

Product components

Components	Component Number	Concentration	4,000 U
Thermostable Mismatch Endonuclease I	RM20571	80,000 U/mL	50 µL
10X Buffer CutB	RM20105	10X	1.25 mL

Product Description

Thermostable Mismatch Endonuclease I is a Mg²⁺ dependent DNA endonuclease that specifically cleaves mismatched base pairs (T/T, T/G, T/C, G/G, and A/G mismatches). The enzyme cleaves at the 3rd phosphodiester bond upstream (5' direction) of the mismatched base on the DNA duplex, resulting in a 5-nucleotide 5' overhang (sticky end).

Product Source

Thermostable Mismatch Endonuclease I was expressed in *E. coli*. and subsequently purified.

Storage

Store at -20°C and avoid repeated freeze-thaw cycles

Unit Definition

One unit is defined as the amount of enzyme required to cleave 50% of 0.2 pmol of fluorescently labeled 60-mer double-stranded oligonucleotide containing a single T:T mismatched after 30 min at 55°C in 1X Buffer CutB.

Reaction Conditions

1X Buffer CutB, incubate at 55°C

1X Buffer CutB

10 mM Tris-HCl, 50 mM NaCl, 10 mM MgCl₂, 100 µg/mL Recombinant Albumin, pH 7.9 @ 25°C

Storage Conditions

10 mM Tris-HCl, 500 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 50 % Glycerol, pH 7.4 @ 25°C

Heat Inactivation

No

Operation Description

1. Set-up the following reaction on ice, noting that the enzyme should be added at the end and then gently mix the reaction by pipetting up and down and microcentrifugation.

Components	50 μ L Reaction
10X Buffer CutB	5 μ L(1X)
Substrate (Mismatch- containing DNA)	to 8 pmol
Thermostable Mismatch Endonuclease I	1 μ L(80 units)
Nuclease-Free-Water	Up to 50 μ L

2. Incubate at 55°C for 30 minutes.
3. Terminate the reaction by adding EDTA to at least 25 mM.

Notes

1. The enzyme exhibits increased activity at higher reaction temperatures.
2. For precise control or partial digestion applications, it is recommended to optimize the enzyme-to-substrate ratio by performing an enzyme titration.