ZYMO RESEARCH

Endura™ Tn5 Transposase

Superior Stability & Compatible with Any Adapter.

Highlights

- **Exceptional Stability:** Optimized novel formulation for superior stability.
- **Guaranteed Batch-to-Batch Consistency:** Enables highly reproducible assays and library preps.
- **Ready-to-Load:** Easily generate transposomes or load with custom adapters with Unloaded format.
- **Ready-to-Tagment:** Immediately tagment DNA for short-read sequencing library prep with Loaded format.

Catalog Numbers:
E1024-20, E1024-100, E1025-20, E1025-100

Scan with your smart-phone camera
to view the online protocol.

tech@zymoresearch.com www.zymoresearch.com Toll Free: (888) 882-9682

Table of Contents

Product Contents

E1024	Quantity	Storage Temperature
Endura™ Tn5 Transposase - Unloaded	20 µl / 100 µl (1 U/µl)	-20 °C[1]
10X Tagmentation Buffer	1 ml	-20 °C
10X Stop Buffer	1 ml	-20 °C

E1025	Quantity	Storage Temperature
Endura™ Tn5 Transposase - Loaded	20 µl / 100 µl (1 U/µl)	-20 °C[1]
10X Tagmentation Buffer	1 ml	-20 °C
10X Stop Buffer	1 ml	-20 °C

1 Note: For long-term storage, store at -80 °C.

Specifications

- **DNA Input:** It is recommended to use 1 to 500 ng of DNA for optimal tagmentation using the standard protocol. The user should optimize enzyme amount to achieve the desired fragment size.

- **Equipment Required:** Thermocycler

- **Processing Time:** Transposome preparation takes ~60 min. A standard tagmentation reaction is ~25 min:
  - 5 min for setup
  - 15 min for tagmentation
  - 5 min to stop the reaction

- **Unit Definition:** One unit (U) of enzyme is defined as the amount of enzyme required to tagment 100 ng of human gDNA to an average size of ~500 bp at 55 °C in 15 min.

- **Unloaded vs. Loaded:** The *unloaded* version can be readily loaded with any adapter or transposon DNA sequence (See **Section 1** and **2** for design criteria). *Loaded* version is preloaded with P5 and P7 adapters compatible for short-read sequencers. The sequences are as follows:

  P5 – 5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-3′
  P7 – 5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-3′

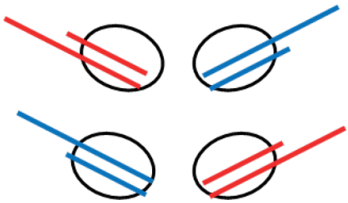
  If using Loaded version, proceed directly to **Section 1.3** for reaction set-up.

Product Description

Endura™ Tn5 Transposase is a recombinant, hyperactive variant of Tn5 transposase with a unique formulation that confers superior stability compared to other Tn5 enzymes. It can be used to simultaneously cut-and-tag (tagment) target DNA with adapter sequences. It can also be used to randomly insert transposon DNA into any target DNA *in vitro*.

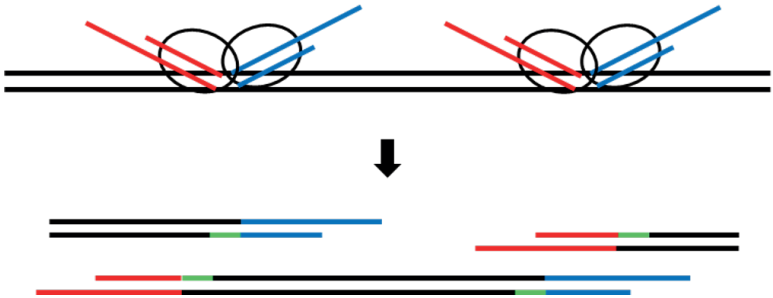
Endura™ Tn5 Transposase must first be assembled into active transposomes by loading it with short adapter sequences or transposon DNA containing a specific 19-bp Mosaic End (ME) sequence.

1. **Transposome Formation**



Transposomes are generated by mixing adapters (red and blue lines) with Endura™ Tn5 Transposase.

2. **Tagmentation and Gap-fill**



Transposomes form dimers and cut-and-tag DNA. This results in a 9-bp duplication gap between the adapter and target DNA. The gap is filled with an elongation step by a DNA polymerase. The green lines indicate the filled gaps.

Protocol

Before Starting:

✓ **NGS Guidelines**

Adapterizing target DNA with Tn5 transposase, a process also known as tagmentation, involves simultaneously cutting DNA via double-stranded break and ligation of adapter sequences. The adapter sequences can be designed to contain PCR primer binding sites, unique secondary structures, and/or non-nucleic acid moieties that are desired for library preparation.

1. Adapter Design Considerations

- Adapters must contain the 19-bp ME sequence that allows Tn5 transposase to bind.
- The remaining adapter sequence should ideally be single stranded.
- Shorter adapters (30 to 50 bp) will result in more efficient tagmentation than longer adapters (>50bp).

2. Target Library Fragment Size

Endura™ Tn5 Transposase does not exhibit catalytic turnover, so the activity can be controlled in a dose-dependent manner. Obtaining the desired library fragment size requires careful consideration of DNA type, size, and amount. Users are encouraged to optimize the enzyme-to-DNA ratio and the tagmentation reaction time based on their specific sample and application requirements.

✓ ***In vitro* Transposition Guidelines**

Transposon DNA typically contains an antibiotic resistance marker linked with an expression cassette. Random transposition into plasmid or genomic DNA can be useful for studying mutations or insertion of a gene-of-interest into a host. A critical component of transposon DNA is the inclusion of the 19-bp ME sequence flanking both ends of the DNA. This is typically appended to the transposon via PCR amplification with primers containing the ME sequence.

Section 1: Adapterization for Sequencing

1.1 Adapter Design and Preparation

Endura™ Tn5 Transposase recognizes a double stranded 19-bp ME sequence that allows the adapter to bind to the enzyme.

1. Adapters are typically composed of annealed oligos but can be longer double stranded DNA. Here are example adapter sequences with P5 (Oligo A) and P7 (Oligo B):[1]

   Oligo A: 5′-TCGTCGGCAGCGTC**AGATGTGTATAAGAGACAG**-3′
   Oligo B: 5′-GTCTCGTGGGCTCGG**AGATGTGTATAAGAGACAG**-3′
   ME Complement Oligo: 5′-/Phos/*CTGTCTCTTATACACATCT*-3′

2. Resuspend the oligos to a final concentration of 200 µM using 10 mM Tris buffer, pH 8.0.

3. Combine and mix the ME complement oligo with each of the Oligo A and B in equal volumes in two separate tubes. Place the tubes into a thermocycler and run the following annealing protocol with a heated lid (100 °C).

   1) 95 °C for 5 minutes
   2) Ramp down 5 °C every 30 seconds until reaching 4 °C

4. Combine annealed adapters in equal volumes. The final stock concentration of adapter mix will be 100 µM.

1 Note: The bolded sequence is the 19-bp ME sequence while the underlined sequence corresponds to the Nextera Read 1 and 2 sequencing primers in Oligo A and B, respectively. The italicized sequence is the reverse complement to the bolded ME sequence. The 5′ phosphate helps facilitate ligation following tagmentation.

1.2 Transposome Assembly – Adapter Loading

1. Prepare the **Transposome Assembly Reaction:**

Components	Volume
Adapters (100 µM)	1.25 µl
Endura™ Tn5 Transposase - Unloaded	10 µl

2. Combine the adapters and Endura™ Tn5 Transposase in a tube, mixing carefully to avoid bubble formation.

3. Incubate at room temperature for 60 min.

The **loaded Endura™ Tn5 Transposome** is ready to use. If desired, it can be diluted by mixing with Tn5 Dilution Buffer (See Appendix A for recipe).

1.3 Tagmentation Reaction

1. Prepare the **Tagmentation Reaction**:

Components	Volume
10X Tagmentation Buffer	2 µl
1 to 500 ng Target DNA	X µl[1]
Loaded Endura™ Tn5 Transposase	Y µl[1]
ddH_2O	to 20 µl

2. Combine and mix each reaction component in a PCR tube, adding the loaded Endura™ Tn5 Transposome last.

3. Incubate at 55 °C for 15 min in a thermocycler with a heated lid (65-100 °C).

4. Add 2 µl of 10X Stop Buffer and incubate at 75 °C for 5 min to stop the reaction.

5. Tagmented DNA can now be cleaned up using commercial size-selection beads to enrich for desired fragment size. If PCR amplification of tagmented DNA is desired, DNA should be purified first using a DNA clean-up kit. See Appendix B: PCR Amplification for PCR protocol recommendations.

1 Note: Target DNA and enzyme amount depend on DNA type and desired library fragment size. We recommend customers titrate DNA and enzyme amount to obtain their desired library size. E.g., 100 ng of human gDNA with 1 µl of loaded enzyme yields fragments with an average size of ~500 bp in 15 min.

Section 2: *In vitro* Transposition

The ME sequence can be appended to any DNA of interest, allowing for *in vitro* transposition into any DNA target.

2.1 Transposon DNA Preparation

1. Design forward and reverse primers to amplify your DNA of interest with the ME sequence (5'-/Phos/CTGTCTCTTATACAC ATCT-3') on the 5' ends of both primers. The 5' phosphate helps improve transposition efficiency.

2. Amplify your DNA of interest with the designed primers containing the ME sequence via PCR with a high-fidelity polymerase.

3. Purify your fragments from the PCR reaction directly using a commercial DNA clean-up kit (E.g., **DNA Clean & Concentrator™ Cat. D4013**) or following agarose gel electrophoresis (E.g., **Zymoclean Gel DNA Recovery Kit Cat. D4001**).

2.2 Transposome Assembly

1. Prepare the **Transposome Assembly reaction:**

Components	Volume
Transposon DNA	X µl (~1 pmol)
Endura™ Tn5 Transposase - Unloaded	5 µl
100% glycerol	X µl[1]

2. Combine the transposon DNA and Endura™ Tn5 Transposase in a tube, mixing carefully to avoid bubble formation.

3. Incubate at room temperature for 60 min.

The **loaded Endura™ Tn5 Transposome** is ready to use. If desired, it can be diluted by mixing with Tn5 Dilution Buffer (See Appendix A for recipe).

1 Note: Volume of glycerol should be the same as the volume of DNA added. This keeps the final concentration of the transposome at 50% glycerol for optimal stability.

2.3 *In vitro* Transposition Reaction and Transformation

1. Prepare the **Transposition Reaction:**

Components	Volume
10X Tagmentation Buffer	1 µl
200 ng Target DNA	X µl[1]
Loaded Endura™ Tn5 Transposase	Y µl[1]
ddH_2O	to 10 µl

2. Combine and mix each reaction component in a PCR tube, adding the loaded Endura™ Tn5 Transposase last.

3. Incubate at 55 °C for 15 min in a thermocycler with a heated lid (65 -100 °C).

4. Add 1 µl of 10X Stop Buffer and incubate at 75 °C for 5 min to stop the reaction.

5. Add 1 µl of the reaction mix immediately into a 50 µl of an electrocompetent strain into an electroporation cuvette.

6. Electroporate, following standard procedures for the bacteria.

7. Recover in 1 ml of desired rich media for outgrowth (if required by the selection marker) and plate onto the corresponding selective media plate.

1 Note: Target DNA and Transposase should be added in equal molar equivalent.

Appendices

Appendix A

Tn5 Dilution Buffer

Components	Concentration
HEPES Buffer, pH 7.5	20 mM
NaCl	100 mM
DTT	1 mM
Triton X-100	0.1% (v/v)
Glycerol	50% (v/v)

Appendix B

PCR Amplification Recommendations

We recommend using a high-fidelity polymerase for the best library quality. Before PCR amplification, libraries will need to undergo an extension step to gap-fill the 9-bp duplication gap that occurs during tagmentation.

Step	Temperature	Time	Cycles
Gap-fill	72 °C	5 min	1
Initial Denaturation	98 °C	30 sec	1
Denaturation	98 °C	10 sec	3-12 Cycles[2]
Annealing	63 °C[1]	30 sec	
Extension	72 °C	60 sec[3]	
-	4 °C	Hold	-

1 Note: Exact annealing temperature depends on the indexing primers used and should be adjusted accordingly.
2 Note: Number of cycles depends on input DNA amount. E.g., for 100 ng input DNA 6-8 cycles total is recommended for a target of ~500 ng of DNA after PCR.
3 Note: Exact extension time depends on the desired library fragment size and the chosen DNA polymerase and should be adjusted accordingly.

Ordering Information

Product Description	Catalog No.	Size
Endura™ Tn5 Transposase – Unloaded (20 U)	E1024-20	20 µl (1 U/µl)
Endura™ Tn5 Transposase – Unloaded (100 U)	E1024-100	100 µl (1 U/µl)
Endura™ Tn5 Transposase – Loaded (20 U)	E1025-20	20 µl (1 U/µl)
Endura™ Tn5 Transposase – Loaded (100 U)	E1025-100	100 µl (1 U/µl)

Notes

Notes

Notes

100% satisfaction guarantee on all Zymo Research products, or your money back.

Zymo Research is committed to simplifying your research with quality products and services. If you are dissatisfied with this product for any reason, please call 1(888) 882-9682.

Integrity of kit components is guaranteed for up to one year from date of purchase.
Reagents are routinely tested on a lot-to-lot basis to ensure they provide the highest performance and reliability.

This product is for research use only and should only be used by trained professionals. It is not for use in diagnostic procedures. Some reagents included with this kit are irritants. Wear protective gloves and eye protection. Follow the safety guidelines and rules enacted by your research institution or facility.

The **BEAUTY** *of* **SCIENCE** *is to Make Things* **SIMPLE**®

tech@zymoresearch.com www.zymoresearch.com Toll Free: (888) 882-9682