

Alkaline Phosphatase, Calf Intestinal (CIP)

F-201S

10 units/ μ l

Store at -20°C

Stable for one year from the assay date

Alkaline phosphatase catalyses the removal of 5' phosphate residues from DNA, RNA and ribo- and deoxyribonucleoside triphosphates. Primarily used to remove 5' phosphates from DNA and RNA prior to labelling the 5' end with ^{32}P and to remove 5' phosphates from DNA fragments to prevent self ligation (1). Finnzymes' alkaline phosphatase is purified free of exonucleases, endonucleases and RNases.

Unit assay conditions

1 M diethanolamine-HCl (pH 9.8)
0.5 mM MgCl_2
10 mM p-nitrophenylphosphate
CIP

Reaction conditions:

10 mM Tris-HCl (pH 7.9 at 25°C)
10 mM MgCl_2
1 mM dithiothreitol
50 mM NaCl

Unit definition: One unit is defined as the amount of enzyme that hydrolyses 1 μ mol of p-nitrophenylphosphate to p-nitrophenol in 1 minute at 37°C in a volume of 1 ml (2).

Concentration and shipping: 10,000 units/ml. Supplied in 10 mM Tris-HCl (pH 8.2), 50 mM KCl, 1 mM MgCl_2 , 0.1 mM ZnCl_2 and 50 % glycerol.

Specific activity: >2000 U/mg (2, 3)

16-hour incubation: A 50 μ l reaction containing 1 μ g of λ DNA and 5 U of enzyme incubated for 16 hours at 37°C resulted in the same DNA band as a reaction produced without the enzyme.

Exonuclease activity: Incubation of 10 U for 4 hours at 37°C in 50 μ l assay buffer with 1 μ g sonicated ^3H DNA (2×10^5 cpm/ μ g) released < 0.5 % of radioactivity.

Endonuclease contamination: Incubation of 10 U with 1 μ g ϕ X174 RFI DNA (4 hours, 37°C, 50 μ l) gave < 5 % conversion to RFI.

RNase contamination: Incubation of 10 U with 1 μ g MS2 RNA (4 hours, 37°C, 50 μ l) resulted in the same pattern of RNA band as produced without the enzyme.

Transformation assay: pUC 19 DNA was linearized with *Hind* III restriction endonuclease. The DNA was then treated with 0.01 U/pmol 5'ends Calf Intestinal Alkaline Phosphatase at 37°C for 60 minutes. After phenol/chloroform extraction and ethanol precipitation the ligation efficiency of the DNA was tested with and without Kinase treatment. Also the ability to ligate an insert to the vector was tested. After ligation the DNAs were transformed into competent cells and plated onto plates containing X-Gal and IPTG.

Reference: (1) Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Laboratory, pp. 134.

(2) Mossner, E., Boll, M. and Pfeiderer, G. (1980) *Hoppe-Seyler's Z. Physiol. Chem.* 361, 543-549.

(3) Bradford, M. (1976) *Anal. Biochem.* 72, 248.

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