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-3’ 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:
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.

1X NEBuffer 2:
- 50 mM NaCl
- 10 mM Tris-HCl
- 10 mM MgCl₂
- 1 mM dithiothreitol
- pH 7.9 @ 25°C

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.

Protein Purity (SDS-PAGE):
- T7 Endonuclease 1 is > 95% pure as determined by SDS-PAGE analysis using Coomassie blue detection.

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References: