T7 Endonuclease I

EN303

Version 22.2

Product Description

T7 Endonuclease I recognizes and cuts mismatched DNA, cruciform DNA, Holliday structures or junctions, and heteroduplex DNA. It can also recognize and cut double-stranded DNA with nicks with a lower speed. The enzyme cuts the first, second or third phosphodiester bond that is 5' to the mismatch. Recombinant T7 Endonuclease I is purified from *E.coli* expression system. It has high purity and can be applicable for gene mutation, SNP and TALEN or CRISPR/Cas9-mediated mutation detection. It can recognize DNA mismatches, resolve four-way junction or branched DNA, detect heteroduplex or nicked DNA and randomly cleave linear DNA for shot-gun cloning.

Components

Components	EN303-01 (250 U)	EN303-02 (1,250 U)
T7 Endonuclease I	25 μl	125 μl
T7 Endonuclease I Reaction Buffer (10 ×)	1 ml	1 ml
Control template*	10 μl	20 μl

*The Control template provided in this kit is an 1:1 mixture of mutant and wild-type PCR products (100 ng each). It needs to be annealed into unpaired heterozygous chains before use.

Storage

Store at -30 ~ -15℃ and transport at ≤0℃.

Applications

It's applicable for gene mutation, SNP and TALEN or CRISPR/Cas9-mediated mutation detection.

Notes

For research use only. Not for use in diagnostic procedures.

1. T7 endonuclease I is a structure-selective enzyme which acts on different DNA substrates with different activities.Therefore, it is essential to control the amount of enzyme and reaction time when cleaving a specific substrate.
2. Nonspecific nuclease activity will be increased when the temperature is above 42℃. Control the temperature and make sure it won't exceed 55℃, or the activity of enzyme will be decreased.

Experiment Process

1. PCR amplification of DNA fragments with mutation sites

▲ DNA fragments with mutation sites (such as the target sites of TALEN or Cas 9) 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 cleavage .

a. Genomic DNA of transfected cells was extracted. Set up the following three PCR:
   Genomic DNA of target cells (with mutation sites)
   Genomic DNA of control cells (without mutation sites)
   ddH_2O (no-template control)

b. After the PCR, a small amount of the PCR products are verified by electrophoresis. If the amplified products showed unique band with correct size, perform the next step.

c. Purify the PCR products with beads or other methods.

d. Quantify the purified PCR products.

▲ Calculation of the mutation rate is facilitated after quantification, otherwise only validation experiments of mutants can be performed.

2. Digestion reaction of T7 Endonuclease I

a. Prepare the reaction mixture according to the following table:

Components	Positive cells	Negative cells	Positive control	No-template control
Target cells PCR products (200 ng)	x μl	0 μl	0 μl	0 μl
Negative cells PCR products (200 ng)	0 μl	x μl	0 μl	0 μl
Control template	0 μl	0 μl	2 μl	0 μl
10 × T7 Endonuclease I Reaction Buffer	2 μl	2 μl	2 μl	2 μl
Nuclease-free ddH_2O	To 19 μl	To 19 μl	To 19 μl	To 19 μl

b. Perform annealing according to the following PCR program:

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

c. Add 1 μl T7 Endonuclease I to the annealing products.

d. Incubate at 37℃ for 15 min.

e. Add 1.5 μl 0.25 M EDTA to terminate the reaction.

▲ Nonspecific cleavage will be generated after putting at room temperature for a long time if EDTA is not added. Please carry out electrophoresis detection as soon as possible.

f. Analyze the products by 2% agarose gel electrophoresis.

▲ Bromophenol blue will cover the 250 bp band, use OG Loading Buffer for electrophoresis.

3. Electrophoresis Results

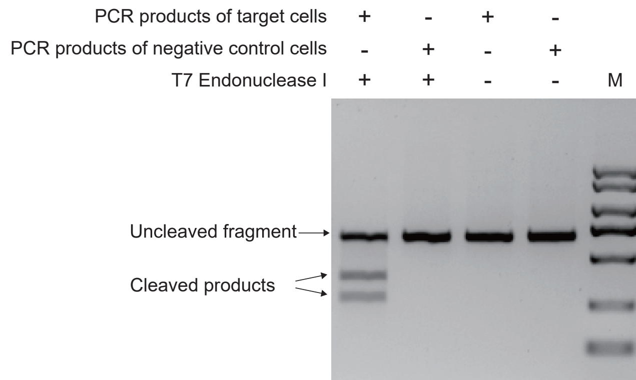

Figure 1. Digestion Products Analyzed by 2% Agarose Gel Electrophoresis

M: DL 2,000 Plus DNA Marker