Exonuclease III

REF: EG20207S

Storage Condition

-20℃

Components

Component	Amount
Exonuclease III (100 U/μl)	50 μl
10×Exo III Buffer	500 μl

Description

Exonuclease III is a nuclease that stepwise removes mononucleotides from the 3'-OH termini of double-stranded DNA (dsDNA), and inactive on single-stranded DNA (ssDNA). The preferred substrates of Exonuclease III are blunt or recessed 3'-termini, although the enzyme also acts at nick sites in double-stranded DNA to generate single-stranded gaps. 3'-overhanging termini are resistant to cleavage; the degree of resistance depends on the length of the extension, with extensions 4 bases or longer being essentially resistant to cleavage. This characteristic can be used to produce strand-specific ssDNA with designed linearized dsDNA having one non-cleavage end (3'-overhang) and the other end designed as a cleavable end (blunt or 5'-overhang), allowing Exonuclease III to digest only one strand.

Exonuclease III also exhibits RNase H, 3'-phosphatase, and apurinic/apyrimidinic-endonuclease activities.

Definition of Activity Unit

One unit is defined as the amount of enzyme required to produce 1 nmol of acid-soluble material from double-stranded DNA as a substrate in 30 minutes at 37℃ .

Applications

1. Unidirectional nested deletion.
2. Site-directed mutagenesis.
3. Generation of strand-specific probes.
4. Generation of single-stranded substrates for dideoxy sequencing.
5. Cleavage of probe with AP site.

Quality Control Assays

Endonuclease Activity

A 50 μl reaction containing 200 ng of supercoiled plasmid and 100 U of Exonuclease III incubated for 4 hours at 37 ℃ results in <10% conversion to the nicked or linearized form as determined by agarose gel electrophoresis.

Protocol

1. Prepare the following reaction mixture on ice:

Reagent	Amount
DNA	~5 μg
10×Exo III Buffer	5 μl (1×)
Exonuclease III	0.5 μl
Nuclease-Free Water	To 50 μl

2. Mix gently and spin down, then incubate at 37℃ for 30 minutes.
3. Add EDTA to a final concentration of 11 mM to stop the reaction.
4. Heat inactivate at 70℃ for 30 minutes.
5. It is recommended to recover the products using one of the following methods:
   a. Clean with DNA purification kit.
   b. Perform agarose gel electrophoresis and recover DNA from the gel.
   c. Perform phenol/chloroform extraction, followed by ethanol precipitation.

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