Exonuclease III (*E. coli*)

Catalogue number: MB43001, 5000 U

Description

Exonuclease III (*E. coli*) is a typical double-stranded DNA specific nuclease that cleaves DNA from the 3' termini of double-stranded DNA with 5' overhangs or blunt ends and 3' overhangs containing less than four bases. The enzyme also initiates cleavage at nicked sites in double-stranded DNA although this is not the preferred substrate. The enzyme is not active on single-stranded DNA. Thus, exonuclease III (*E. coli*) is an enzyme that catalyses the removal of nucleotides from linear or nicked double-stranded DNA in the 3' to 5' direction. Exonuclease III (*E. coli*) has also been reported to display residual RNase H, 3´-phosphatase and AP-endonuclease activities.

Storage conditions

Exonuclease III (*E. coli*) should be stored at -20 °C in a constant temperature freezer. The protein will remain stable till the expiry date if stored as specified.

Unit definition

One unit of enzyme activity is defined as the amount of enzyme required to produce 1 nmol of acid-soluble total nucleotide in a total reaction volume of 50 µL in 30 minutes at 37 °C.

Enzyme concentration: 100 U/ µL

Inactivation

Exonuclease III (*E. coli*) is heat inactivated at 70 °C for 20 min.

System components and Reaction conditions

Exonuclease III (*E. coli*) is provided with a dedicated and highly optimized NZYtech reaction buffer and displays an optimum temperature of 37 °C.

Standard protocol

The following standard protocol serves as a general guideline for the degradation of nicked and linear dsDNA (with blunt or 5' overhangs) from 3' to 5' direction with Exonuclease III (*E. coli*), leaving supercoiled dsDNA. Preferably the enzyme should be added last.

1. Prepare the following 50 µL reaction:

Component	Volume
Substrate DNA	≤ 5 µg
Exonuclease III reaction buffer (10x)	5 µL
Exonuclease III	0.5 µL (50 U)
Nuclease-free H_2O (Cat. No. MB11101)	up to 50 µL

Note: *It may be required to titrate the enzyme or test different incubation periods for more specific results or partial digestions.*

2. Gently mix and pulse.
3. Incubate at 37 °C for 30 minutes.
4. If required, heat inactivation 70 °C for 30 minutes.
5. To obtain a highly pure product, perform a column purification step using NZYGelpure kit (Cat. No. MB011). Best results may be achieved by separating cleaved DNA through agarose gel electrophoresis prior to DNA clean-up.

Quality Control Assays

Purity

Exonuclease III (*E. coli*) is >95% pure as judged by SDS polyacrylamide gel electrophoresis followed by BlueSafe staining (NZYtech, Cat. No. MB15201).

Nucleases assays

To test for DNase contamination, 0.2-0.3 µg of supercoiled pNZY28 plasmid DNA are incubated with 100 U of Exonuclease III (*E. coli*) for 14-16 hours at 37 °C. To test for RNase contamination, 1 µg of RNA is incubated with 100 U of Exonuclease III (*E. coli*) for 1 hour at 37 °C. Following incubation, the nucleic acids are visualized on a GreenSafe-stained agarose gel. There must be no visible nicking or cutting of the nucleic acids.

Functional assay

Exonuclease III *(E. coli*) is assayed in a reaction containing 100 ng of a double stranded DNA fragment of 1 kb under standard conditions. Activity is measured by monitoring reduction of absorbance (A260nm) over time.

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