Exonuclease I (*E. coli*)

Catalogue number: MB42901, 3000 U

Description

Exonuclease I (*E. coli*) is a typical DNA specific exonuclease that catalyses the removal of nucleotides from linear single-stranded DNA in the 3' to 5' direction. The enzyme may be used to remove single-stranded primers in PCR reactions prior to Sanger DNA sequencing or SNP analysis nested PCR reactions. Exonuclease I (*E. coli*) may be used to generally remove linear single-stranded DNA, leaving behind double-stranded DNA in the sample.

Storage conditions

Exonuclease I (*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 10 nmol of acid-soluble nucleotide in a total reaction volume of 50 µL in 30 minutes at 37 °C.

Enzyme concentration: 20 U/ µL

Inactivation

Exonuclease I (*E. coli*) is heat inactivated at 80 °C for 15 min.

System components and Reaction conditions

Exonuclease I (*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 to proceed to an enzymatic PCR clean-up to remove unincorporated primers and nucleotides with Exonuclease I (*E. coli*). Preferably the enzyme should be added last.

1. Prepare the following reaction mixture:

Component	Volume
PCR product	9 µL
Exonuclease I (*E. coli*)	1 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 15 minutes.
4. Inactivate Exonuclease I (*E. coli*) at 80 °C for 15 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 I (*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 20 U of Exonuclease I (*E. coli*) for 14-16 hours at 37 °C. To test for RNase contamination, 1 µg of RNA is incubated with 20 U of Exonuclease I (*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 I (*E. coli*) is tested for activity by measuring their capacity to degrade oligonucleotide primers used in a PCR reaction following standard protocols.

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