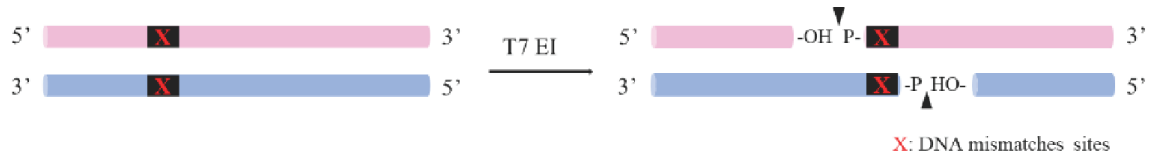
T7 Endonuclease I

Product description

T7 Endonuclease I (T7EI) is a special DNA Endonuclease, derived from the recombinant T7 Endonuclease I gene expressed by E.coli. It can cleave the non-perfectly matched DNA, cruciform DNA structures, Holliday structured or junctions DNA, and heteroduplex DNA, as well as cleave nicked double-stranded DNA to a lesser extent. Note that the length of insertion, deletion or mutation fragment for T7EI recognition must be ≥ 2 bp, and the cleavage site is the first, second or third phosphodiester bond at the 5 'end of the mismatch base. This product is commonly used for the detection of mutants formed by CRISPR/Cas9, TALEN or other gene editing tools, the detection of gene point mutation and SNP, and the random cleavage of linear DNA for shotgun cloning.



T7 Endonuclease I recognize and cleave the DNA mismatches

Components and storage conditions

Components \ Size	250 U	1250 U
T7 Endonuclease I (10 U/μL)	0.025 mL	0.125 mL
10X T7 Endonuclease I Reaction Buffer	0.25 mL	1.25 mL
Store the components at -20 °C.		

Experimental operation

Take the digestion of base-mismatched DNA fragments for example

1. Configure the reaction system on ice according to the following table:

Total Reaction Volume	20 μL	
DNA fragment	X μL	~ 200 ng
10X T7 Endonuclease I Reaction Buffer	2 μL	
T7 Endonuclease I	1 μL	
Nuclease-free Water	X μL	To 20 μL

2. Mix the reaction system Gently, then centrifuge rapidly to collect the residual liquid from the tube wall.
3. Incubate at 37°C for 15-30 min.
4. Heat at 80 °C for 15 min or add 1.5 μL of 0.25 M EDTA to terminate the digestion reaction.
5. Lysing bands can be examined by agarose gel electrophoresis.

Notes

1. T7 Endonuclease I should be added last when configuring the reaction system. Keep the enzyme on ice when removing it from the freezer.
2. Mix the components by repeat pipetting or "flicking" the tube. Do not mix the reactants by vortexing.
3. T7 endonuclease I is structurally selective and acts on different DNA substrates with different activities. Therefore, for specific substrate cleavage, the enzyme dosage and reaction time must be controlled.
4. Excessively high reaction temperature increase nonspecific nuclease activity (>42°C) and decrease T7 Endonuclease I activity (>55°C).
5. For the detection of mutation sites, it should be avoided that the mutation site is in the middle position of DNA, otherwise the subsequent electrophoresis cleavage bands may not be distinguished.

Product characteristics

1. Enzyme activity unit (U) definition: The amount of enzyme required to digest 90% of 1 ug supercoiled cruciform pUC(AT) at 37°C for 1 h in a 50 μL reaction containing T7 Endonuclease I Reaction Buffer.
2. Stored solution composition: 20 mM Tris-HCl (pH 7.5), 200 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 0.2% Triton X-100, 50% Glycerol.
3. 10X T7 Endonuclease I Reaction Buffer: 100 mM Tris-HCl(pH 7.9 @ 25°C), 500 mM NaCl, 100 mM $MgCl_2$, 10 mM DTT.
4. Quality assurance:
   - Purity: SDS-PAGE detection purity > 95%.
   - No non-specific nuclease, endonuclease or exonuclease.
5. Inactivation conditions: T7 Endonuclease I can be inactivated by heating at 80 °C for 15 minutes.
6. This product is for scientific use only.

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