

## Anti-rabbit Tn5

### Product description

Anti-rabbit Tn5 is a fusion protein combining a nanobody with the Tn5 transposase, designed for use in Multi-CUT&Tag experiments. This product is compatible with rabbit primary antibodies in CUT&Tag assays. It is supplied as a bare enzyme, and users must assemble their own adapter sequences.

### Specifications

|         |                                   |
|---------|-----------------------------------|
| Cat NO. | 12594ES04 / 12594ES12 / 12594ES48 |
| Size    | 4 T / 12 T / 48 T                 |

### Components

| Components No. | Name             | 12594ES04 | 12594ES12 | 12594ES48 |
|----------------|------------------|-----------|-----------|-----------|
| 12594-A        | Anti-rabbit Tn5  | 4 µL      | 12 µL     | 48 µL     |
| 12594-B        | Annealing Buffer | 1000 µL   | 1000 µL   | 1000 µL   |
| 12594-C        | Assemble Buffer  | 4 µL      | 12 µL     | 48 µL     |

### Storage

Shipped on dry ice. This product should be stored at -85~-65°C for one year.

### Notes

1. The transposase is temperature-sensitive. Upon receipt, promptly complete adapter loading to generate active transposomes. The assembled transposomes can be stored at -25°C to -15°C for up to 1 year.
2. For your safety and health, please wear a lab coat and disposable gloves during handling.
3. This product is for research use only.

### Instructions

The following protocol uses high-throughput sequencing library preparation as an example. Please read these instructions carefully before use.

#### 1. Adapter Preparation (User-supplied Sequences Required)

##### 1) Reference primer names and sequences for Illumina platforms:

Primer A-1:

5'-AATGATACGGCGACCACCGAGATCTACACATATCCTTCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-3'

Primer A-2:

5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-3'

Primer B:

5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-3'

Primer C:

5'-phos-CTGTCTCTTATACACATCT-3'

2) Dissolve Primer A, Primer B, and Primer C in Annealing Buffer to a final concentration of 200  $\mu$ M.

3) Prepare the following two annealing reactions:

| Component              | Reaction 1 |
|------------------------|------------|
| Primer B (200 $\mu$ M) | 10 $\mu$ L |
| Primer C (200 $\mu$ M) | 10 $\mu$ L |
| Total                  | 20 $\mu$ L |

| Component                       | Reaction 2 |
|---------------------------------|------------|
| Primer A-1 or A-2 (200 $\mu$ M) | 10 $\mu$ L |
| Primer C (200 $\mu$ M)          | 10 $\mu$ L |
| Total                           | 20 $\mu$ L |

4) Vortex each reaction thoroughly and briefly centrifuge to collect liquid at the bottom of the tube. Place tubes in a thermal cycler and run the following program:

Heated lid: ON at 105°C

94°C for 2 min

Immediately stop the program after heating. Do not open the lid. Allow tubes to cool naturally inside the instrument for 2 hours, then incubate at 4°C for 5 min.

5) After annealing, mix Reaction 1 and Reaction 2 in equal volumes. Mix well and label as Adapter Mix. Store at –25°C to –15°C.

## 2. Transposome Assembly

1) Prepare the following assembly reaction:

| Component                | Volume ( $\mu$ L) |
|--------------------------|-------------------|
| Anti-rabbit Tn5          | 4                 |
| Adapter Mix (50 $\mu$ M) | 0.6               |
| Assemble Buffer          | 3.4               |

2) Gently pipette up and down to mix thoroughly. Incubate at 25°C for 1 hour (with heated lid OFF). The resulting transposome can be used directly in library construction or stored at –25°C to –15°C.