### Alkaline Phosphatase, Calf Intestinal (CIP)

**Description:** Alkaline Phosphatase, Calf Intestinal (CIP) nonspecifically catalyzes the dephosphorylation of 5’ and 3’ ends of DNA and RNA phosphomonoesters. Also, CIP 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 re-circularization 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

**Reagents Supplied with Enzyme:**
- 10X CutSmart™ Reaction Buffer
- Reagents Supplied with Enzyme:
  - CutSmart Buffer (10X)
  - 1 unit
  - H₂O, purified

### Protocol for Dephosphorylation of 5’-ends of DNA

1. Prepare a 20 µl reaction as follows:
   - DNA 1 pmol of DNA ends* 2 µl
   - CutSmart Buffer (10X) 2 µl
   - CIP 1 unit
   - H₂O, purified 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.

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

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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* 2 µl
   - CutSmart Buffer (10X) 2 µl
   - CIP 1 unit
   - H₂O, purified 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.

3. Purify DNA by gel purification, spin-column or phenol extraction.
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
   - H₂O, 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 30–60 minutes.

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

5. Proceed with ligation.

Usage Notes:
1. CIP, as are most alkaline phosphatases, is a Zn²⁺ and Mg²⁺-dependent enzyme. Our formulation of its storage buffer provides Zn²⁺ and Mg²⁺, which does not require supplemental zinc or other additives in reactions with CIP.

2. 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.

3. CIP activity is enhanced in the presence of monovalent salts.

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

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 [³²P] E. coli DNA for 4 hours at 37°C released < 0.1% of the total radioactivity.

Endonuclease Activity: Incubation of a 50 µl reaction containing 50 units of CIP for 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: Incubation of a 10 µl reaction containing 10 units of CIP with 40 ng of fluorescein labeled RNA transcript for 4 hours at 37°C resulted in < 10% degradation of the RNA as determined by gel electrophoresis using fluorescence detection.

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

Heat Inactivation: No

References:

Companion Products Sold Separately:
- T4 DNA Ligase
- Quick Ligation™ Kit
- Instant Sticky-end Ligase Master Mix
- Blunt/TA Ligase Master Mix

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