Product Contents

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 $MgCl_2$, 0.1mM $ZnCl_2$, 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 $MgCl_2$, 0.1mM $ZnCl_2$ 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 $MgCl_2$ (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.

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®-3Zf(+) 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.

Part# 9PIM182

Revised 1/07

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Part# 9PIM182
Printed in USA. Revised 1/07

Promega

Usage Information

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 0.01u/µl. Each picomole of DNA ends will require 0.01u CIAP. (1µg 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

1. Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
2. Seeburg, P.H. *et al.* (1977) Nucleotide sequence and amplification in bacteria of structural gene for rat growth hormone. *Nature* **270**, 486–94.
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4. Meyerowitz, E.M. *et al.* (1980) A new high-capacity cosmid vector and its use. *Gene* **11**, 271–82.
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6. *Protocols and Applications Guide,* Third Edition (1996) Promega Corporation.
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Part# 9PIM182
Printed in USA. Revised 1/07