Tn5 Transposase

Cat. No. : RK20547

Product components

Components	Component number	Size-1 20 μg	Size-2 100 μg
Tn5 Transposase	RM21303	20 μL	100 μL
1X Tn5 Dilution Buffer	RM20188	1 mL	2 × 1 mL
Assemble Buffer	RM20187	200 μL	1 mL
5X Tagment Buffer	RM20250	200 μL	1 mL
Termination Buffer	RM20251	200 μL	1 mL
Annealing Buffer	RM20821	200 μL	1 mL

Product Description

Tn5 Transposase is a highly active, mutant form of Tn5 transposase. This efficient enzyme inserts Tn5 transposons into any target DNA in vitro. The monomeric molecular weight of Tn5 Transposase is approximately 55 kD. It recognizes the Inside End (IE), Outside End (OE), and Mosaic End (ME) sequences of Tn5 transposons, though it exhibits the highest transposition efficiency when recognizing ME sequences.

This product is a mutant Tn5 transposase that recognizes ME sequences, demonstrating ~1000-fold higher i*n vitro* transposition efficiency compared to the wild-type enzyme. Tn5 transposase binds to the 19 bp ME sequence to form a Transposome. This transposome then randomly attacks and cleaves the phosphodiester bonds of target DNA. Finally, Tn5 transposase catalyzes the formation of a phosphodiester bond between the 3'-OH end of the ME and the exposed 5'-phosphorylated end of the target DNA, thereby completing the transposition reaction (DNA fragmentation or DNA insertion). The transposition reaction generates a 9 bp gap on the complementary DNA strand opposite the insertion site.

Tn5 Transposomes possess highly random insertion activity and are widely used in fields such as in vitro transgenics (integration of exogenous genes into host cells) and NGS library preparation.

Product Source

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

Storage

-20℃

Application

In vitro transgenesis (integration of foreign genes into host cells)

Next-generation sequencing (NGS) library construction

Construction of a transposon random insertion library

Molecular Weight

55 kD

Reaction Conditions

React in 1X Tagment Buffer at 37℃ or 55℃* (Use different reaction temperatures for different applications).

***Note: Recommended reaction temperatures vary for different applications. For *in vitro* experiments, reaction at 55℃ is recommended; for *in vivo* experiments, reaction at 37℃ is recommended.**

Termination Reaction

In Termination Buffer, the transposition reaction can be terminated.

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Operation Description

1. Prepare Adapter Mixes

1.1. Synthesize Tn5 adapter sequences compatible with the Illumina sequencing platform.

MErev: 5′-phos-CTGTCTCTTATACACATCT-3′

ME-A: 5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-3′

ME-B: 5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-3′

1.2. Dilute primers to 100 μM each using 1X TE Buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

1.3. Prepare the following reaction mixtures separately:

MixA	10 μL	MixB	10 μL
Annealing Buffer	2 μL	Annealing Buffer	2 μL
ME-A (100 μM)*	4 μL	ME-B (100 μM)*	4 μL
MErev (100 μM)	4 μL	MErev (100 μM)	4 μL

*Note: The reaction system may be modified and adapter sequences for ME-A and ME-B designed as needed.

1.4. Mixed thoroughly. After short-time centrifugation, follow the reaction procedure.

Temp	Time	The rate of change in temperature	Cycles
95 ℃	2 min	1℃/s	1
22 ℃	5 min	0.1℃/s	1
4 ℃	hold	1℃/s	1

1.5. After the reaction, combine equal volumes of MixA and MixB and mix thoroughly to prepare the Adapter Mix (40 μM). Store at -30℃ to -15℃.

2. Prepare Assembled Transposome

2.1. Prepare the following reaction system (scale up/down according to actual input amount):

Components	30 μL
Adapter mix (40 μM)	2.62 μL
Tn5 Transposase *	8.25 μL
Assemble Buffer	10.5 μL
ddH_2O	Up to 30 μL

*Note: The molarity of Tn5 Transposase is approximately 18.2 uM; The assembly ratio of proteins and connectors can be adjusted according to experimental needs;

2.2. Mix thoroughly, then incubate in a metal bath or thermal cycler at 35℃ for 2 hours. After incubation, remove and place on ice. The assembled product can be directly used for subsequent DNA fragmentation reactions.

2.3. For long-term storage at -20℃, adjust the final glycerol concentration in the assembled product to 50% （Replace ddH2O in the assembly system with glycerol）. Note that 5X Assemble Buffer contains 20% glycerol, and Tn5 Transposase is stored in 50% glycerol buffer.

3. Transposome Activity Assay

3.1. DNA Tagmentation

3.1.1. Prepare the following DNA fragmentation system in a sterile PCR tube (scale up/down proportionally as needed):

Components	20 μL
5X Tagment Buffer	4 μL
gDNA *	50-100 ng
Transposome/assembly product *	1 μL

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ddH_2O	Up to 20 μL

*Note: The input amount of assembled transposome should be optimized based on both template DNA quantity and transposome concentration used during assembly. 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. Titration adjustments should be performed based on experimental requirements for fragment size distribution.

3.1.2. Pipette up and down to mix thoroughly.

3.1.3. Place PCR tubes in a thermal cycler (with heated lid set to 75℃) and run the following program:

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

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

3.2. Termination

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

3.2.2. 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.

4. Amplification

4.1. Prepare the PCR reaction in a sterile PCR tube:

Components	50 μL
Tagmented DNA (Step 3.2.2)	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 RM20239, RM20242 (ABclonal).

4.2. Mix thoroughly, centrifuge briefly, then place in a thermal cycler (with heated lid set to 105°C) 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	
The final Extension	72 ℃	5 min	1
Hold	4-12 ℃	hold	1

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

**The number of amplification cycles is adjusted based on the initial amount of DNA input.

Optimization

1. For suboptimal fragmentation results (e.g., oversized/undersized fragments): Optimize adapter and Tn5 transposase inputs during transposome assembly, or adjust transposome input during the fragmentation reaction.

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2. For larger-than-desired library fragments: Increase transposase and adapter inputs.
3. For smaller-than-desired library fragments: Reduce assembled transposome input.

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