# TIANSeq End Repair/dA-Tailing Module

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# TIANSeq End Repair/dA-Tailing Module

**Cat.no. 4992352/4992353**

## Kit Contents

| Contents | 4992352 (24 rxn) | 4992353 (96 rxn) |
|---|---|---|
| 5×ERA Enzyme Mix | 240 µl | 960 µl |
| 10×ERA Buffer | 120 µl | 480 µl |
| Nuclease-Free dd$H_2O$ | 1 ml | 4×1 ml |
| Handbook | 1 | 1 |

## Storage Conditions

TIANSeq End Repair/dA-Tailing Module could be stored at -30~-15°C for one year. Avoid repeated freezing and thawing.

## Product Description

TIANSeq End Repair/dA-Tailing Module is an optimized enzyme mix to perform DNA end-repair and dA-tailing in one step for a library construction of illumina platform. The ends of double-stranded DNA, fragmented by covaries, chemical method or enzymatic method, can all form blunt ends using this kit. And 5'-P and 3' dA will be added at the both blunt ends of fragmented DNA. The obtained product could proceed directly to adapter ligation using TIANseq Fast Ligation Module(Cat# 4992354/4992355) without purification.

The kit adopts a one-step reaction process, eliminating the need for a multi-step purification step, which can perform efficient, rapid end-repair and dA-tailing on very low input DNA samples. This kit ensures simpler operation and more efficient library construction. Application: ideal choice for end-repair and 3'-end dA-tailing of double-stranded DNA for the NGS library construction of illumina high throughput sequencing platform.

DNA Input amount: 0.25 ng-1 μg DNA.

## Recommend Alternative Reagents

1. TIANseq Fast Ligation Module(Cat# 4992354/4992355)
2. TIANSeq NGS Library Amplification Module (Cat# 4992373/4992374)
3. TIANSeq Single-Indexed Adapter (Illumina)(Cat# 4992641/4992642/4992378)
4. TIANSeq Size Selection DNA beads(Cat# 4992358/4992359/4992979)

## Product Highlights

1. Enzyme-based reaction in a single tube. End-repair of double-stranded DNA fragments and dA-tailing reaction can be achieved in one step.
2. High library construction efficiency can be achieved with the DNA input as low as 0.25 ng.

## Precautions <u>Please carefully read these precautions before using this kit.</u>

1. Attention should be paid in the operating process to avoid cross-contamination between nucleic acid samples and products.
2. Please use RNase- or DNase-free pipette tips or EP tubes for the experiment.
3. Before starting, wipe down work area and pipettes with an RNase and DNase cleaning product such as RNase Away® (Molecular BioProducts, Inc)
4. Before proceeding related operation, make sure the thermal cycler is calibrated

and in a stable state.

5. Please read the protocol carefully before the experiment. If test suspension is needed or the downstream test is not needed to be carried out immediately, the test products can be frozen and stored at -20°C based on the recommendation of the instruction, and the subsequent test can be planned accordingly.

## Protocol:

Before the experiment, it is critical to know the concentration and purity of the input DNA. The recommended DNA input is 0.25 ng-1 μg. DNA needs to be dissolved in the buffer as below: water, 10 mM Tris, Buffer EB or LoTE (0.1× TE).

1. Place the reagents on ice. Once thawed, mix the 5× ERA Enzyme Mix by finger flicking (do not vortex to mix). The remaining reagents can be mixed by quick vortexting.

2. Prepare the following program (table below) into a thermal cycler. Turn on the hot lid and set the temperature to 70°C.

| Step | Temperature | Time |
|---|---|---|
| 1 | 4°C | 1 min |
| 2 | 20°C | 30 min |
| 3 | 65°C | 30 min |
| 4 | 4°C | Hold |

3. Prepare the reaction mix on ice according to the following table in a 200 μl thin-walled tube. Mix well by gently pipetting. Do not vortex to mix.

| Components | Volume (μl) |
|---|---|
| 10×ERA buffer | 5 |
| DNA sample | X |
| Nuclease-Free dd$H_2O$ | 35-X |
| Total volume | 40 |

4. Add 10 μl 5× ERA Enzyme Mix to the thin-walled tube from Step 3, and gently mix by pipetting up and down for 10 times. Do not vortex to mix.

   **Note: This step needs to be operated on ice the whole time.**

5. Pulse-spin the thin-walled tube, and immediately put it in the thermal cycler that has been pre-cooled to 4°C, and start the cycling program.

6. When the cycling program is completed, remove the thin-walled tube from block and put it on ice.

7. Proceed to the adapter ligation step immediately. To achieve optimal ligation efficiencies, we recommend using TIANSeq Fast Ligation Module(Cat# 4992354/ 4992355).