

T7 Endonuclease I

Please read the manual carefully before use

Cat. No. LE101

Version No. Version 1.1

Storage: at -20°C for two years

Concentration: 10,000 units/ml

Description

This product is purified from *E. coli* expressing the recombinant T7 Endonuclease I (T7EI) gene. It recognizes and cleaves non-perfectly matched DNA, cruciform DNA structures, Holliday junctions, heteroduplex DNA, and randomly cleaves single-stranded DNA. The cleavage site is at the first, second, or third phosphodiester bond that is 5' to the mismatch.

Features

High efficiency and high specificity.

Application

- Gene mutation and SNP detection for result from TALEN and CRISPR/CAS9
- Recognition and cleavage for non-perfectly matched DNA and Holliday junctions
- Randomly cleave single-stranded DNA

Kit Contents

Component	LE101-01	LE101-02
T7 Endonuclease I	250 units	5×250 units
10×T7 Endonuclease I Buffer	200 µl	1 ml
T7 Endonuclease I Control Template (40 ng/µl)	20 µl	20 µl
10×DNA Loading Buffer	1 ml	1 ml

Definition of Activity Unit

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.

Quality control

Exonuclease activity: 10 units of enzyme are incubated with 1 µg of [³H] labeled DNA (purified PCR products) at 37°C for 2 hour in 100 µl reaction system, and < 5% radioactive material is released.

Endonuclease activity: 5 units of enzyme are incubated with 1 µg of pBR322 DNA at 37°C for 2 hours in 50 µl reaction system, and the proportion of linearized DNA should be no more than 20%.

10×T7 Endonuclease I Buffer: 200 mM Tris-HCl pH 7.9, 500 mM NaCl, 100 mM MgCl₂, 10 mM DTT



Reaction volume (Take 20 μ l as an example)

Component	Volume
Special DNA structures*	≤ 250 ng
10 $\times$ T7 Endonuclease I Buffer	2 μ l
T7 Endonuclease I	1 μ l
Nuclease-free Water	Variable
Total volume	20 μ l

* Special DNA structures: hybrid DNA formed by annealing wild type DNA and mutation DNA.

Working conditions

Incubate at 37°C for 15-30 minutes and terminate the reaction by adding 10 $\times$ DNA loading buffer to 1 $\times$ or heating at 80°C for 15 minutes.

Precautions

- T7EI exhibits substrate structure selectivity, with varying activities on different DNA substrates. It is strongly recommended to control the enzyme amount and reaction time. Non-specific digestion by T7EI increases when the temperature is above 42°C, and its enzymatic activity decreases when the temperature is over 55°C.
- When a mutation site is detected, it should be avoided to be at the center of DNA.

For research use only, not for clinical diagnosis

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