

## Certificate of Analysis

### Alkaline Phosphatase, Calf Intestinal (CIAP):

Part No.	Conc. (u/μl)	Size (units)
M182A	1	1,000
M282A	20	1,000

**Description:** Alkaline Phosphatase catalyzes the hydrolysis of 5'-phosphate groups from DNA, RNA and both ribo- and deoxyribonucleoside triphosphates.

**Enzyme Storage Buffer:** Alkaline Phosphatase, Calf Intestinal (CIAP), is supplied in 10mM Tris-HCl (pH 8.0), 1mM MgCl<sub>2</sub>, 0.1mM ZnCl<sub>2</sub>, 50mM KCl and 50% glycerol.

**Alkaline Phosphatase 10X Reaction Buffer (M183A):** When the 10X Reaction Buffer supplied with this enzyme is diluted 1:10, it has a composition of 50mM Tris-HCl (pH 9.3 at 25°C), 1mM MgCl<sub>2</sub>, 0.1mM ZnCl<sub>2</sub> and 1mM spermidine.

**Source:** Calf intestinal mucosa.

**Unit Definition:** One unit is defined as the amount of enzyme required to catalyze the hydrolysis of 1μmol of 4-nitrophenyl phosphate per minute at 37°C in 1M diethanolamine, 10.9mM 4-nitrophenyl phosphate, 0.5mM MgCl<sub>2</sub> (pH 9.8). See the unit concentration on the Product Information Label.

**Storage Temperature:** For long-term storage (infrequent use; 1–2 times per month), store at –70°C. For daily/weekly use, store at –20°C. Avoid multiple freeze-thaw cycles. See the expiration date on the Product Information Label.

Part# 9PIM182

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**Promega**

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## Quality Control Assays

### Contaminant Activity

**Endonuclease Assay:** To test for endonuclease activity, 1μg of Type I supercoiled plasmid DNA is incubated with 5 units of Calf Intestinal Alkaline Phosphatase in 1X Reaction Buffer for one hour at 37°C. Following incubation, the supercoiled DNA is visualized on an ethidium bromide-stained agarose gel to verify the absence of visible nicking or cutting.

**DNase and RNase Assay:** To test for nuclease activity, 50ng of radiolabeled DNA or radiolabeled RNA is incubated with 5 units of Calf Intestinal Alkaline Phosphatase in 1X Reaction Buffer for one hour at 37°C, and the release of radiolabeled nucleotides is monitored by scintillation counting of TCA-soluble material. Minimum passing specification is ≤3% release for DNase and ≤3% release for RNase.

**Blue/White Assay:** pGEM®-3Z(+/-) Vector is linearized with three different restriction enzymes, in separate reactions, to generate three different types of termini: 5'-overhangs, 3'-overhangs or blunt ends. Each microgram of cut plasmid is treated with 1 unit of Calf Intestinal Alkaline Phosphatase for 2 hours at 37°C, kinased and ligated. The religated plasmid is then transformed into JM109 cells that are plated on X-Gal/IPTG/Ampicillin plates. White colonies result from transformation with ligated plasmids with damaged ends. These white colonies represent the number of false positives expected in a typical cloning experiment. Enzymes that generate overhangs must produce fewer than 2% white colonies, and blunt-cutting enzymes must produce fewer than 5% white colonies.

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Signed by:

J. Stevens, Quality Assurance

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## I. Description

Calf intestinal alkaline phosphatase (CIAP) catalyzes the hydrolysis of 5'-phosphate groups from DNA, RNA, and ribo- and deoxyribonucleoside triphosphates. This enzyme is used to prevent recircularization and religation of linearized cloning vehicle DNA by removing phosphate groups from both 5'-termini (1-5).

## II. Reaction Conditions

### A. Dephosphorylation of 5' Overhangs

#### Reagents to Be Supplied by the User

(Solution compositions are provided in Section III.)

- 10mM Tris-HCl (pH 8.0)
- CIAP stop buffer
- TE-saturated phenol:chloroform
- chloroform:isoamyl alcohol (24:1)
- 7.5M ammonium acetate (pH 5.5)
- ethanol, 100% and 70%

1. Dilute sufficient CIAP for immediate use in CIAP 1X Reaction Buffer to a final concentration of 0.01 u/μl. Each picomole of DNA ends will require 0.01u CIAP. (1 μg of 1,000bp DNA = 1.52pmol DNA = 3.03pmol of ends.)

2. Purify the DNA to be dephosphorylated by ethanol precipitation, and resuspend the pellet in 40μl of 10mM Tris-HCl (pH 8.0). Set up the following reaction:

DNA (up to 10 pmol of 5'-ends)	40μl
CIAP 10X Reaction Buffer	5μl
Diluted CIAP (0.01u/μl)	up to 5μl
	50μl

3. Incubate at 37°C for 30 minutes.
4. Add another aliquot of diluted CIAP (equivalent to the amount used in Step 2), and continue incubation at 37°C for an additional 30 minutes.
5. Add 300μl of CIAP stop buffer. Phenol:chloroform extract and ethanol precipitate by adding 0.5 volume 7.5M ammonium acetate (pH 5.5) and 2 volumes of 100% ethanol to the final aqueous phase.

**Note:** CIAP may be added directly to digested DNA. Add 5μl CIAP 10X Reaction Buffer, 0.01u CIAP/pmol of ends and deionized water to a final volume of 50μl (6).

### B. Dephosphorylation of 5' Recessed or Blunt Ends

When 5' recessed or blunt end DNA fragments are used as substrate, incubate at 37°C for 15 minutes and then at 56°C for 15 minutes. Then add a second aliquot of CIAP, and repeat the incubations at both temperatures. The higher temperature ensures accessibility of the recessed end (7).

## III. Composition of Buffers and Solutions

#### CIAP stop buffer

10mM	Tris-HCl (pH 7.5)
1mM	EDTA (pH 7.5)
200mM	NaCl
0.5%	SDS

#### TE buffer

10mM	Tris-HCl (pH 8.0)
1mM	EDTA

#### TE-saturated phenol:chloroform

Mix equal parts of TE buffer and phenol and allow the phases to separate. Then mix 1 part of the lower, phenol phase with 1 part of chloroform:isoamyl alcohol (24:1).

## IV. References

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