

# Phosphatase, alkaline

From calf intestine

*Orthophosphoric-monoester phosphohydrolase*  
(alkaline optimum), EC 3.1.3.1

Special quality for molecular biology

Cat. No. 713 023 1000 units  
Cat. No. 1 097 075 1000 units, high concentration

Version 3, June 1999

Store at 2-8° C

## Product description

**Volume activities** Cat. No. 713 023: ca.  $1 \times 10^3$  units/ml.  
Cat. No. 1 097 075: ca.  $20 \times 10^3$  units/ml.  
Alkaline phosphatase is assayed according to (1).  
One unit of alkaline phosphatase is the enzyme activity which hydrolyzes 1  $\mu$ mol 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** 25 mM Tris-HCl, 1 mM MgCl<sub>2</sub>, 0.1 mM ZnCl<sub>2</sub>, 50% glycerol (w/v), pH 7.6 (4° C).

**Activity determination** The activity determination is performed according to (1) at 37° C in 1 M diethanolamine buffer, 10 mM 4-nitrophenyl phosphate, 0.5 mM MgCl<sub>2</sub>, pH 9.8.

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

**Stability** stable at 2-8° C.

**Supplied buffer** Dephosphorylation buffer, 10-times concentrated: 0.5 M Tris-HCl, 1 mM EDTA, pH 8.5 (20° C).

**Incubation procedure (3, 4)** The reaction assay is adjusted with  $1/10$  volume  $10 \times$  dephosphorylation buffer.

- 1 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
- 1 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 200 mM EGTA, 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  $\mu$ g  $\lambda$ DNA is incubated with alkaline phosphatase for 1 h at 37° C in 25  $\mu$ l dephosphorylation buffer. The number of enzyme units which shows no degradation of  $\lambda$ DNA after agarose gel electrophoresis is stated under "DNase(1)".
  - 1  $\mu$ g *Eco* RI/*Hind* III fragments of  $\lambda$ DNA is incubated with alkaline phosphatase for 1 h at 37° C in 25  $\mu$ l 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  $\mu$ g supercoiled pBR322 DNA is incubated with alkaline phosphatase for 1 h at 37° C in 25  $\mu$ l 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

15 nmol of sonicated [<sup>3</sup>H]-DNA (ca. 100 000 cpm/ $\mu$ g) from calf-thymus are incubated with alkaline phosphatase for 4 h at 37° C in 100  $\mu$ l buffer (50 mM Tris-HCl, 10 mM MgCl<sub>2</sub>, 1 mM dithioerythritol, pH 7.5). The number of enzyme units that do not liberate radioactivity is stated under "Exo".

## Absence of ribonucleases

5  $\mu$ g MS2 RNA are incubated with alkaline phosphatase for 1 h at 50° C in 50  $\mu$ l 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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