EURx® MOLECULAR BIOLOGY PRODUCTS

T7 Endonuclease I

(Escherichia coli)

Cat. No.	size
E1125-01	250 units
E1125-02	1250 units

Concentration: 10 u/µl

Unit Definition: One unit is defined as the amount of enzyme required to convert over 90% of 1 µg of supercoiled cruciform pUC(AT) to linear form in a 50 µl volume in 1 hour at 37°C.

Note: T7 Endonuclease I is a structure selective enzyme. It acts on a variety of DNA substrates with different specific activity. It is important to control the amount of enzyme and the reaction time. Temperatures above 42°C and excess units cause an increase in nonspecific nuclease activity and should be avoided.

Storage Conditions: Store at -20°C.

Storage Buffer:

20 mM Tris-HCl (pH 7.5), 200 mM NaCl, 0.15% Tergitol™ TMN, 1 mM dithiothreitol and 50% glycerol.

10 x Reaction Buffer:

100 mM Tris-HCl (pH 7.9 at 25°C), 500 mM NaCl, 100 mM $MgCl_2$, 10 mM dithiothreitol.

Quality Control:

All preparations are assayed for contaminating 3'- and 5'-exonuclease. Typical preparations are greater than 95% pure, as judged by SDS polyacrylamide gel electrophoresis.

T7 Endonuclease I is an ultrapure recombinant enzyme, the product of T7 gene 3, purified from *E. coli*. It is structure-selective enzyme that recoginzes and cleaves mismatched DNA, heteroduplex DNA, cruciform DNA structures, Holliday structures or junctions and more slowly, nicked dsDNA. The cleavage site is the first, second or third phosphodiester bond that is 5' to the mismatch. The enzyme has a preference for single stranded over double stranded DNA.

Application:

- Recognition of mismatched DNA.
- Resolve four-way junction or branched DNA.
- Detect or cleave heteroduplex and nicked DNA.
- Randomly cleave linear DNA for shot-gun cloning.

Protocol for mismatch cleavage assay:

Step 1. PCR

Set up 50 µl reaction using genomic DNA as a template. For each amplicon set up 2 PCR reactions:

- Wild type genomic DNA from not transfected cells
- Mutated genomic DNA from Cas9 or TALEN transfected cells

It is higly recommended to use high fidelity enzyme like Hybrid DNA Polymerase (E2950).

Analyze a small amount of the PCR product on 1.5-2% agarose gel. If a single band of expected size is present, proceed with the hybridization step. T7 Endonuclease I works well in most PCR buffers suplemented with Recation Buffer, so there is no need to purify the reactions.

Step 2. Hybridization

Mix the following componetns:

DNA fragments:	~ 200 ng total
10 x Reaction Buffer:	2 µl
Nuclease-free Water:	variable
Total volume:	19 µl

Hybridization conditions:

Step	Temperature	Ramp Rate	Time
Denaturation	95˚C		5 minutes
Annealing	95-85˚C	-2˚C/second	
	85-25˚C	-0.1˚C/second	
Final Extension	4˚C		Hold

Step 3. T7 Endonuclease I reaction

Mix the following componetns:

Annealed PCR product:	19 µl
T7 Endonuclease I:	1 µl
Total volume:	20 µl

Incubate in a thermal cycler for 15 minutes at 37°C.

Step 4. Detection

Add loading buffer directly to the reaction mixture and detect the cleavage efficiency by agarose gel electrophoresis.

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