Tn5 Transposase User Manual

Tn5 Transposase

Cat. No: PC047 (20 µl) , PC048(100 µl)

User Manual

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Poly(A) Polymerase

I. Description

Tn5 Transposase efficiently catalyzes the random insertion of Tn5 transposons into target sequences, exhibiting extremely high transposition insertion efficiency for both eukaryotic and prokaryotic DNA.

The enzyme specifically recognizes the inverted repeat Mosaic End (ME) sequences flanking the transposon. It recognizes DNA fragments bearing chimeric ME sequences at both ends (including primers containing ME sequences) and forms a Tn5 transposome. This transposome can randomly bind target DNA, cleave it, and insert the carried DNA fragment. Tn5 Transposase is widely used in in vitro transgenesis and next-generation sequencing (NGS) library preparation.

■ Source

Recombinant protein expressed in E. coli.

■ Quality Assurance

Free of host DNA contamination. Free of endonuclease and exonuclease contamination.

■ Applications

✧ Fragmentation and adapter addition for next-generation sequencing library preparation.
✧ Construction of random transposon insertion mutant libraries in vitro or in vivo.
✧ Rapid sequencing of large DNA molecules (e.g., BAC clones).
✧ Insertion of antibiotic resistance markers into target DNA.

II. Contents and Storage

Cat. No.	Contents	Part No.	Quantity
PC047	Tn5 Transposase	PC047-01	20 μl
	5× Tn5 Reaction Buffer	PC047-02	200 μl
	5×Stop Buffer	PC047-03	200 μl
PC048	Tn5 Transposase	PC047-01	20 μl×5
	5× Tn5 Reaction Buffer	PC047-02	200 μl×5
	5×Stop Buffer	PC047-03	200 μl×5

Store all components at -20℃ (stable for at least 12 months).Avoid repeated freezing/ thawing.

III. Procedures

1.In Vitro Transposon Insertion Reaction

1. Tn5 transposon DNA is the inclusion of the 19-bp Me sequence flanking both ends of the DNA.This is appened to the transposon via PCR amplification with primers containing5′-phosphorylated ME-sequence .
   ME sequence: 5′-pCTGTCTCTTATACACATCT-3′

2. Prepare the transposon insertion mixture as follows:

Reagent	Volume
5× Reaction buffer	4 µl
Target DNA *	200-400ng
Tn5 Transposase	1 µl
Equimolar transposon	X µl
ddH2O	To 10 µl

Note: **Calculate the molar amount of target DNA in the reaction and add an equimolar amount of the Tn5 transposon fragment to avoid excess insertions.*

µmol target DNA = µg target DNA / [(base pairs in target DNA) × 660]

Example: 0.2 µg of a 1000-bp target DNA = 0.2µg/ [1000bp×660]=0.3×10^{-6}µmol = 0.3pmol.

Target DNA must be free of exogenous nucleic acid contamination.

3. Mix thoroughly and incubate at 37°C for 2 hours.

4. Add 2 µl of 5× Stop Buffer (thaw at room temperature), mix, and incubate at 55°C for 10 min to terminate the reaction..

2. Construction of Transposome Complex and Fragmentation for Next-Generation Sequencing

1. Construction of the Transposome Complex

① ME and adapter sequences:

Adapter 1: 5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-3′

Adapter 2: 5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-3′

ME: 5′-pCTGTCTCTTATACACATCT-NH_2-3′ (5′-phosphorylated, 3′-amino modifier)

② Preparation of the two adapters:

Mix equal volumes of 100 µM ME and 100 µM Adapter 1; separately, mix equal volumes of 100 µM ME and 100 µM Adapter 2. Anneal by slow cooling in a thermal cycler to obtain Adapter 1 and Adapter 2, each at 50 µM.

PCR program: 95°C for 3 min; ramp from 95°C to 25°C over 45 min; hold at 4°C.

③ Transposome assembly:

Mix equal volumes of Adapter 1 and Adapter 2 to obtain the adapter mix. Gently mix the enzyme and the adapter mix by pipetting and incubate at room temperature for 1 h.

A 1:1 molar ratio of enzyme to adapter is typically used; the adapter proportion may be increased if needed.

For a 1:1 molar ratio, prepare as follows:

Reagent	Volume
Double-stranded adapter mix (50 pmol/μl)	4 μl
Tn5 Transposase (10pmol/μl)	20 μl

The assembled transposome can be used directly for fragmentation assays or stored at -20°C.

2. Fragmentation Reaction

Set up a 20 μl fragmentation reaction:

Reagent	Volume
5× Reaction buffer	4 μl
DNA	50-100ng
Transposome complex	1 μl
ddH_2O	To 20 μl

3. Mix by pipetting and incubate at 55°C for 10 min (use a heated lid).
4. After the reaction, add 5 μl of 5× Stop Buffer (thaw at room temperature), mix, and incubate at 55°C for 5 min to terminate the reaction.

The fragmented products can be analyzed directly or purified for library construction. If the resulting fragments are too large, increase the amount of transposome complex to reduce fragment size; conversely, decrease the amount of transposome complex if fragments are too small.

FAQ:

Q1: The fragment size distribution is unsatisfactory-fragments are too large or too small.

A: Test a gradient of enzyme amounts and incubation times to determine the optimal conditions for the desired fragment size.

Q2: Low transposition efficiency and insufficient library yield.

A: Improper storage or repeated freeze-thaw cycles of Tn5 may reduce activity; therefore, aliquot storage and avoidance of repeated freeze-thaw are recommended. Alternatively, inhibitors (e.g., SDS, EDTA, high salt concentrations) may be present in the sample-purify the sample before proceeding.s.

IV. Limited Use License and Warranty

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