T7 Endonuclease I

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

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.

Reagents Supplied with Enzyme: 10X NEBuffer 2

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)
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: