

# T7 Endonuclease I

## 1 Packing list

Components	HY-KE8005-250U
T7 Endonuclease I (10 U/ $\mu$ L)	25 $\mu$ L
5 $\times$ T7 Endonuclease I Reaction Buffer	1 mL
Control primer (10 $\mu$ M)	20 $\mu$ L
Control template C (30 ng/ $\mu$ L)	10 $\mu$ L
Control template D (30 ng/ $\mu$ L)	10 $\mu$ L

## 2 Introduction

T7 Endonuclease I recognizes and cleaves imperfectly paired DNA, cruciform structure DNA, Holliday structure or crossover DNA, heteroduplex DNA, or cleaves nicked double-stranded DNA at a slower rate. This enzyme cleaves the first, second, or third phosphodiester bond at the 5' end of the mismatched base.

## 3 Unit definition

1 unit refers to the amount of enzyme required to convert more than 90% of 1  $\mu$ g of supercoiled cross-shaped structure pUC(AT)\* into a linear DNA structure in 1 hour at 37°C in a 50  $\mu$ L reaction system.

## 4 General Protocol

1) Using mutant DNA and wild-type DNA as templates, PCR amplify the fragment containing the mutation site. The length of the fragment is about 500 bp. The mutation site should be avoided in the middle of the fragment to facilitate the differentiation of the bands after cutting.

2) Denatured annealed PCR product:

Components	Adding amount
10 $\times$ T7 Endonuclease I Reaction Buffer	1 $\mu$ L
PCR product	0.3 $\mu$ g
Water(RNase-Free)	Up to 9 $\mu$ L

3) Enzyme digestion reaction: Add 0.5  $\mu$ L T7 E1 enzyme to the reaction solution in the previous step, and incubate at 37°C for 15-30min. Immediately add DNA loading buffer to terminate the reaction. After mixing, incubate at 65°C for 10min. Check the enzyme digestion results by agarose gel electrophoresis.

## 5 Storage

-20°C, 1 years

## 6 Precautions

1. Use T7 endonuclease I, an enzyme with substrate structure selectivity. The enzyme acts on different DNA substrates with different activities. When cleaving a specific substrate, the amount of enzyme and reaction time must be controlled. When the reaction temperature exceeds 42°C, non-specific nuclease activity will increase. Avoid reaction temperatures exceeding 55°C, as this will lead to a decrease in enzyme activity.
2. This product is for R&D use only, not for drug, household, or other uses.
3. For your safety and health, please wear a lab coat and disposable gloves to operate.