

## Alkaline Phosphatase, Calf Intestinal (CIP)



1-800-632-7799  
info@neb.com  
www.neb.com



M0290S 067160318031

# M0290S

37°

1,000 units Lot: 0671603 Exp: 3/18

10,000 U/ml Store at -20°C

**Description:** Alkaline Phosphatase, Calf Intestinal (CIP) nonspecifically catalyzes the dephosphorylation of 5' and 3' ends of DNA and RNA phosphomonoesters. CIP also hydrolyses ribo-, as well as deoxyribonucleoside triphosphates (NTPs and dNTPs). CIP is useful in many molecular biology applications such as the removal of phosphorylated ends of DNA and RNA for subsequent use in cloning or end-labeling of probes. In cloning, dephosphorylation prevents

religation of linearized plasmid DNA. The enzyme acts on 5' protruding, 5' recessed and blunt ends. CIP may also be used to degrade unincorporated dNTPs in PCR reactions to prepare templates for DNA sequencing or SNP analysis.

**Source:** Calf intestinal mucosa

**Specific Activity:** ~ 3,000 units/mg

**Molecular Weight:** CIP is a homodimer. The molecular weight of the monomer is 69 kDa.

### Applications:

- Dephosphorylation of cloning vector DNA to prevent recircularization during ligation
- Dephosphorylation of DNA prior to end-labeling using T4 Polynucleotide Kinase
- Treatment of dNTPs in PCR reactions prior to sequencing or SNP analysis
- Dephosphorylation of DNA and RNA

Supplied in: 50 mM KCl, 10 mM Tris-HCl (pH 8.2 @ 25°C), 1 mM MgCl<sub>2</sub>, 0.1 mM ZnCl<sub>2</sub> and 50% glycerol.

### Reagents Supplied with Enzyme:

10X CutSmart® Buffer

**Reaction Conditions:** 1X CutSmart Buffer.

Incubate at 37°C.

### 1X CutSmart Buffer:

50 mM Potassium acetate  
20 mM Tris-acetate  
10 mM Magnesium acetate  
100 µg/ml BSA  
pH 7.9 @ 25°C

**Unit Definition:** One unit is defined as the amount of enzyme that hydrolyzes 1 µmol of *p*-Nitrophenyl Phosphate, PNPP (NEB #P0757) in a total reaction volume of 1 ml in 1 minute at 37°C.

**Unit Assay Conditions:** 1 M diethanolamine-HCl (pH 9.8), 0.5 mM MgCl<sub>2</sub>, 50 mM *p*-Nitrophenyl Phosphate and enzyme. These conditions are only used for quantitating enzyme activity.

**Heat Inactivation:** No

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**Functional Assay:** Dephosphorylation with CIP of a restriction enzyme-digested vector DNA with 5' recessed ends, the least favorable type for dephosphorylation, reduces re-ligation to < 0.5% compared to untreated control as measured by transformation into *E. coli*. CIP has been functionally tested in the following protocol:

### Protocol for Dephosphorylation of 5'-ends of DNA using CIP

1. Prepare a 20 µl reaction as follows:

|                            |                     |
|----------------------------|---------------------|
| DNA                        | 1 pmol of DNA ends* |
| CutSmart Buffer (10X)      | 2 µl                |
| CIP                        | 1 unit              |
| H <sub>2</sub> O, purified | to 20 µl**          |

2. Incubate at 37°C for 30 minutes.

\* Note: 1 pmol of DNA ends is about 1 µg of a 3 kb plasmid.

\*\* Scale larger reaction volumes proportionally.

3. Purify DNA by gel purification, spin-column or phenol extraction.

CERTIFICATE OF ANALYSIS

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CERTIFICATE OF ANALYSIS

### Protocol for Dephosphorylation of 5'-ends of DNA using CIP in Restriction Enzyme Reaction

1. Digest 1–5 µg of plasmid DNA in a 20 µl reaction as follows:

|                                 |          |
|---------------------------------|----------|
| DNA                             | ≥ 1 µl   |
| Restriction Enzyme Buffer (10X) | 2 µl     |
| Restriction Endonuclease        | 1 µl     |
| H <sub>2</sub> O, purified      | to 20 µl |

*Note: Scale larger reaction volumes proportionally.*

2. Incubate at 37°C for 60 minutes or follow manufacturer's recommendations.
3. Add 1 unit of CIP for every 1 pmol of DNA ends (about 1 µg of a 3 kb plasmid) and incubate at 37°C for 30 minutes.
4. Purify DNA by gel purification, spin-column or phenol extraction.
5. Proceed with ligation.

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5. Proceed with ligation.

### Usage Notes:

CIP, as are most alkaline phosphatases, is a Zn<sup>2+</sup> and Mg<sup>2+</sup>-dependent enzyme. Our formulation of its storage buffer provides Zn<sup>2+</sup> and Mg<sup>2+</sup>, which does not require supplemental zinc or other additives in reactions with CIP.

CIP is also active in 1X NEBuffers 1.1, 2.1, 3.1, as well as NEBuffers 1, 2, 3, 4 and unique NEBuffer for EcoRI.

CIP activity is enhanced in the presence of monovalent salts.

CIP is inhibited by metal chelators (e.g. EDTA), inorganic phosphate and phosphate analogs.

The Antarctic Phosphatase activity is decreased in the presence of reducing agents (DTT, β-ME).

### Quality Controls Assays

**Exonuclease Activity:** Incubation of a 50 µl reaction containing 50 units of CIP with 1 µg of a mixture of single and double-stranded [<sup>3</sup>H] *E. coli* DNA for 4 hours at 37°C released < 0.1% of the total radioactivity.

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**Endonuclease Activity:** Incubation of a 50 µl reaction containing 50 units of CIP with 1 µg of φX174 RF I DNA for 4 hours at 37°C resulted in < 10% conversion to RF II as determined by agarose gel electrophoresis.

**RNase Activity (Extended Digestion):** A 10 µl reaction in CutSmart Buffer containing 40 ng of fluorescein labeled RNA transcript and 10 units of CIP is incubated at 37°C. After incubation for 4 hours, > 90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.

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

### 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 Pfeleiderer, G. (1980) *Hoppe-Seyler's Z. Physiol. Chem.* 361, 543–549.

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### Companion Products Sold Separately:

|                            |               |
|----------------------------|---------------|
| CutSmart Buffer<br>#B7204S | 5 ml          |
| T4 DNA Ligase<br>#M0202S   | 20,000 units  |
| #M0202L                    | 100,000 units |
| #M0202T                    | 20,000 units  |
| #M0202M                    | 100,000 units |

|                                |          |
|--------------------------------|----------|
| Quick Ligation™ Kit<br>#M2200S | 30 rxns  |
| #M2200L                        | 150 rxns |

|   |          |
|---|----------|
| Instant Sticky-end Ligase Master Mix<br>#M0370S | 50 rxns  |
| #M0370L   | 250 rxns |



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This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

### Companion Products Sold Separately:

|                            |               |
|----------------------------|---------------|
| CutSmart Buffer<br>#B7204S | 5 ml          |
| T4 DNA Ligase<br>#M0202S   | 20,000 units  |
| #M0202L                    | 100,000 units |
| #M0202T                    | 20,000 units  |
| #M0202M                    | 100,000 units |

|                                |          |
|--------------------------------|----------|
| Quick Ligation™ Kit<br>#M2200S | 30 rxns  |
| #M2200L                        | 150 rxns |

|   |          |
|---|----------|
| Instant Sticky-end Ligase Master Mix<br>#M0370S | 50 rxns  |
| #M0370L   | 250 rxns |



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