pAG-Tn5 Transposome

Cat. No. : RK20264

Product components

Components	Component number	Size-1 12 μL	Size-2 48 μL
pAG-Tn5 Transposome	RM20677	12 μL	48 μL

Product Description

The pAG-Tn5 Transposase for CUT&Tag is an innovative fusion enzyme combining the functionalities of both Tn5 transposase and Protein A/G. With a monomeric molecular weight of approximately 75 kD, this enzyme exhibits dual activities attributed to both Tn5 transposase and Protein A/G. It effectively recognizes the inside end (IE), outside end (OE), and mosaic end (ME) sequences of the Tn5 transposon, demonstrating the highest transposition efficiency at the ME sequence.

The mutated variant of Tn5 transposase integrated into this product specifically targets the ME sequence, resulting in a remarkable in vitro transposition efficiency that is 1000 times higher than the wild type. Tn5 Transposase catalyzes a multi-step "cut and paste" transposition reaction. Initially, the enzyme binds the 19-bp ME of the transposon to form a Transposome. The transposome then randomly attacks and cleaves the phosphodiester backbone of the target DNA. Finally, the Tn5 Transposase catalyzes the covalent linkage of the 3'-OH ends of the transposon to the exposed 5'-phosphorylated ends of the target DNA. Transposition creates a 9-bp sequence duplication immediately flanking the transposon insertion site.

Moreover, the fused Protein A/G predominantly interacts with the Fc region of immunoglobulins (Ig), enabling it to bind to IgG molecules from various mammalian species. Consequently, this fusion enzyme is well-suited for conducting Protein-Genome interaction studies utilizing the CUT&Tag technique.

Product Source

pAG-Tn5 transposase gene was induced and expressed in *E. coli* and obtained by separation and purification.

Storage

-20℃

Application

Construction of random library for second-generation sequencing

ATAC-seq

CUT&Tag

Molecular Weight

75 kD

Concentration

Molar concentration: 4 μM

Reaction Conditions

In 1X Tagment Buffer reaction, 37℃ or 55℃ * reaction (reaction temperature varies with product applications)

*** Note: The recommended reaction temperature varies with product applications. The recommended reaction temperature is 55℃ for in vitro experiments and 37℃ for in vivo experiments.**

Termination Reaction

In 1X Termination Buffer, the transposition reaction can be terminated

QC

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No DNase and RNase activity

No exonuclease and endonuclease activity

There is no microbial genome residue in PCR detection

SDS-PAGE detection of protein purity greater than 95%

Product Recommendations

ABclonal offers index primer sets compatible with the Illumina platform for library amplification, available for ordering according to your needs.

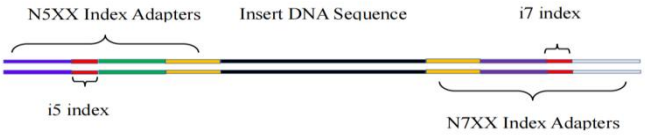
RK20290: Dual DNA Adapter 96 Kit for One-step DNA Lib Prep

RK20265: CUT&Tag Assay Kit (pAG-Tn5) for Illumina

Sequences for N5XX and N7XX:

N5XX 5′-AATGATACGGCGACCACCGAGATCTACAC[XXXXXXXX]TCGTCGGCAGCGTC-3′

N7XX 5′-CAAGCAGAAGACGGCATACGAGAT[XXXXXXXX]GTCTCGTGGGCTCGG-3′



Tn5 linker sequences for illumina sequencing platform

MErev: 5′-phos-CTGTCTCTTATACACATCT-3′

ME-A: 5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-3′

ME-B: 5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-3′

Examples of Applications

1. Transposome activity assay

1.1 DNA Tagmentation

1.1.1 Prepare the following DNA fragmentation system in a sterile PCR tube (you can wait as needed scaling up/down interrupt system):

Components	20 μL
5X Tagment Buffer（RM20250)	4 μL
gDNA *	X ng
pAG-Tn5 Transposome **	1 μL
ddH2O	up to 20 μL

*Note: The amount of gDNA should be adjusted according to experimental requirements. **Note: The amount of pAG-Tn5 Transposome added should be calculated based on the input amount of the template. If subsequent results show excessively large fragmented pieces, it indicates insufficient input of the transposome complex. Conversely, if the fragments are too small, it suggests an excess of the transposome complex input. Adjustments can be made accordingly based on the experimental needs for fragment size.

1.1.2 Use a pipette to pipette up and down to mix thoroughly.

1.1.3 Adjust the hot lid temperature to 75°C，and put the PCR tube on the PCR instrument and perform the following reaction procedure:

Temp	Time	Cycles
55 ℃*	5-15 min *	1
12 ℃	hold	1

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* Note: The reaction time can be appropriately extended according to the needs of the experiment to ensure full reaction.The recommended reaction temperature varies with product applications. The recommended reaction temperature is 55℃ for in vitro experiments and 37℃ for in vivo experiments.

1.2 Termination

3.2.1 When the reaction is over, add 2 μL 6X Termination Buffer(RM20251), vortex to mix up and down to mix well, and incubate at room temperature for 5 min.

*Note: This step is to terminate the fragmentation reaction and separate the transposase and DNA fragments from each other; failure to perform this step will result in lower library yields.

1.3 Amplification

1.3.1 Prepare the PCR reaction in a sterile PCR tube

Components	50 μL
Tagment Products (Step 3.2.1)	22 μL
PCR Mix*	23 μL
N5 Primer（10 μM）	2.5 μL
N7 Primer（10 μM）	2.5 μL
ddH2O	up to 50 μL

* PCR mix requires non-heat-initiated Taq enzymes , such as RM20239, RM20242(ABclonal).

1.3.2 Mix thoroughly by pipetting. Centrifuge quickly, and place in the PCR machine to start the circulation program (hot lid temperature 105 ℃)

The recommended PCR procedure

Step	Temp	Time	Cycles
Fill the Gap	72 ℃	3 min*	1
Predenaturation	98 ℃	30 s	1
Denaturation	98 ℃	15s	
Annealing	60 ℃	30 s	N**
Extension	72 ℃	1min	
The final Extension	72 ℃	5 min	1
Hold	4-12 ℃	hold	1

*Incubation at 72 ℃ for 3 minutes is a process for gap repair, and this step cannot be omitted.

**The number of amplification cycles is adjusted based on the initial amount of DNA input. 13-15cycles for 1ng；11-12cycles for 5ng，7-8cycles for 50ng.

Optimization

1) If the result of the fragmentation reaction is unsatisfactory (such as larger/smaller fragments), you can adjust the amount of assembly adapter and Tn5 transposase in the transposome assembly step, or adjust the amount of transposome in the fragmentation reaction step.

2) The library construction fragments are too large: increase the amount of assembly enzyme and adapter.

3) Small fragments for library construction: reduce the amount of assembly product added, or dilute the Transposome Complex with 1X Tn5 Dilution Buffer, and then interrupt and prepare the library. The volume of theTransposome Complex and the dilution factor are adjusted according to the amount of experimental substrate.

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