

T7 Endonuclease I



1-800-632-7799
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M0302S 002130615061

M0302S

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.

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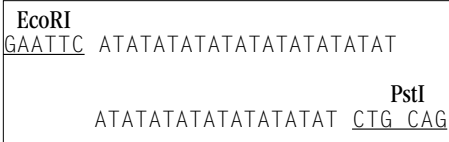
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Incubate at 37°C.



1X NEBuffer 2:

50 mM NaCl
10 mM Tris-HCl
10 mM MgCl₂
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.



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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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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

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.
4. Hadden, J.M. et al. (2001) *Nat. Struct. Biol.* 8, 62–67.

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