T7 Endonuclease I

Cat. No. : RK20554

Product components

Components	Component Number	250 U	1250 U
T7 Endonuclease I (10,000 U/mL)	RM20536	25 μL	125 μL
10X Buffer CutB	RM20105	1.25 mL	1.25 mL

Product Description

T7 Endonuclease I (T7 Endo I, T7EI), can recognize and cleave non-perfectly mismatched DNA, cruciform DNA structures, holliday structures or junctions and Heteroduplex DNA.The cleavage site is at the first, second or third phosphodiester bond that is 5′ end to the mismatch. This product is commonly used for detection of gene mutations caused by CRISPR/Cas9, etc.

Product Source

The protein is the product of T7 gene 3. It is expressed in *E. coli* and obtained by isolation and purification.

Storage

-20℃

Protein Inactivation

Heating at 80℃ for 15 minutes or adding EDTA to a final concentration of 20 mM.

Experiment Process

1. Primer design and amplification of fragment with mutation sites

1.1 Use high-fidelity DNA polymerase to amplify DNA fragments with mutation sites. To avoid similar sizes of fragments after enzyme digestion, please do not design the mutation site in the middle of the fragment. The recommended size of the amplified fragment is 0.5-1 kb.

1.2 PCR product was detected by electrophoresis, and if the size of the product is correct and the band is single, enzyme digestion detection can be performed.

2. Enzyme digestion analysis

If PCR product has correct size and clear band, purification is not necessary, and the product can be directly denatured and annealed. Use Nanodrop or Qubit to quantify PCR product. The amount of each mutant and wild-type PCR product in the reaction should be between 50-250 ng. Insufficient PCR products quantity will lead to weak bands during subsequent electrophoresis, while excessive PCR products quantity will lead to incomplete enzymatic digestion.

2.1 Prepare the reaction mixture according to the following table.

Components	19 μL
10X Buffer CutB	2 μL
PCR Product (WT)	50-250 ng
PCR Product (Mutant)	50-250 ng（same as the WT）
Nuclease-free Water	to 19 μL

2.2 Perform annealing according to the following PCR program.

Step	Temperature	Time
Denaturation	95℃	5 min
Annealing	95-85℃	-2℃/sec
	85-25℃	-0.1℃/sec
Hold	4℃	-

2.3 Add 1 μL T7 Endonuclease I (10,000 U/mL) to the obtained annealing products.

2.4 Incubate at 37 ℃ for 15 min.

Note: The recommended digestion time is 15 minutes, depending on the length and starting amount of different PCR products, this time can be adjusted to achieve better electrophoresis results. However, please note that T7 Endonuclease I has weak nonspecific nuclease activity. If the digestion time is too long, it may cause smear or trailing bands in the electrophoresis gel.

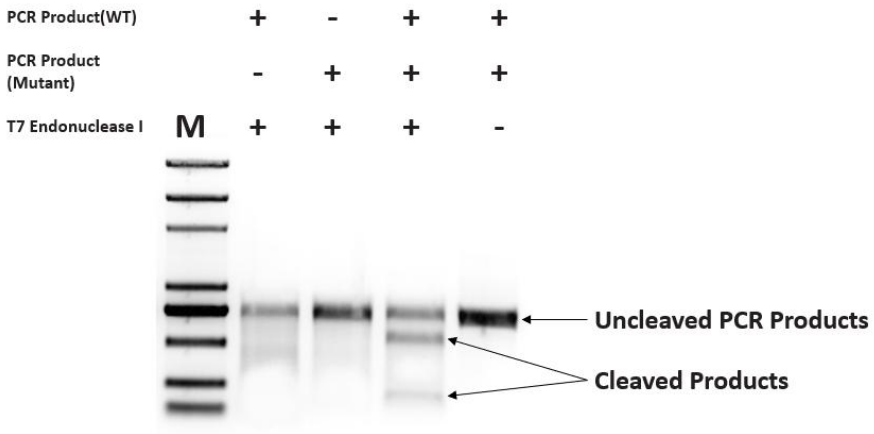
2.5 Add 1.5 μL 0.25 M EDTA to terminate the reaction. Or heat it at 80℃ for 15 minutes to inactivate the enzyme. It is recommended to proceed with gel electrophoresis immediately.

Note: If gel electrophoresis cannot be performed immediately, the terminated samples can be stored at -20℃.

2.6 Analyze the products by 2% agarose gel electrophoresis.

Electrophoresis Results

1. Example of hybrid dsDNA



M: 100 bp、250 bp、500 bp、750 bp、1000 bp、2000 bp、3000 bp、5000 bp

The mismatched T7EI Control Template was digested by T7 Endonuclease I,so in the fig. above it shows two new bands of 190 bp and 550 bp.

The WT PCR Product and Mutant PCR Product do not have any mismatches, such PCR product had been slightly degradated and exhibited smear bands due to the nonspecific nuclease activity.

Notes

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. When the reaction temperature exceeds 42℃, it will increase the non-specific endonuclease activity of T7 Endonuclease I. When the reaction temperature exceeds 55℃, enzyme activity will be decreased.

3. Manganese ions significantly increase non-specific endonuclease activity of T7 Endonuclease I. It is advised to avoid the presence of manganese ions in the PCR buffer.

4. T7 Endonuclease I had been tested to be compatible with various PCR buffers. PCR products can be used for enzyme digestion detection without purification. If digestion results are abnormal, user can try purifying the PCR products.