Tinzyme Co., Limited

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Exonuclease III

Product Number: EN03

Description

Exonuclease III is an enzyme that acts on double-stranded DNA, progressively removing single nucleotides from the 3'-OH terminus. The optimal substrates for this enzyme are blunt-ended or 5' overhanging DNA, but it can also act on double-stranded DNA nick sites to produce single-strand nicks. Since it is inactive on single-stranded DNA, it has difficulty cleaving 3' overhanging ends. The 3' to 5' exonuclease activity of Exonuclease III varies with the length of the 3' overhang; ends with four or more nucleotides are difficult to cleave. This characteristic can be used to produce single-stranded DNA in a specific direction. For example, linearized DNA can be designed with one end being an uncleavable terminus (3' overhang) and the other end being a cleavable terminus (blunt end or 5' overhang). In this case, Exonuclease III will digest only one strand.

The activity of Exonuclease III is partly dependent on the DNA double helix structure and varies with the sequence (C > A = T > G). Additionally, this enzyme also exhibits RNase H, 3'-phosphatase, and apurinic/apyrimidinic (AP) site-specific endonuclease activities.

Components

Component	EN03-01	EN03-02	EN03-03
Exonuclease III (100 U/μL)	5 KU	25 KU	100 KU

Storage

Store at -30 to -15°C. Shipping conditions: ≤0°C.

Unit Definition

One unit (U) of enzyme activity is defined as the amount of enzyme required to produce 1 nmol of acid-soluble material from double-stranded DNA at 37°C for 30 minutes.

Applications

1. Unidirectional nested deletion
2. Site-directed mutagenesis
3. Preparation of single-strand-specific probes
4. Preparation of single-strand substrates for dideoxy sequencing

Protocol

1. Prepare the reaction mixture as suggested below (on ice):

DNA:	~5 μg
10×Exo III Buffer:	5 μl
Exonuclease III:	0.5 μl
Nuclease-Free Water:	To 50 μl

2. Incubate at 37°C for 30 minutes.
3. Terminate the reaction by adding EDTA to a final concentration of 11 mM, or inactivate Exonuclease III by incubating at 70°C for 30 minutes.
4. Analyze the double-stranded DNA digestion results by agarose gel electrophoresis.

Notes

Exonuclease III cannot cleave thiophosphate bonds. Therefore, a DNA molecule can be protected at one end by introducing an

α-thiophosphate nucleotide, allowing for unidirectional digestion. This characteristic can be used for unidirectional digestion of linear DNA molecules with one end being a resistant terminus (3' overhang) and the other end being a sensitive terminus (blunt end or 5' overhang).