T7 Endonuclease I

**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:** An E. coli strain that carries a fusion of maltose binding protein and T7 Endonuclease I (T7 Endo I).

**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:**
- 1X 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: 50 mM NaCl, 10 mM Tris-HCI, 10 mM MgCl₂, 1 mM dithiothreitol pH 7.9 @ 25°C

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

**Quality Control Assays**

**Ligation and Recutting (Terminal Integrity, Digested DNA):** A 400 µl reaction in NEBuffer 2 containing 16 µg of HaeIII digested φX174 DNA and a minimum of 40 units of T7 Endonuclease I incubated for 2 hours at 37°C, 50% of the DNA fragments can be ligated with T4 DNA ligase in 5 minutes at 25°C. Of these ligated fragments, > 95% can be recut with HaeIII.

**Protein Purity (SDS-PAGE):** T7 Endonuclease I is > 95% pure as determined by SDS-PAGE analysis using Coomassie blue detection.

**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.

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.

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**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).

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