the function of ConA magnetic beads?

ConA magnetic beads are coated with concanavalin A and bound to glycoproteins on the cell membrane to adsorb cells. It can visualize cells to reduce cell loss during processing.

◇ How to choose TD901 and TD902?

The TD901 kit provides a transposon with Protein G and Tn5 fusion.

The TD902 kit provides a transposon with Protein A and Tn5 fusion.

For most antibodies, both Protein G and Protein A have broad applicability, but Protein A has a high affinity for rabbit-derived antibodies, and Protein G has a high affinity for some mammalian antibodies such as mice. Please select according to the affinity of the antibody species to Protein A or Protein G.

◇ Is CUT&Tag only suitable for Illumina platform?

The transposons provided by TD901 and TD902 are designed for the Illumina platform.

For other sequencing platforms, it is recommended to use Vazyme #S602 or #S603 to replace the adaptor and Index amplification primers.