GIBCO BRL

LIFE TECHNOLOGIES™

Calf Intestinal Alkaline Phosphatase (CIAP)

Cat. No. 18009-019 **Size: 1,000 units**
Lot No.
Conc.: U/µl
Exp. Date: **Store at -20°C.**

Description:
NOTE: DILUTION BUFFER FORMULATION HAS BEEN CHANGED. THIS PRODUCT IS NO LONGER COMPATIBLE WITH 18059-014.
Calf intestinal alkaline phosphatase (CIAP) is a phosphomonoesterase purified from calf intestinal mucosa that hydrolyzes 5'-phosphate groups from DNA, RNA, and nucleotides. CIAP is used to dephosphorylate linearized vector DNA prior to insert ligation and to remove 5'- phosphate groups prior to 5'-end labeling of nucleic acids with T4 polynucleotide kinase.

Components:

18009-019	CIAP	Lot No
Y01371	CIAP 10X Buffer	Lot No
50839	CIAP Dilution Buffer	Lot No.

Unit Definition: One unit of CIAP hydrolyzes 1 mmol of p-nitrophenyl phosphate in 1 min at 37°C.

Dilution Buffer:	Unit Assay Conditions
25 mM Tris-HCl (pH 7.6)	1 M Diethanolamine (pH 9.8)
1 mM $MgCl_2$	0.25 mM $MgCl_2$
0.1 mM $ZnCl_2$	10 mM p-nitrophenyl phosphate
50% glycerol (v/v)	

Dephosphorylation Buffer (1X concentration): 50 mM Tris-HCl (pH 8.5), 0.1 mM EDTA. The Dephosphorylation buffer is supplied as the 10X concentrate and should be diluted, 1:10 (1 part Dephosphorylation buffer + 9 parts other components = 10 parts final reaction volume.)

Quality Control Assays:
No detectable contaminating activity is observed in endodeoxyribonuclease, exodeoxyribonuclease, and ribonuclease assays. The enclosed buffers were assayed with the enzyme and met quality control specifications.
Doc. Rev. 091499

Traditional Protocol:

This protocol dephosphorylates 1 pmol of 5′- DNA termini from purified DNA. DNA dephosphorylated by this method is suitable for cloning or for labeling by T4 polynucleotide kinase using the Forward Reaction:

1. Determine the mass of DNA required for 1 pmol of the type of DNA 5′ end.
2. To a 1.5-ml microcentrifuge tube, add 4 µl of CIAP 10X Buffer and 1 pmol of DNA ends.
3. Add autoclaved, distilled water to 39 µl.
4. Dilute CIAP in dilution buffer such that 1 µl contains the amount of enzyme required for the appropriate 5′ end (*i.e.*, 1 unit for 5′-recessed and blunt ends and 0.01 units for a 5′ overhang).
5. For 5′-recessed and blunt-ended DNA, incubate at 50°C for 60 min. For DNA with a 5′ overhang, incubate at 37°C for 30 min.
6. Inactivate/remove the CIAP according to the protocol described below.

Simplified Protocol:

This protocol allows for the dephoshporylation of DNA directly in restriction endonuclease buffer in the presence of the restriction endonuclease. This is a convenient way of preparing DNA for cloning.

1. Restriction endonuclease digest the vector DNA. (**NOTE:** Heat inactivation of the restriction endonuclease and subsequent purification of the vector DNA are not necessary.)
2. Add 1 unit of CIAP to the restriction endonuclease digest.
3. For 5′-recessed and blunt-ended DNA, incubate at 50°C for 5 min. For DNA with a 5′ overhang, incubate at 37°C for 5 min.
4. Inactivate/remove the CIAP according to the protocol described below.

Inactivation/Removal of Calf Intestinal Alkaline Phosphatase (3 methods):

1. Heat Inactivation: Note the $MgCl_2$ concentration in the reaction and add EDTA (pH 8.0) to an equal final concentration. Incubate the reaction at 65°C for 15 min.
2. Organic Extraction: Add an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1). Vortex thoroughly and centrifuge at 14,000 × *g* at room temperature for 5 min. Carefully remove the upper, aqueous phase and transfer it to a fresh microcentrifuge tube. Add 0.1 volume of 3 M sodium acetate. Vortex. Add 2.5 volumes of 100% EtOH. (**NOTE:** Do not substitute NH_4OAc for NaOAc because NH_4 ions inhibit T4 polynucleotide kinase.) Vortex the mixture thoroughly and centrifuge at 14,000 × *g* at room temperature for 5 min.
3. CONCERT™ Gel Extraction Systems: Following electrophoresis of the dephosphorylated DNA on an agarose gel, use the protocols supplied with the CONCERT Gel Extraction Systems to purify the DNA.