

T7 Endonuclease I (10 U/μL)

Product description

T7 Endonuclease I is encoded by gene 3 of bacteriophage T7. It recognizes and cleaves mismatched DNA, cruciform DNA structures, Holliday junctions (four-way DNA junctions), heteroduplex DNA, and also cleaves nicked double-stranded DNA at a slower rate. Cleavage occurs at the first, second, or third phosphodiester bond on the 5' side of the mismatched base.

Specifications

Cat NO.	14548ES72 / 14548ES86
Size	250 U / 2500 U
Source	Recombinant expression in Escherichia coli of gene 3 from bacteriophage T7
Concentration	10 U/μL
Purity	≥95%

Components

Components No.	Name	14548ES72	14548ES86
14548-A	T7 Endonuclease I (10 U/μL)	25 μL	250 μL
14548-B	10× T7 Endonuclease I Reaction Buffer	1 mL	1 mL

Applications

Detection of mutations following genome editing (e.g., CRISPR/Cas9 or TALEN-induced indels)

Resolution of four-way DNA junctions or branched DNA structures

Detection or cleavage of heteroduplex DNA and nicked DNA

Random fragmentation of linear DNA for shotgun cloning

Storage

This product should be stored at -25~-15°C for 2 years.

Instructions

1. PCR Amplification of Target DNA Containing Mutation Sites

Amplify the genomic region surrounding the mutation site (e.g., TALEN or Cas9 target site) using a high-fidelity DNA polymerase. It is recommended that the amplicon length be 0.5–1 kb, and the mutation site should not be positioned at the exact center of the fragment to allow clear resolution of two unequal cleavage products by gel electrophoresis.

a. Extract genomic DNA from transfected cells. Set up the following three PCR reactions:

- Genomic DNA from edited (mutant) cells
- Genomic DNA from unedited (wild-type) control cells
- Nuclease-free water (no DNA template control)

- b. After PCR, run products on an agarose gel. Proceed only if a single, correctly sized band is observed.
- c. Purify the PCR products using magnetic beads or another purification method.
- d. Quantify the purified PCR products (quantification enables accurate mutation frequency calculation; otherwise, only qualitative validation is possible).

2. T7 Endonuclease I Digestion Assay

2.1 Prepare the following reaction setup:

Component	Mutant Sample	Wild-Type Control	No-Template Control
PCR product from mutant cells (200 ng)	X μ L	0 μ L	0 μ L
PCR product from wild-type cells (200 ng)	0 μ L	X μ L	0 μ L
10 $\times$ T7 Endonuclease I Reaction Buffer	2 μ L	2 μ L	2 μ L
ddH ₂ O	to 19 μ L	to 19 μ L	to 19 μ L

2.2 Perform the following annealing program in a thermal cycler:

Temperature	Ramp Rate / Time
95°C	5 min
95 $\rightarrow$ 85°C	~2°C/sec
85 $\rightarrow$ 25°C	~0.1°C/sec
4°C	Hold (∞)

2.3 Add 1 μ L of T7 Endonuclease I to the annealed DNA.

2.4 Incubate at 37°C for 15 minutes.

2.5 Stop the reaction by adding 1.5 μ L of 0.25 M EDTA.

[Note]: Without EDTA quenching, non-specific cleavage may occur during prolonged room-temperature storage. Proceed to electrophoresis promptly.

2.6 Analyze the digestion products directly by 2% agarose gel electrophoresis.

Notes

1. Enzyme amount and incubation time must be optimized for specific substrates to avoid over-digestion.
2. Do not exceed 42°C during the reaction, as higher temperatures may increase non-specific nuclease activity.
3. For your safety and health, wear a lab coat and disposable gloves during handling.
4. This product is for research use only (RUO).