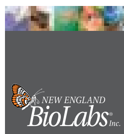
Alkaline Phosphatase, Calf Intestinal (CIP)



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M0290S

37°

1,000 units **Lot: 0631306** **Exp: 6/15**
10,000 U/ml **Store at –20°C**

Description: Alkaline Phosphatase catalyzes the removal of 5´ phosphate groups from DNA, RNA and 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

Molecular Weight: 69 kDa

Applications:
- Removing 5´ phosphates from DNA, RNA, rNTPs and dNTPs
- Preparation of templates for 5´end labeling
- Prevention of recircularization of cloning vectors
- Dephosphorylation of serine, threonine and tyrosine residues in proteins

Supplied in: 50 mM KCl, 10 mM Tris-HCl (pH 8.2), 1 mM $MgCl_2$, 0.1 mM $ZnCl_2$ and 50% glycerol.

Reagents Supplied with Enzyme:
10X NEBuffer 3.

Reaction Conditions: 1X NEBuffer 3.
CIP is also active in NEBuffers 2 or 4 as well as the NEBuffer for EcoRI.
Incubate at 37°C.

1X NEBuffer 3:
100 mM NaCl
50 mM Tris-HCl
10 mM $MgCl_2$
1 mM dithiothreitol
pH 7.9 @ 25°C

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

Unit Definition: One unit is defined as the amount of enzyme that hydrolyzes 1 µmol of *p*-nitrophenylphosphate to *p*-nitrophenol in a total reaction volume of 1 ml in 1 minute at 37°C (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.

Quality Controls Assays

Exonuclease Activity: Incubation of 50 units of CIP with a 1 µg mixture of sonicated single and double-stranded [^{3}H] DNA (200,000 cpm/µg), in a reaction volume of 0.05 ml, released < 0.1% of the total radioactivity in 4 hours at 37°C.

Endonuclease Activity: Incubation of 50 units of CIP with 1 µg of φX174 RF I DNA for 4 hours at 37°C in 50 µl reaction buffer resulted in < 5% conversion to RF II.

RNase Activity: Incubation of 50 units of CIP with 1 µg RNA Transcript for 4 hours at 37°C resulted in the same banding pattern as a sample with no enzyme.

Physical Purity: Purified to > 95% homogeneity as determined by SDS-PAGE analysis using Coomassie Blue detection.

Heat Inactivation: No

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.

CERTIFICATE OF ANALYSIS

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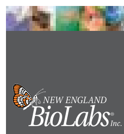

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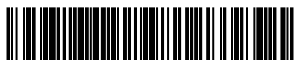

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