Product Manual

Product Name	pAG-Tn5 Transposase for CUT&Tag
Source	Recombinant expression in Escherichia coli
Catalog Number	CSB-DEM070
Physical Form	Liquid
Storage Conditions	-20° C
Molecular Weight	75 kDa
Storage Buffer	50mM HEPES (pH 7.2), 100mM NaCl, 0.1mM EDTA, 1mM DTT, 0.1% Triton X-100, 50% (v/v) Glycerol
Quality Control	No residual nucleases and exonucleases
Shelf Life	12 months

Product Description

pAG-Tn5 Transposase is a fusion enzyme that combines Protein A/G with modified high-activity Tn5 transposase. It forms a novel fusion enzyme that possesses both transposase and Protein A/G activities. It is suitable for CUT&Tag technology, which is used for protein-genome interaction studies.

CUT&Tag is a new method for studying protein-DNA interactions and offers several advantages over traditional ChIP-seq, including time efficiency, minimal cell requirements, low background signal, high reproducibility, and even applicability in single-cell sequencing. CUT&Tag is a powerful tool for studying protein-chromatin DNA interactions and has significant implications for research in gene regulation and epigenetics.

The Protein A/G fusion in this product mainly interacts with the Fc region of immunoglobulins and can bind to IgG from most mammals.

Product Components

Label	Components	Specifications
1	pAG-Tn5 Transposase(2 mg/mL)	10μL
2	Annealing Buffer	500μL
3	Tagment Buffer (5X)	300μL
4	Stop Buffer (5X)	300μL

Application

(1) Adapter Preparation

1) Synthesize the adapter sequences

ME: 5'-phos-CTGTCTCTTATACACATCT-NH2-3'

P1: 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-3'
P2: 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-3'
Dissolve ME, P1, and P2 in Annealing Buffer to a final concentration of 100μM. Vortex and mix thoroughly to ensure complete dissolution.
2) Prepare Adaptor1 (ME+P1) and Adaptor2 (ME+P2) in the following proportions.

Components	Adaptor1
ME (100μM)	5 μL
P1 (100μM)	5 μL
Total	10μL

Components	Adaptor2
ME (100μM)	5 μL
P2 (100μM)	5 μL
Total	10 μL

Place the tubes containing Adaptor1 and Adaptor2 in a PCR machine with the following settings: 95°C for 3 min; ramp from 95°C to 25°C over 40 min; hold at 4°C. After the reaction, mix equal volumes of Adaptor1 and Adaptor2, name it as "Assembly Adapter Mix," and store at -20°C.

(2) Transposome Generation

Prepare the reaction system as shown in the table below.

Components	Volume
pAG-Tn5 Transposase（2 mg/mL）	5 μL
Adapter Mix（50μM）	3 μL

Gently pipette and mix thoroughly. Incubate at 25°C for 1 hour. The reaction product is named pAG-Tn5 Mix and can be directly used for CUT&Tag experiments or stored at -20°C.

(3) **DNA Fragmentation**

(4) Prepare a 25μl fragmentation Reaction.

Components	Reaction
DNA	50-100 ng
pAG-Tn5 Mix	1 uL
Tagment Buffer (5X)	5 uL
ddH2O	To 25 uL

Gently pipette and mix thoroughly. Incubate in a PCR machine at 55°C for 10 min, followed by holding at 4°C. Then add 5μl of Stop Buffer (5X) and mix thoroughly, followed by incubation at 55°C for 5 min to stop the reaction. The fragmented product can be used for detection or library construction after purification. If the fragmented fragments are too long, increase the amount of transposome to reduce the fragment size, or vice versa.

(4) Amplification of Fragmented Products

1) Prepare the following components in a PCR tube.

Components	Volume
Fragmented DNA	30 μL
5×Pfu buffer	10 μL
N5 Primer（10 μM）	2.5 μL
N7 Primer（10 μM）	2.5 μL
Pfu DNA Polymerasea[a]	1 μL

Total	50 µL

Note:a. PCR reaction requires a non-hot start Taq enzyme, such as CSB-DEM035, CSB-DEM034, CSB-DEM023.

2) Gently pipette and mix thoroughly for 10 times. Perform the following reaction in a PCR machine:

Temperature	Time	Cycle Number
72°C	3 min	1
98°C	30 s	1
98°C 60°C 72°C	15 s 30 s 1 min	X[b]
72°C	5 min	1
4°C	Hold	---

Note: b.The number of amplification cycles can be adjusted based on the input DNA amount and experimental requirements.

(5) Library Length Distribution Analysis

Use 2% agarose gel electrophoresis or Agilent 2100 Bioanalyzer to analyze the length distribution of the library.

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