invitrogen™

Calf Intestinal Alkaline Phosphatase (CIAP)

Cat. No.	Size	Conc.
18009-019	1,000 U	20 U/µl
18009-027	1,000 U	1 U/µl
		Store at -20°C

Description

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

Component	20 U/µl kit	1 U/µl kit
CIAP	50 µl	1 ml
10X Dephosphorylation Buffer	1 ml	1 ml
Dilution Buffer	1 ml	1 ml

Unit Definition

One unit of CIAP hydrolyzes 1 µmol of p-nitrophenyl phosphate in 1 min at 37°C. Unit assay conditions: 1 M Diethanolamine (pH 9.8), 0.25 mM $MgCl_2$, 10 mM p-nitrophenyl phosphate

Use Limitation

This product must be for *in vitro* use only and not used in cell culture technology or for the production of veterinary biologics or pharmaceuticals. Do not use this product in a manner that could result in direct or indirect exposure to animals. Any unused product must be destroyed by autoclaving, incineration, or burial in a landfill.

For technical support, email tech_support@invitrogen.com.
For country-specific contact information, visit ***www.invitrogen.com***.

Dilution Buffer

25 mM Tris-HCl (pH 7.6), 1 mM $MgCl_2$, 0.1 mM $ZnCl_2$, 50% glycerol (v/v)

10X Dephosphorylation Buffer

Final 1X concentration: 50 mM Tris-HCl (pH 8.5), 0.1 mM EDTA

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 10X Dephosphorylation Buffer, 1 pmol of DNA ends, and 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 as described on page 4.

Simplified Protocol

This protocol allows for the dephosporylation 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. Digest the vector DNA with restriction endonuclease. (**Note:** Heat inactivation of the endonuclease and 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 as described on page 4.

Quality Control

The enzyme exhibits no detectable contaminating activity in endodeoxyribonuclease, exodeoxyribonuclease, and ribonuclease assays.

Inactivation/Removal of Calf Intestinal Alkaline Phosphatase
The following three methods are available for inactivating or removing CIAP:

Heat Inactivation

1. Note the $MgCl_2$ concentration in the reaction and add EDTA (pH 8.0) to an equal final concentration.
2. Incubate the reaction at 65°C for 15 min.

Organic Extraction

1. Add an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1).
2. Vortex thoroughly and centrifuge at 14,000 × *g* at room temperature for 5 min.
3. Carefully remove the upper, aqueous phase and transfer it to a fresh microcentrifuge tube.
4. Add 0.1 volume of 3 M sodium acetate and vortex.
5. Add 2.5 volumes of 100% EtOH. (**Note:** Do not substitute NH_4OAc for NaOAc because NH_4 ions inhibit T4 polynucleotide kinase.)
6. Vortex the mixture thoroughly and centrifuge at 14,000 × *g* at room temperature for 5 min.

S.N.A.P.™ Gel Purification

Following agarose gel electrophoresis of the dephosphorylated DNA, use the protocols in the S.N.A.P.™ Gel Purification Kit (Cat. no K1999-25) to purify the DNA.