# T7 Endonuclease I

1-800-632-7799
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M0302S 002130615061

## M0302S

RR NEB2 37°

**250 units** **10,000 U/ml** **Lot: 0021306**

**RECOMBINANT** **Store at –20°C** **Exp: 6/15**

**Description:** T7 Endonuclease I recognizes and cleaves non-perfectly matched DNA, cruciform DNA structures, Holliday structures or junctions, heteroduplex DNA and more slowly, nicked double-stranded DNA. The cleavage site is at first, second or third phosphodiester bond that is 5´ to the mismatch. The protein is the product of T7 gene 3.

**Source:** A fusion of maltose binding protein and truncated, inactive T7 Endonuclease I (tT7 Endo I) possessing a C-terminal thioester is purified to near homogeneity using the IMPACT-TWIN system.

The full-length, active T7 Endo I is generated *in vitro* by ligating a synthetic peptide, consisting of the truncated amino acid residues, to the thioester-tagged tT7 Endo I (1).

**Applications:**

- Resolve four-way junction or branched DNA
- Detect or cleave heteroduplex and nicked DNA
- Randomly cleave linear DNA for shot-gun cloning

Supplied in: 200 mM NaCl, 20 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, 1 mM dithiothreitol, 0.15% Triton X-100 and 50% glycerol.

**Reagents Supplied with Enzyme:**
10X NEBuffer 2

**Note:** pUC(AT) is derived from pUC19 with a modification of the polylinker between the EcoRI site and the PstI site:

**Reaction Conditions:** 1X NEBuffer 2.
Incubate at 37°C.

EcoRI
GAATTC ATATATATATATATATATAT
PstI
ATATATATATATATATAT CTG CAG

**1X NEBuffer 2:**
50 mM NaCl
10 mM Tris-HCl
10 mM $MgCl_2$
1 mM dithiothreitol
pH 7.9 @ 25°C

**Unit Definition:** One unit is defined as the amount of enzyme required to convert > 90% of 1 µg of supercoiled cruciform pUC(AT) to > 90% linear form in a total reaction volume of 50 µl in 1 hour at 37°C.

**Diluent Compatibility:** Diluent Buffer C
250 mM NaCl, 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM dithiothreitol, 0.15% Triton X-100, 200 µg ml BSA and 50% glycerol (pH 7.4 @ 25°C).

**Quality Assurance:** Purified free of contaminating exonucleases and endonucleases.

## Quality Control Assays

**Ligation:** 16 µg of Hae III digested ϕX174 DNA was incubated with 40 units of T7 Endonuclease I in a total reaction volume of 400 µl for 2 hours at 37°C. The DNA was ethanol precipitated and ligated with T4 DNA Ligase and then recut with Hae III. Approximately 50% of the T7 Endo I treated fragments were ligated and of those, > 95% were recut with Hae III.

## Enzyme Properties

**Activity in NEBuffers:**

| | |
|---|---|
| NEBuffer 1 | 50% |
| NEBuffer 2 | **100%** |
| NEBuffer 3 | 100% |
| NEBuffer 4 | 100% |

When using a buffer other than the optimal (supplied) NEBuffer, it may be necessary to add more enzyme to achieve complete digestion.

**Notes On Use:** 10–15 units will cut 1 µg pUC19 in 4 hours resulting in equal amounts of linear and nicked DNA.

*(See other side)*

CERTIFICATE OF ANALYSIS

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*(See other side)*

CERTIFICATE OF ANALYSIS

T7 Endonuclease I is a structure-selective enzyme. It acts on a variety of DNA substrates with different specific activities. It is important to control the amount of enzyme and the reaction time used for cleavage of a particular substrate.

This enzyme is not recommended to be used at 55°C, as the activity is decreased.

**References:**


1. Xu, M. -Q. and Evans, T.C. (2001) *Methods* 24, 257–277.
2. Parkinson, M.J. and Lilley. D.M.J. (1997) *J. Mol. Biol.* 270, 169–178.
3. White, M.F. et al. (1997) *J. Mol. Biol.* 269, 647–664.
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