T7 Endonuclease I

Cat.No. LE101

Storage: at -20°C for one year

Concentration: 10, 000 units/ml

Description

T7 Endonuclease I is a junction-resolving enzyme of 149 amino acid residue, encoded by gene 3 of bacteriophage T7, existing as a stable dimer. Not only does it selectively bind and cleave four-way DNA (Holliday) junctions with high specificity for branched structures in double-stranded DNA, such as cruciform DNA, but also it has a strong preference for cutting single-stranded DNA. It requires metal ions such as magnesium for activity. This product is purified from *E. coli* expressing the recombinant T7 Endonuclease I (T7EI) gene.

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

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 [^{3}H] 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 ratio of RF I to RF II is no more than 20%.

Storage buffer: 20 mM Tris-HCl (pH8.0)，200 mM NaCl，0.1 mM EDTA，1 mM DTT，0.15% Triton X-100, 50% Glycerol

10×T7 Endonuclease I Buffer: 200 mM Tris-HCl, 500 mM NaCl, 100 mM $MgCl_2$, 10 mM DTT, pH 7.9

Reaction volume (Take 20 µl as an example)

Component	Volume
Special DNA structures*	< 250 ng
10×T7 Endonuclease I Buffer	2 µl
T7 Endonuclease I	1 µl
Nuclease-free Water	Variable
Total volume	20 µl

* 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×DNA loading buffer to 1× or heating at 80°C for 15 minutes.

Precautions

- As T7 Endonuclease I can selectively cleave various conformation of DNA with different specificity, it is strongly recommended to control the reaction condition, such as enzyme amount, reaction time, and temperature. The suggested temperature is 42°C-55°C.
- When a mutation site is detected, it should be avoided to be at the center of DNA.

Reference

- M. Janine Parkinson& David M. J. Lilley. The Junction-resolving Enzyme T7 Endonuclease I: Quaternary Structure and Interaction with DNA. J. Mol. Biol. (1997) 270, 169-178.
- Alasdair D. J. Freeman, Anne-Cécile Déclais and David M. J. Lilley. The Importance of the N-Terminus of T7 Endonuclease I in the Interaction with DNA Junctions. J. Mol. Biol. (2013) 425, 395-410.
- Bernard de Massy, Robert A. Weisberg, F.William Studier. Gene 3 endonuclease of bacteriophage T7 resolves conformationally branched structures in double-stranded DNA. J. Mol. Biol. (1987) Volume 193, Issue 2, 359-376.
- Anne-CeÂ cile DeÂ clais1, Jonathan Hadden, *et al*. The Active Site of the Junction-resolving Enzyme T7 Endonuclease I. J. Mol. Biol. (2001) 307, 1145-1158.

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