Tinzyme Co., Limited

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PA-Tn5 Transposase

Product Number: TN5T01

Shipping and Storage

Store at -80℃. Transportation conditions: ≤ 0℃.

Components

Component	200pmol	1000pmol
PA-Tn5 Transposase, (10pmol/μL)	20μL	100μL
5× Reaction Buffer	1mL	1mL
5 ×Stop buffer	1mL	1mL

Enzyme storage solution: 50mM HEPES (pH7.2), 100mM NaCl, 0.1mM EDTA, 1mM DTT, 0.1% Triton X-100, 50% (v/v) Glycerol.

Reaction Buffer (5×): 50mM HEPES (pH7.2), 500mM NaCl, 50mM $MgCl_2$.

Stop Buffer (5×): 50mM EDTA (pH8.0).

Description

PA-Tn5 Transposase， It is a novel fusion enzyme (pA-Tn5 Transposase) that combines Protein A with a modified ultra-high activity Tn5 transposase to form a dual activity enzyme. It can efficiently insert Tn5 transposons randomly into target sequences. PA-Tn5 Transposase can specifically recognize DNA fragments containing chimeric end sequences (ME) at both ends (including primers containing ME sequences), ultimately forming Tn5 Transposomes, which can randomly bind to target DNA and cleave and insert DNA fragments carried by it. Tn5 transposase is widely used in fields such as in vitro transgenic (integration of exogenous genes into host cells) and next-generation sequencing (NGS) library construction.

Application

This product can be used for fragmentation and adapter addition during the construction of next-generation sequencing (NGS) libraries; Introducing sequencing primers into cloned DNA or plasmids; Establishment of bacterial gene knockout library; Engineering transformation of new bacterial strains; Insertion inactivation of target genes; Insert T7 transcription promoter, resistance markers, etc. into target DNA, etc.

Unit Definition

PA-Tn5 transposase refers to the amount of enzyme required to completely cleave 1ug of DNA fragments containing recognition sequences under 37℃ conditions for 1 hour, defined as 1 unit (U).

Protocol

1. Constructing a second-generation sequencing library:
   1.1. Constructing transposable complexes
      1.1.1. Freeze dried powder dissolution: Freeze dried powders of single chain ME, single chain adapter1, and single chain adapter2 were centrifuged at high speed for 1 minute and dissolved in annealed Annealing Buffer at a concentration of 100μM/L. Vortex and mix thoroughly to ensure that the joint is fully dissolved.
      1.1.2. Prepare two types of adapters: equal volume mixing of ME and adapter1, equal volume mixing of ME and adapter2, and slow cooling annealing of the PCR instrument to obtain adapters 1 and 2 with a concentration of 50μmol. PCR setting: 95℃ for 3 minutes; 95℃ to 25℃, 45 minutes; 4℃Hold.
      1.1.3. Composite preparation: Mix joint 1 and joint 2 in equal volumes to obtain a mixed joint; Mix the enzyme and adapter evenly, and incubate at room temperature for 1 hour. The usual molar ratio of enzyme to adapter is 1:1,

and the incubation ratio of adapter can be increased as needed. If the molar ratio of enzyme to linker is 1:1, the following configuration is used:

Component	Volume
Double chain joint mixture (50pmol/μL)	4μL
PA-Tn5 Transposase (10pmol/μL)	20μL

The prepared transposon can be used for fragmentation experiments and can also be stored at -20℃.

1.2. Fragmentation effect testing

Configure a 20μL fragmented system

Component	Volume
5× Reaction buffer	4μL
DNA	50-100ng
Transposable complex	1μL
ddH_2O	To 20μL

After blowing and mixing the system, react at 55℃ for 10 minutes, then add 5μL of 5 × Stop Buffer. After mixing, react at 55℃ for 5 minutes to terminate the reaction. Fragmentation products can be used for detection or purification before library construction. If the interrupted fragment is too long, the amount of transposable complex can be increased to reduce the fragment size, otherwise, the amount of transposable complex can be reduced.

Sequence of ME and adapters:

ME: 5'-pCTGTCTCTTATACACATCT-3'

Primer 1: 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-3'

Primer 2: 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-3'

Note: This connector sequence refers to Nextera® DNA Sample Preparation Kit (Illumina, FC-121-1030), It can also be designed according to the requirements of different high-throughput sequencing.