Alkaline Phosphatase (*E. coli*)

Catalogue number: MB01801, 200 U

Description

Alkaline Phosphatase (*E. coli*) non-specifically catalyses the removal of most phosphomonoester bonds from the 5' and 3' termini of DNA and RNA without degrading diphosphate or triphosphate linkages. The enzyme is suitable for removal of terminal monoesterified phosphates from deoxyribo-oligonucleotides. The enzyme is generally used for the removal of single phosphate groups from 5´-ends of linear vectors to prevent re-circularization during cloning or to dephosphorylate DNA prior to kinase labelling protocols.

Storage temperature

Alkaline Phosphatase 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 liberates 1 µmol of p-nitrophenol per minute at 25 °C, pH 8.0 with p-nitrophenyl phosphate as the substrate.

Enzyme concentration: 0.5 U/µL

Inactivation

Alkaline Phosphatase (*E. coli*) is highly resistant to heat inactivation. Thus, alternative protocols should be considered when requiring removing the enzyme from reactions, such as DNA silica column purification or phenol/chloroform extraction.

System components and Reaction conditions

Alkaline Phosphatase (*E. coli*) is provided with a dedicated highly optimized NZYtech reaction buffer, which includes Zn^{2+} is essential for enzyme activity. The optimum reaction temperature is 37 °C.

Protocol for dephosphorylation of 5´-ends of DNA

1. Perform a typical dephosphorylation reaction using 1.0 pmol of DNA termini (1.0 µg of a 3.0 kb plasmid) and using between 0.1 to 1 U of Alkaline Phosphatase (higher amounts for 5´-recessed terminus) in 1× Reaction buffer (provided).

2. Incubate at 37 °C for 1 hour.

3. After the reaction is complete, perform a DNA purification step using NZYGelpure (NZYtech, Cat. No. MB011).

Quality Control Assays

Purity

Alkaline Phosphatase (*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 pNZY28 plasmid DNA are incubated with 1 U of Alkaline Phosphatase for 14-16 hours at 37 °C. To test for RNase contamination, 1 µg of RNA is incubated with 1 U of Alkaline Phosphatase 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

Alkaline Phosphatase (*E. coli*) is assayed in a dephosphorylating reaction followed by ligation and transformation. The efficiency of enzyme reaction is evaluated by the number of bacterial colonies transformed with the cloning product.

Related products

Product name	Cat. No.
Water for Molecular Biology	MB111
T4 Polynucleotide Kinase	MB008
Klenow Fragment of DNA Polymerase I	MB00901
Speedy Ligase	MB130
NZY5α competent cells	MB004

V2401

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