pAG-Tn5 Transposome

Cat. No. : RM20677

Product components

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

Product Description

pAG-Tn5 is a fusion protein combining Protein A/Protein G with a hyperactive mutant form of Tn5 transposase, thereby possessing the functionalities of both Tn5 transposase and Protein A/Protein G. The monomeric molecular weight of pAG-Tn5 transposase is approximately 75 kDa. It is capable of recognizing the Inside End (IE), Outside End (OE), and Mosaic End (ME) sequences of the Tn5 transposon enzyme, though it exhibits the highest transposition efficiency when recognizing the ME sequence fragment.

This product incorporates a mutant form of Tn5 transposase that recognizes the ME sequence, demonstrating an *in vitro* transposition efficiency approximately 1000-fold higher than the wild-type enzyme. The Tn5 transposase binds to the 19 bp ME sequence to form a transpososome. This transpososome then randomly attacks and cleaves the phosphodiester bonds of target DNA. Finally, the Tn5 transposase catalyzes the formation of a phosphodiester bond between the 3'-OH end of the ME sequence and the exposed 5 ' -phosphorylated end of the target DNA, completing the transposition reaction (DNA fragmentation or DNA insertion). The transposition reaction generates a 9 bp gap in the complementary DNA strand at the insertion site.

The fused Protein A/Protein G primarily interacts with the Fc region of immunoglobulins (Ig), enabling it to bind to IgG from most mammals. This product is a pre-assembled pAG-Tn5 transposase complex ready for direct use as a core enzyme replacement in CUT&Tag experiments.

Product Source

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

Storage

-20℃

Application

Lib preparation for NGS

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 is 55℃ for *in vitro* experiments and 37℃ for *in vivo* experiments.**

Termination Reaction

In Termination Buffer, the transposition reaction can be terminated.

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Product Recommendations

ABclonal offers index primer kit compatible with the Illumina platform for library amplification:

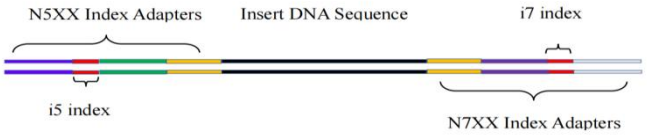
RK20290: Dual DNA Adapter 96 Kit for One-step DNA Lib Prep

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′

Operation Description

1. DNA Tagmentation

(1) Prepare the following DNA fragmentation reaction in a sterile PCR tube (User can proportionally scaling up/down the reaction volume):

Components	20 μL
5X Tagment Buffer（RM20250)	4 μL
gDNA *	X μL
pAG-Tn5 Transposome **	1 μL
ddH_2O	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 resulting fragments are larger than desired, this indicates excessive template DNA or insufficient transposome. Conversely, if fragments are too small, increase template DNA or decrease transposome input. User can adjust input of transoposome complex based on the target fragment size.

(2) Mix thoroughly by pipetting up and down.

(3) Place PCR tubes in a thermal cycler (with heated lid set to 75°C) and run the following program:

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

*Note: The recommended reaction temperature is 55°C for *in vitro* experiments and 37°C for *in vivo* experiments.

**Note: Reaction time can be appropriately extended as needed to ensure complete reaction.

2. Termination

(1) Immediately after fragmentation, add 2 μL of Termination Buffer (RM20251) and mix thoroughly by vortexing or pipetting up and down.

(2) Incubate at room temperature for 5 min to stabilize fragment size distribution.

*Note: This step terminates the fragmentation reaction by dissociating the transposase from the DNA fragments. Skipping this step will result in reduced library yield.

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3. Amplification

(1) Prepare the PCR reaction in a sterile PCR tube:

Components	50 μL
Tagmented DNA	22 μL
PCR Mix *	23 μL
N5 Primer (10 μM)	2.5 μL
N7 Primer (10 μM)	2.5 μL
ddH_2O	up to 50 μL

*Note: The PCR Mix must use a non-hot-start polymerase , such as RM20242 (ABclonal).

(2) Mix thoroughly, centrifuge briefly, then place in a thermal cycler (with heated lid set to 105℃) and initiate the cycling program.

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

*Note: Incubation at 72℃ for 3 min is a process for gap repair, and this step can not be omitted.

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

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