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DATA SHEET

Version: 03
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1. Identification

Product name **Calf Intestinal Alkaline Phosphatase (CIAP)**
(20U/uL) 1000U

Cat. No **P0244**

2. Description

Alkaline phosphatase (AP) is a membrane bound enzyme which occurs in almost all living organisms. The AP superfamily comprises a family of highly promiscuous metallohydrolases that are similar in active site architecture and substrate preference, but show limited sequence homology .The members of this superfamily catalyze the hydrolytic cleavage of phosphate groups from many types of molecules, including nucleotides and proteins.

Individual mammalian alkaline phosphatases differ in their heat stabilities and uncompetitive inhibition properties. This is the result of their nonhomologous disulphide bonds, their structural significance, and nonconserved residues. Four isoenzymes have developed during evolution and are coded by up to four genes. These four isoenzymes can be found in various tissues, three isoenzymes of AP are specific for certain tissues: they are intestinal alkaline phosphatase ,placental alkaline phosphatase, and germ cell alkaline phosphatase, which are expressed by embryonic cells but also carcinoma cells and The fourth isoenzyme is ubiquitous, and it is tissue nonspecific alkaline phosphatase.

Alkaline Phosphatase, Calf Intestinal (CIAP), catalyzes the hydrolysis of 5´-phosphate groups from DNA, RNA, and ribo- and deoxyribonucleoside triphosphates. CIAP is frequently used 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. CIAP may also be used to degrade unincorporated dNTPs in PCR reactions to prepare templates for DNA sequencing or SNP analysis.

3. Composition

Item	Quantity
Alkaline Phosphatase (CIAP)	1000 U
10X Alkaline Phosphatase Buffer	1 ml

4. Unit Definition

One unit is defined as the amount of enzyme that hydrolyzes 1 µmol of p-Nitrophenyl Phosphate (PNPP) in a total reaction volume of 1 ml in 1 minute, at 37˚C.

5. Storage specifications

Store at -20 ˚C.

6. Quality Control

Tested for contaminating non-specific endonucleases, exonucleases, and ribonucleases.

7. Storage Buffer

10mM Tris-HCl (pH 8.0), 1mM MgCl2, 0.1mM ZnCl2, 50mM KCl, 50% (v/v) glycerol.

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8. Procedure: Dephosphorylation of DNA

1. Dissolve DNA in 1X Alkaline Phosphatase Buffer (0.5µg DNA/10 µl).

2. For 5' overhang DNA add 0.1 units/pmol CIAP; for 3' overhang or blunt end DNA add 1 unit/pmol.

3. Incubate at 37 ˚C for 60 minutes.

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

Inactivation

Add EDTA to a final concentration of 5 mM to chelate the required metal cofactors.

Note: EDTA inhibits CIAP activity but does not remove or denature the enzyme. For complete enzyme removal, perform a phenol-chloroform extraction or use a standard DNA cleanup column.

9. Further information

Product Use Limitations	This product is developed, designed and sold exclusively only for research purposes use. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.
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