Hyperactive Tn5 Transposase

User Manual | Recombinant, Research Grade

Cat. No. EV-MOL-009 | Version 1.0 | April 2026

Cat. No.	EV-MOL-009	**Size**	50 µg / 250 µg	**Storage**	-20°C

1. Overview

Hyperactive Tn5 Transposase (EV-MOL-009) is a recombinant, tagmentation-grade enzyme produced in E. coli Rosetta(DE3) carrying the hyperactive E54K and L372P mutations. These substitutions overcome Tn5's natural self-inhibition, increasing transposition frequency by more than 1,000-fold relative to wild-type. The 470 amino acid, ~53.3 kDa enzyme catalyzes tagmentation: the simultaneous fragmentation and adapter ligation of double-stranded DNA in a single, rapid enzymatic step at 55°C.

EV-MOL-009 is supplied as apo-enzyme to allow users to load custom mosaic-end (ME) adapter sequences, providing full flexibility across all published ATAC-seq, CUT&Tag;, ChIPmentation, and Nextera-compatible workflows. Each lot is tested for tagmentation efficiency, fragment size distribution, and adapter incorporation fidelity before release.

2. Catalytic Mechanism

Tn5 Transposase operates as a dimer on pre-loaded ME adapter oligonucleotides. In the synaptic complex, two Tn5 monomers each carry one ME-loaded adapter and together execute a concerted double-strand cleavage and strand-transfer reaction at a single genomic locus. The DDE mechanism (Asp97, Asp188, Glu326) coordinates Mg^{2+} for catalysis. The staggered cut leaves 9-nt single-stranded gaps that are filled and repaired by the PCR polymerase in subsequent library amplification. The E54K and L372P mutations eliminate the inhibitory end-to-end interaction that suppresses wild-type Tn5 activity, resulting in quantitative and unbiased tagmentation of input DNA.

3. Substrate Specificity

Substrate Type	Efficiency	Conditions
Purified genomic dsDNA	Very High	55°C, 5–10 min; 1–50 ng input
Open chromatin (ATAC-seq)	Very High	37°C in nuclei; Tn5:nuclei ratio per published protocol
ChIP DNA (ChIPmentation)	High	55°C, 5–10 min; 1–10 ng input
In vitro-assembled nucleosomes (CUT&Tag)	High	As per published CUT&Tag protocol; antibody-tethered

4. Reaction Conditions & Protocol

4.1 Recommended Reaction Setup

Component	Volume
Input dsDNA (1–50 ng)	x µL
2× Tagmentation Buffer (EV-MOL-009-TB)	10 µL
Pre-loaded Tn5 (EV-MOL-009 + ME adapters)	variable (optimize per ng input)
Nuclease-free water	to 20 µL

- Pre-load ME adapters onto Tn5 before use: mix apo-enzyme with ME oligos (1:1 molar ratio each arm); 30 min at room temperature
- Tagmentation: 55°C for 5–10 min (adjust time to tune fragment size)
- Stop reaction: add Stop Buffer (provided) or 0.2% SDS; 55°C for 15 min
- Purify tagmented DNA (SPRI beads 1.0×) before PCR amplification

5. Unit Definition

Tn5 Transposase is quantified by protein mass (µg/µL) as determined by BCA assay and absorbance at 280 nm. Activity is expressed as tagmentation efficiency (fraction of input DNA fragmented to 150–1,000 bp in 5 min at 55°C) under standard conditions with pre-loaded ME adapters.

6. Quality Control

Test	Specification
Purity (SDS-PAGE)	>95% (single band at ~53.3 kDa)
Molecular Weight	~53.3 kDa (SDS-PAGE)
Concentration	1 µg/µL
Tagmentation Activity	Fragment distribution 150–1,000 bp from 10 ng genomic DNA (gel analysis)
Adapter Incorporation Fidelity	>95% reads with correct ME adapter sequence (sequencing QC)
Unbiased Coverage	GC-AT bias <0.1 in genome-wide sequencing (normalized)
Exonuclease Contamination	No detectable degradation (λDNA, 200 U, 4 h, 37°C)
Endonuclease Activity (non-specific)	No non-specific nicking of supercoiled pUC19 (200 U, 4 h, 37°C)
RNase Activity	No degradation of 5 µg RNA (200 U, 2 h, 37°C)
pH (formulation buffer)	7.0–7.4
Sterility	No microbial growth (7-day incubation)

7. Storage & Stability

- **Storage temperature:** -20°C (avoid repeated freeze-thaw; aliquot upon receipt)
- **Supplied in:** 100 mM HEPES pH 7.2, 0.2 M NaCl, 0.2 mM EDTA, 1 mM DTT, 0.1% Triton X-100, 10% glycerol
- **Stability:** 24 months from date of manufacture when stored correctly
- **Shipping:** On dry ice

8. Applications

- ATAC-seq for genome-wide chromatin accessibility profiling
- Nextera-compatible NGS library preparation from purified genomic DNA
- CUT&Tag; for genome-wide mapping of histone modifications and transcription factors
- ChIPmentation (ChIP-seq with Tn5 tagmentation)
- Single-cell ATAC-seq (sci-ATAC-seq, 10× Genomics ATAC-compatible)
- Low-input whole-genome sequencing library preparation

9. Troubleshooting

Problem	Possible Cause	Suggested Action
Over-tagmentation (fragments too small)	Excessive enzyme or long incubation	Reduce enzyme amount; shorten incubation to 5 min at 55°C
Under-tagmentation (fragments too large)	Insufficient enzyme or cold reaction	Increase enzyme; confirm 55°C reaction temperature
Low library complexity	Input DNA degraded or insufficient	Use high-quality DNA (OD 260/280 ~1.8); increase input to 50 ng
Adapter dimers in library	Excess free adapter	Increase SPRI bead ratio to 1.5× post-tagmentation cleanup

10. Safety Information

This product is intended for research use only. Handle in accordance with standard laboratory safety guidelines. Refer to the accompanying Safety Data Sheet (SDS) for full hazard information. Avoid ingestion, inhalation, or contact with eyes and skin. Dispose of in accordance with local, state, and federal regulations.