# Tn5 Transposase for Tagmentation

## Cat. No. E094

Store E094-1 at -80°C. All other components store at -20°C.

## Product Description

**abm**'s **Tn5 Transposase for Tagmentation** is comprised of our hyperactive Protein A-tagged Tn5 transposase for use in generating Next Generation Sequencing (NGS) libraries. This enzyme **has the unique ability to fragment DNA (genomic DNA, plasmid DNA, and PCR amplicons) and add Illumina-compatible sequencing adaptors.** The technology shows a high rate of reproducibility, requires low DNA input, and can be applied for various research applications, including stem cells, tumors, epigenetics, etc.

| Product Component | Quantity | Part No. |
|---|---|---|
| Hyperactive pA-Tn5 Transposase (500ng/µl) | 60µl (100 rxn) | E094-1 |
| Annealing Buffer | 1.25ml | E094-2 |
| Coupling Buffer | 200µl | E094-3 |
| 5X Tagmentation Buffer | 500µl | E094-4 |

## Additional Materials (User Provided)

1. A fully functioning DNA transposase consists of an enzyme flanked by terminal inverted repeat oligonucleotides. For added flexibility, the Hyperactive pA-Tn5 Transposase is not pre-loaded with the oligonucleotides (not included). The sequences contain 19-mer ends that are recognized by the transposase and can be amplified with Illumina-compatible barcoded i7/i5 primers.

   Primer A：5'-phos-CTGTCTCTTATACACATCT-NH2-3'
   Primer B：5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-3'
   Primer C：5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-3'

2. DNA Magnetic Purification Beads (Cat. No. G950/G951) and magnetic stand
3. Ethanol
4. MegaFi™ Pro Fidelity 2X PCR MasterMix (Cat. No. G887)
5. Illumina-compatible i7 and i5 index primers (5µM)



Example:

N701: 5'-CAAGCAGAAGACGGCATACGAGATTCGCCTTA GTCTCGTGGGCTCGG-3'

N501: 5'-AATGATACGGCGACCACCGAGATCTACACTAGATCGCTCGTCGGCAGCGTC-3'

## Protocol

### A. Preparation of Adaptor Mix

1. Dissolve Primer A, Primer B and Primer C with Annealing Buffer to 100µM.

2. Set up the following mixtures in separate PCR tubes:

| Mixture 1 | Volume |
|---|---|
| Primer A (100µM) | 10µl |
| Primer B (100µM) | 10µl |
| Total | 20µl |

| Mixture 2 | Volume |
|---|---|
| Primer A (100µM) | 10µl |
| Primer C (100µM) | 10µl |
| Total | 20µl |

3. Briefly vortex mixtures thoroughly and spin down. Place the tubes in a thermocycler and run the following annealing program:

| Temperature | Time |
|---|---|
| 75°C | 15 min |
| 60°C | 10 min |
| 50°C | 10 min |
| 40°C | 10 min |
| 20°C | 30 min |

4. Combine equal volumes of annealed Mixture 1 and Mixture 2. This Adaptor Mix can be stored at -20°C for up to 1 month.

### B. Transposon Loading/Assembly

1. Briefly flick the tubes of Coupling Buffer and the Hyperactive pA-Tn5 Transposase to mix well before use, and keep the enzyme on ice.

2. Add the following to a new PCR tube to make the Coupling Mixture:

| Component | 2µg preparation (7 reactions) | 10µg preparation (35 reactions) |
|---|---|---|
| Adaptor Mix | 0.7µl | 3.5µl |
| Hyperactive pA-Tn5 Transposase (500ng/µl) | 4.0µl | 20µl |
| Coupling Buffer | 2.8µl | 14µl |
| Total | 7.5µl | 37.5µl |

3. Mix thoroughly by pipetting 20 times, then briefly spin down.

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4. Incubate in a thermocycler at 16°C for 1 hour to generate the Coupling Mixture.
5. The Coupling Mixture can be used immediately, or stored at -20°C for up to 1 month.

**C. Transposon Reaction**

1. Thaw the 5X Tagmentation Buffer at room temperature, and briefly mix and spin down.
2. Add the following components in order into a new PCR tube for DNA tagmentation:

| Component | Volume |
|---|---|
| 5X Tagmentation Buffer | 4.0µl |
| DNA | Variable (50-100ng) |
| Coupling Mixture | 1.0µl |
| Nuclease-Free Water | Up to 20µl |

3. Mix thoroughly by pipetting up and down 20 times.
4. Incubate at 55°C for 8 minutes in a thermocycler, followed by cooling to 4°C.

**D. Purify Tagmented DNA**

1. Bring the DNA Magnetic Purification Beads to room temperature before use.
2. Freshly prepare 350µl of 80% ethanol for each sample.
3. Vortex Purification Beads until fully homogenized.
4. Add 40µl of beads to each sample (2:1 Beads:Sample ratio) and pipette mix thoroughly.
5. Incubate at room temperature for 10 minutes, then place on a magnetic stand until the liquid is clear.
6. Use a pipette to remove all supernatant from each well.
7. Add 150µl of 80% ethanol to each tube, incubate for 30 seconds and remove the supernatant. Repeat this wash step.
8. Remove excess ethanol with a pipette and allow the beads to air dry for 2-5 minutes. Do not over-dry.
9. Remove the tube from the magnetic stand and add 27µl of nuclease-free water.
10. Pipette mix thoroughly to resuspend the beads and incubate at room temperature for 8 minutes to elute the fragmented DNA.
11. Place the tubes on the magnetic stand and incubate for 2 minutes until the liquid is clear.
12. Transfer 25µl of the supernatant to a new tube.

**E. PCR Amplification of Tagmented Product**

1. Add the following components to the purified tagmented product:

| Component | Volume |
|---|---|
| Purified Tagmented DNA | 25µl |
| Index Primer with Adaptor i7 (5µM) | 5.0µl |
| Index Primer with Adaptor i5 (5µM) | 5.0µl |
| 2X PCR MasterMix | 35µl |
| Total | 70µl |



2. Run the following program on the thermocycler with a 100°C preheated lid:

| Step | Temperature | Time | Cycles |
|---|---|---|---|
| Adaptor Extension | 72°C | 3 min | 1 |
| Initial Denaturation | 95°C | 3 min | 1 |
| Denaturation | 95°C | 10 sec | 8-12 |
| Annealing | 60°C | 30 sec | |
| Extension | 72°C | 30 sec | |
| Final Extension | 72°C | 5 min | 1 |
| Hold | 10°C | ∞ | ∞ |

3. Purify Amplified DNA libraries using the same procedure as outlined in Step 4, but with 56µl of beads per sample (0.8:1 Beads:Sample ratio). Elute the final library in 25µl nuclease-free water or 10mM Tris-HCl, pH 8.0.

4. Analyze libraries with a Qubit Fluorometer and Agilent 2100 Bioanalyzer to confirm quantity, size distribution and quality prior to sequencing. The library size distribution should be between 300bp to 700bp and free of adaptor dimers (~100bp) for ideal sequencing.

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