Vol Act: 1 unit / µl ; 1.000 ml
D Nase (1) + (2) > 50 units
Exo: > 50 units
Nick:> 50 u ; R Nase:> 50 units
S.A.: 3308 units / mg
Boehringer Mannheim GmbH

3/3/92

Phosphatase, alkaline

from calf intestine

Orthophosphoric-monoester phosphohydrolase (alkaline optimum), EC 3.1.3.1

Special quality for molecular biology
1 000 units, Cat. No. 713 023
1 000 units, Cat. No. 1097 075, high concentration

Product description

Volume activities:
Cat. No. 713 023: ca. 1 × 10^3 units/ml.
Cat. No. 1097 075: ca. 20 × 10^3 units/ml.
Alkaline phosphatase is assayed according to (1). One unit of alkaline phosphatase is the enzyme activity which hydrolyzes 1 µmol of 4-nitrophenyl phosphate in 1 min at 37 °C under assay conditions. Note: According to (1) 5 units alkaline phosphatase (37 °C; diethanolamine buffer) correspond to 1 unit alkaline phosphatase (25 °C; glycine/NaOH buffer).
See data label for lot-specific values.

Storage buffer: Triethanolamine, 30 mmol/l; NaCl, 3 mol/l; $MgCl_2$, 1 mmol/l; $ZnCl_2$, 0.1 mmol/l; pH ca. 7.6.

Activity determination: The activity determination is performed according to (1) at 37 °C in diethanolamine buffer, 1 mol/l; 4-nitrophenyl phosphate, 10 mmol/l; $MgCl_2$, 0.5 mmol/l; pH 9.8.

Specific activity: ca. 2000 units/mg according to (1) and (2). See data label for lot-specific values.

Stability: stable at +4 °C.

Supplied buffer:
Dephosphorylation buffer, 10-times concentrated: Tris-HCl, 0.5 mol/l; EDTA, 1 mmol/l; pH 8.5 (20 °C).

Incubation procedure (3, 4): The reaction assay is adjusted with 1/10 volume 10 × dephosphorylation buffer.

a) 50 pmol 5'terminal phosphorylated DNA fragments (3'-recessed, 5'-recessed or blunt-ended) are incubated with 1 unit alkaline phosphatase at **37 °C** for 60 min **or**

b) 50 pmol 5'terminal phosphorylated RNA fragments are incubated with 1 unit alkaline phosphatase at **50 °C** for 60 min.

Inactivation of alkaline phosphatase (4, 5): Add 1/10 volume of EGTA, 200 mmol/l, to the reaction assay and heat to 65 °C for 10 min. To achieve complete inactivation of alkaline phosphatase, an extraction with phenol/chloroform/isoamylalcohol (50 : 48 : 2) should be performed.

Quality control

See data label for lot-specific values.

Absence of deoxyribonucleases:
1) 1 µg λDNA is incubated with alkaline phosphatase for 1 h at 37 °C in 25µl dephosphorylation buffer. The number of enzyme units which shows no degradation of λDNA after agarose gel electrophoresis is stated under "DNase(1)".
2) 1 µg *Eco* RI/*Hind* III fragments of λDNA is incubated with alkaline phosphatase for 1 h at 37 °C in 25 µl dephosphorylation buffer. The number of enzyme units which shows no change in the banding pattern after agarose gel electrophoresis is stated under "DNase(2)".

Absence of nicking activities: 1 µg supercoiled pBR322 DNA is incubated with alkaline phosphatase for 1 h at 37 °C in 25 µl dephosphorylation buffer. The number of enzyme units which shows no relaxing of the supercoiled structure of pBR322 DNA after agarose gel electrophoresis is stated under "Nick".

Absence of exonucleases: A 40-mer oligonucleotide is incubated with increasing amounts of alkaline phosphatase for 4 h at 37 °C and checked for exonuclease contamination by ^{32}P-labeling with polynucleotide kinase* and subsequent separation on a 8% polyacrylamide sequencing gel. The number of enzyme units which shows no degradation of the oligonucleotide is stated under „Exo".

Absence of ribonucleases: 5 µg MS2 RNA are incubated with alkaline phosphatase for 1 h at 50 °C in 50 µl dephosphorylation buffer. The number of enzyme units which shows no change of MS2 RNA after agarose gel electrophoresis is stated under "RNase".

References

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3 Maxam, A. M. & Gilbert, W. (1980) Methods Enzymol. **65,** 499.
4 Chaconas, G. & v. d. Sande, J. H. (1980) Methods Enzymol. **65,** 75.
5 Simsek, M. et al. (1973) Proc. Natl. Acad. Sci. USA **70,** 1041.

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