# T7 Endonuclease I

**Cat# ER1005 – 250U**

**Storage at -20 ℃**

## INTRODUCTION

**T7 Endonuclease I** recognize and cuts mismatched DNA, cruciform DNA, Holliday structures or junctions, and heteroduplex DNA. It can also recognize and cut double-strand DNA with nicks with a lower speed. The enzyme cuts the first, second or third phosphodiester bond at 5' end of the mismatch site. This product is a high purity T7 Endonuclease I protein with high activity, which stems from the expression of a recombinant T7 Endonuclease I (T7 Endonuclease I) gene in E. coli expression system.

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| No | Component | **EP1005 – 100 U** |
|---|---|---|
| BA | T7 Endonuclease I | 25 µl |
| BB | 10X Reaction Buffer | 1 ml |

## UNIT DEFINITION

One unit (U) is defined as the amount of enzyme that required to cut more than 90% of 1 µg of supercoiled cross-structured pUC (AT) plasmid into more than 90% of linearized DNA at 37 ℃ within 1 hour in a 50 µl reaction system.

## REACTION CONDITIONS

1 × T7 Endonuclease I Reaction Buffer, incubate at 37 ℃.

## APPLICATION

1. Detection of gene mutation, SNP, mutations produced by TALEN and CRISPR/CAS9.
2. Recognition of mismatched DNA, resolve four-way junction or branched DNA.
3. Detecting or cutting heterogeneous dimer of DNA.
4. Random cutting of linear DNA for shot-gun cloning.

## Strategy for detection of mutants with T7 Endonuclease I

### A. PCR amplification of DNA fragments with mutation sites

1. Extract genomic DNA of cells after transfection.
2. DNA fragments with mutation sites, such as the target sites of TALEN or Cas9, are amplified through PCR, which should be performed with high fidelity DNA polymerase. The recommended size of the amplified fragment is 0.5-1 kb. Please do not set the mutation point in the middle of the amplified fragment, in order to produce two fragments with obviously different sizes after the cutting. Three PCR reactions should be set up for each sample with different templates as follows:

   **a**: Genomic DNA of target cells (such as TALEN or Cas9 transfected cells)

   **b**: Genomic DNA of negative control cells (cells without transfection of specific target sequence)

   **c**: Water (the non-template control)
3. After the PCR reaction, do agarose gel electrophoresis analysis with a small amount of the PCR product. If the amplified products showed unique band with correct size, perform the next experiments.
4. Purify the PCR products with beads or PCR product purification kits.
5. Quantify the purified PCR products.

### B. Digestion Reaction of T7 Endonuclease I.

1. Prepare the reaction mixture with the purified PCR product in order as follows:

| No. | 1 | 2 |
|---|---|---|
| Target Cell PCR products (200 ng) | x μl | - |
| Negative control PCR products (200 ng) | - | x μl |
| 10 × T7 Endonuclease I Reaction Buffer | 2 μl | 2 μl |
| Nuclease-free Water | To 19 μl | To 19 μl |

2. Denaturation and annealing with a PCR machine using the following program:

| | |
|---|---|
| 95 ℃ | 5 min |
| 95-85 ℃ | -2 ℃/sec |
| 85-25 ℃ | -0.1 ℃/sec |
| Hold | 4 ℃ |

3. Add T7 Endonuclease I to the annealed PCR products:

| | |
|---|---|
| Annealed PCR products | 19 μl |
| T7 Endonuclease I | 1 μl |

4. Incubate at 37 ℃ for 15 min.
5. After the reaction, add 1.5 μl of 0.25 M EDTA to terminate the reaction.
6. After termination, the products of digestion can be analyzed directly by 2% agarose gel electrophoresis.

Notes:

1. T7 Endonuclease I is a substrate-structure selective enzyme. The enzyme acts on different DNA substrates with different activity. Therefore, the dosage of the enzyme and reaction time must be adjusted according to the types of substrates.
2. The nonspecific nuclease activity will increase when the reaction temperature exceeds 42 ℃. Keep the reaction temperature under 55 ℃, otherwise the nuclease activity will decrease.

## PRODUCT USE LIMITATION

These products are intended for research use only.