

## Product Manual

|                    |                                                                                             |
|--------------------|---------------------------------------------------------------------------------------------|
| Product Name       | Tn5 Transposase                                                                             |
| Source             | Recombinant expression in Escherichia coli                                                  |
| Catalog Number     | CSB-DEM076                                                                                  |
| Physical Form      | Liquid                                                                                      |
| Storage Conditions | -20° C                                                                                      |
| Molecular Weight   | 67.9 k kDa                                                                                  |
| Storage Buffer     | 50mM HEPES (pH 7.2), 100mM NaCl, 0.1mM EDTA, 1mM DTT, 0.1% Triton X-100, 50% (v/v) Glycerol |
| Quality Control    | No residual nucleases and exonucleases                                                      |
| Shelf Life         | 12 months                                                                                   |

### Product Description

Tn5 Transposase is a highly active mutant variant derived from E.coli that has been modified to efficiently insert Tn5 transposons into target sequences. It exhibits high transposition insertion efficiency for both eukaryotic and prokaryotic DNA. Tn5 Transposase specifically recognizes DNA fragments with chimeric end sequences, forming Tn5 transposomes. These transposomes randomly bind to target DNA and cleave to insert their carried DNA fragments. This product is also used for constructing second-generation sequencing library fragments during the fragmentation and adapter ligation steps.

### Product Components

| Label | Components                 | Specifications |
|-------|----------------------------|----------------|
| 1     | Tn5 Transposase (10μM)     | 20μL           |
| 2     | Tagment Buffer (5X)        | 200μL          |
| 3     | Stop Buffer (5X)100mM EDTA | 300μL          |

### Application

Construction of Tn5 transposomes required for second-generation sequencing

#### (1) Adapter Preparation

Synthesize the adapter sequences

ME: 5'-phos-CTGTCTTATACACATCT-NH2-3'

P1: 5'-TCGTCCGCGATGATGTGTATAAGAGACAG-3'

P2: 5'-GTCTCGTGGCTCGGAGATGTGTATAAGAGACAG-3'

Dissolve all sequences in a concentration of 100 μM. Vortex thoroughly to ensure complete dissolution

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## (2) Prepare Adapter 1 and Adapter 2

Adaptor1 (ME+P1) and Adaptor2 (ME+P2) should be prepared according to the proportions in Table below.

| Component                      | Concentration | Volume       |
|--------------------------------|---------------|--------------|
| ME (100 $\mu$ M)               | 40 $\mu$ M    | 10 $\mu$ L   |
| P1 or P2 (100 $\mu$ M)         | 40 $\mu$ M    | 10 $\mu$ L   |
| Tris-HCl buffer (pH 8.0) (1 M) | 10 mM         | 0.25 $\mu$ L |
| Total Volume                   |               | 25 $\mu$ L   |

Set up the annealing reaction program on a PCR machine: 95 $^{\circ}$ C, 3 min, gradually decrease to 25 $^{\circ}$ C at a rate of 0.1 $^{\circ}$ C/s.

## (3) Preparation of Tn5 transposomes

Mix Tn5 Transposase, Adapter 1, and Adapter 2 in a molar ratio of 1:0.5:0.5 according to the table below. Vortex and incubate at room temperature for 1 hour. Adjust the mixing ratio of Tn5 Transposase as needed, but ensure the concentrations of Adapter 1 and Adapter 2 remain consistent. Prepared Tn5 transposomes can be directly used for DNA fragmentation experiments or stored at -20 $^{\circ}$ C.

| Component                    | Volume    |
|------------------------------|-----------|
| Tn5 Transposase (10 $\mu$ M) | 8 $\mu$ L |
| Adapter 1 (40 $\mu$ M)       | 1 $\mu$ L |
| Adapter 2 (40 $\mu$ M)       | 1 $\mu$ L |

## (4) Fragmentation efficiency test

Set up a 25  $\mu$ L fragmentation reaction.

| Component           | Volume    |
|---------------------|-----------|
| DNA                 | 50-100 ng |
| Tn5 transposomes    | 0.5-2 uL  |
| Tagment Buffer (5X) | 5 uL      |
| ddH <sub>2</sub> O  | To 25 uL  |

After vortexing, react at 55 $^{\circ}$ C for 10 minutes, then add 5  $\mu$ L of 5 $\times$ Stop Buffer, mix, and continue the reaction at 55 $^{\circ}$ C for 5 minutes to terminate the reaction. Fragmentation products can be detected by agarose gel electrophoresis or library construction after purification. If the fragments are too long, increase the amount of transposome to reduce the fragment size, and vice versa, decrease the amount of transposome.