Exonuclease VII (*E. coli*)

Catalogue number: MB43201, 200 U

Description

Exonuclease VII (*E. coli*) is a typical DNA specific exonuclease that cleaves linear single-stranded DNA in both the 3' to 5' and 5' to 3' directions. The enzyme maybe used for the removal of primers with or without 3' or 5' terminal phosphorothioate bonds or to map positions of introns in genomic DNA. Generally, Exonuclease VII (*E. coli*) is used to remove single-stranded DNA, leaving behind the double-stranded DNA in a sample, in particular in PCR reactions. This enzyme is not active on linear or circular dsDNA.

Storage conditions

Exonuclease VII (*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 that will catalyse the release of 1 nmol of acid-soluble nucleotide in a total reaction volume of 50 µL in 30 minutes at 37 °C.

Enzyme concentration: 10 U/ µL

Inactivation

Exonuclease VII (*E. coli*) is heat inactivated at 95 °C for 10 min.

System components and Reaction conditions

Exonuclease VII (*E. coli*) is provided with a dedicated 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 removing primers with or without 3' modifications from a PCR reaction with Exonuclease VII (*E. coli*). Preferably the enzyme should be added last.

1. Prepare the following reaction mixture:

Component	Volume
PCR Product	7 µL
Exonuclease VII (E. coli)	2 µL (20 U)

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. Heat inactivation 95°C for 10 minutes.

5. To obtain a highly pure product, perform a column purification step using NZYGelpure kit (NZYTech, 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 VII (*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 10 U of Exonuclease VII (*E. coli*) for 14-16 hours at 37 °C. To test for RNase contamination, 1 µg of RNA is incubated with 10 U of Exonuclease VII (*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 VII (*E. coli*) is tested for activity by measuring their capacity to degrade oligonucleotide primers used in a PCR reaction.

V2101

Certificate of Analysis

Test	Result
Enzyme purity	Pass
Nucleases contamination	Pass
Functional assay	Pass

Approved by:

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