T7 Endonuclease I

Catalogue number: MB21201, 250 U

Description

T7 Endonuclease I is an ultrapure recombinant enzyme purified from *Escherichia coli*. This endonuclease is the product of phage T7 gene 3. 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 the first, second or third phosphodiester bond that is 5´ to the mismatch. The enzyme has a variety of applications, including: (1) resolving four-way junction or branched DNA; (2) detection or cleavage of heteroduplex and nicked DNA; (3) random cleavage of linear DNA for shot-gun cloning.

Storage temperature

T7 Endonuclease I should be stored at -20 °C in a constant temperature freezer. The protein will remain stable till the expiry date if stored as specified.

Unit definition

One unit is defined as the amount of enzyme required to cleave 1 pmol of a 34-mer oligonucleotide duplex containing a single deoxyinosine site in a reaction volume of 10 µL in 1 hour at 37 °C.

Enzyme concentration: 10 U/µL

Inactivation

T7 Endonuclease I is heat inactivated at 65 °C for 20 min.

System components and Reaction conditions

T7 Endonuclease I is provided with a dedicated and highly optimized NZYtech reaction buffer and displays an optimum temperature of 37 °C.

Standard Protocol

The following standard protocol serves as a general guideline for the cleavage of DNA using T7 Endonuclease I. which should result in equal amounts of linear and nicked nucleic acid.

1. Combine 1 µg of the nucleic acid with 10–15 units of T7 Endonuclease in 1x reaction buffer provided.

2. Incubate at 37 °C for 4 hours

Note: *T7 Endonuclease I is a structure-selective enzyme. Thus, the enzyme 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.*

Quality control assays

Purity

Recombinant T7 Endonuclease I is >95% pure as judged by SDS polyacrylamide gel electrophoresis followed by BlueSafe staining (NZYtech, Cat. No. MB15201).

Nuclease assays

0.2-0.3 µg of pNZY28 plasmid DNA are incubated with 5 U of T7 Endonuclease I in 1× Reaction buffer for 14-16 hours at 37 °C. Following incubation, the DNA is visualized on a GreenSafe Premium (NZYtech, Cat. No. MB132)-stained agarose gel. There must be no visible nicking or cutting of the DNA.

Functional assay

T7 Endonuclease I is tested for activity in a standard digestion reaction of DNA fragments containing DNA mismatches. Efficiency of enzyme's reaction is analyzed through agarose gel.

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