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Alkaline Phosphatase, Calf Intestinal (CIP)

#M0290S 1,000 units ..... $58 (USA)
#M0290L 5,000 units ..... $232 (USA)

Description
Alkaline phosphatase catalyzes the removal of 5´ phosphate groups from DNA, RNA, ribo- and deoxyribonucleoside triphosphates. Since CIP-treated fragments lack the 5´ phosphoryl termini required by ligases, they cannot self-ligate (1). This property can be used to decrease the vector background in cloning strategies.

Source
Calf intestinal mucosa

Applications
- Removing of 5´ and 3´ phosphoryl groups from nucleic acids
- Preparing of templates for 5´ end labeling
- Preventing fragments from self ligating
- Dephosphorylation of proteins

Reaction Buffer
10X NEBuffer 3:
[100 mM NaCl, 50 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM dithiothreitol (pH 7.9 @ 25°C)].

CIP is also active in NEBuffers 2 or 4 as well as the NEBuffers for EcoR I, BamH I and Sal I.

Concentration
10,000 units/ml

Storage Conditions
50 mM KCl, 10 mM Tris-HCl (pH 8.2), 1 mM $MgCl_2$, 0.1 mM $ZnCl_2$ and 50% glycerol. Store at –20°C.

Unit Definition
One unit is defined as the amount of enzyme that hydrolyzes 1 µmole of p-nitrophenylphosphate to p-nitrophenol in 1 minute at 37°C in a volume of 1 ml (2).

Unit Assay Conditions
1 M diethanolamine-HCl (pH 9.8), 0.5 mM $MgCl_2$, 10 mM p-nitrophenylphosphate and enzyme. These conditions are only used for quantitating enzyme activity.

Dephosphorylating with CIP:
1. Suspend DNA in 1X NEBuffer (0.5 µg/10 µl).
2. Add 0.5 unit/µg vector DNA.
3. Incubate 60 minutes at 37°C.
4. Purify DNA by gel purification, spin-column purification or phenol extraction.

Note
Slightly lower activity may be observed in any reaction buffer containing a total salt concentration of less than 50 mM, under these conditions a 2-fold excess of CIP is recommended.

References
1. Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual*, (2nd ed.), p 5.72, Cold Spring Harbor: Cold Spring Harbor Laboratory Press.
2. Mossner, E., Boll, M. and Pfleiderer, G. (1980) *Hoppe Seyler's Z. Physiol. Chem*. 361, 543–549.