T7 Endonuclease I

REF: EG24209-S/M

Storage Condition

-20℃

Components

Component	EG24209S	EG24209M
T7 Endonuclease I (10 U/μl)	25 μl	125 μl
10× Cut Buffer G	1.25 ml	1.25 ml
Control Template (T7EI)	20 μl	20 μl

Description

T7 Endonuclease I (T7 Endo I, T7EI), can recognize and cleave imperfectly matched DNA, cruciform DNA structures, Holliday junctions ,DNA branch points, and heteroduplex DNA. The cleavage sites are located at the first, second, or third phosphodiester bonds on the 5' side of the mismatch site. Additionally, T7EI can cleave nicked double-stranded DNA, albeit with reduced efficiency. It is noteworthy that T7EI can recognize DNA mismatches resulting from insertions, deletions, or mutations of two or more base pairs (bp) in length, but cannot recognize 1 bp insertions, deletions, or mutations. Furthermore, T7 Endonuclease I does not recognize all types of DNA mismatches and works best with C mismatches.

This product is a high-purity protein obtained through cloning and recombinant expression of the T7 Endonuclease I gene in Escherichia coli, followed by purification. It is free of contamination from other endonucleases or exonucleases. (This product is a high-purity protein expressed and purified from E. coli after cloning and expressing the T7 Endonuclease I gene, free from contamination by other endonucleases or exonucleases.)

Applications

1. T7E1 is commonly used to detect mutations introduced by CRISPR/Cas9, TALEN, or other gene editing technologies, and to detect SNP sites.
2. Detect or cleave heteroduplex and nicked DNA.
3. Randomly cleave linear DNA for shot-gun cloning and sequencing.

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℃ .

Quality Control Assays

Protein Purity

The enzyme is ≥95% pure as determined by SDS-PAGE analysis using Coomassie Blue staining.

Endonuclease Activity

A 20 μl reaction containing 200 ng of supercoiled plasmid and 10 U of T7 Endonuclease I incubated for 2 hours at 37℃ results in <20% conversion to the nicked or linearized form as determined by agarose gel electrophoresis.

Non-specific Nuclease Activity

A 20 μl reaction containing 15 ng of dsDNA fragments and 10 U of T7 Endonuclease I incubated for 2 hours at 37℃ results in no detectable degradation of the dsDNA fragments as determined by agarose gel electrophoresis.

Protocol

1. Annealing

Reagent	Experimental group	Negative control 2	Negative control 2	Positive control
PCR Product (Wild Type)	200 ng	200 ng	/	/
PCR Product (Mutant)	200 ng	/	200 ng	/
Control Template (T7EI)[a]	/	/	/	2 μl
10× Cut Buffer G	2 μl	2 μl	2 μl	2 μl
Nuclease-free water	To 19 μl	To 19 μl	To 19 μl	To 19 μl

a. The Control Template is a mixture of mutant and wild-type PCR products at a 1:1 ratio (each 100 ng). Before use, it needs to be annealed to form heteroduplexes containing mismatches. After annealing, digestion with T7EI enzyme produces bands of 620 bp and 175 bp.

2. Annealing with PCR instrument

Temperature	Time
95℃	5 min
95~85℃	-2℃ /s
85~25℃	-0.1℃ /s
4℃	∞

3. T7 Endonuclease I Enzyme digestion

Reagent	Amount
"Step 1" annealing reaction product	19 μl
T7 Endonuclease I	1 μl

① Incubation at 37℃ for 15~30 min.

② Stop the reaction by incubation at 85℃ for 15 min, or by adding 1.5 μl of 0.25 M EDTA.

4. The digestion products were detected by electrophoresis using 2% agarose gel.

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Notice

1. T7E1 is a structure-dependent enzyme that performs selective digestion and displays different activity on different DNA substrates. Therefore, it is necessary to optimize the amount of T7E1 and incubation time for a specific DNA substrate.

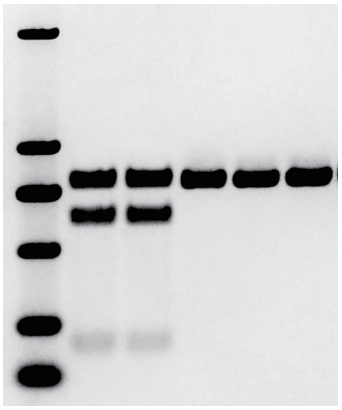
2. Non-specific digestion by T7E1 increases when the temperature is above 42℃. T7E1 enzyme activity decreases when the temperature is over 55℃.

3. Mn^{2+} significantly increases the non-specific nuclease activity of T7 Endonuclease I. Please use a PCR Buffer without Mn^{2+} for PCR amplification.

4. T7 Endonuclease I is compatible with various PCR Buffers. PCR products can be used directly for enzyme digestion without purification. If the digestion results are abnormal, the PCR products should be purified before experiment.

Control Template (T7EI) digestion diagram

+	+	+	-	+	Wild Type PCR product
+	+	-	+	+	Mutant PCR product
+	+	+	+	-	T7 Endonucleasel
M	1	2	3	4	5



M. FY2000 Marker
1. Yugong T7EI
2. A company T7EI
3. Wild Type PCR product
4. Mutant PCR product
5. Wild-type and mutant PCR products in a 1:1 ratio (without T7E1)

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