T7 Endonuclease 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 Pstl 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

Quality Assurance:
- Purified free of contaminating exonuclease and endonuclease.

Quality Control Assays
- Ligation and Recutting (Terminal Integrity, Digested DNA): A 400 µl reaction in NEBuffer 2 containing 16 µg of Haell1 digested qX174 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 HaellI.

Enzyme Properties
- Activity in NEBuffers:
  - NEBuffer 1: 50%
  - NEBuffer 2: 100%
  - NEBuffer 3: 100%
  - NEBuffer 4: 100%

Notes On Use:
- 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 I is > 95% pure as determined by SDS-PAGE analysis using Coomassie blue detection.

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