

Product Information

Alkaline Phosphatase

from calf intestine

Catalog Number **P4978**

Storage Temperature $-20\text{ }^{\circ}\text{C}$

EC 3.1.3.1

Synonyms: Calf Intestinal Alkaline Phosphatase, (CIP)

Product Description

Calf Intestinal Alkaline Phosphatase (CIP) is commonly used to remove 5'-phosphate groups from DNA, RNA, and both ribo and deoxy-ribonucleoside triphosphates. Removal of 5'-phosphates is very useful in preventing self-ligation of cleaved DNA vectors. This property greatly reduces background (plasmids without insert) in cloning procedures.

This product is provided in a solution of 10 mM Tris-HCl, pH 8.2, 50 mM KCl, 1 mM MgCl_2 , 0.1 mM ZnCl_2 , and 50 % glycerol (storage buffer).

Activity: ~10,000 units/ml

Unit Definition: One unit will hydrolyze 1 μmole of *p*-nitrophenyl phosphate per minute at $37\text{ }^{\circ}\text{C}$ in diethanolamine buffer.

DNase: None detected

Nickase: None detected

RNase: None detected

Components

Alkaline Phosphatase 1 vial
(Catalog Number P4978)

10 \times CIP Buffer 1 vial
1 M NaCl, 0.5 M Tris-HCl, 0.1 M MgCl_2 ,
and 0.01 M dithiothreitol, pH 7.9 at $25\text{ }^{\circ}\text{C}$.
(Catalog Number C3225)

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Store the product at $-20\text{ }^{\circ}\text{C}$.

Procedures

Dephosphorylation of DNA

1. Prepare 1 \times CIP Reaction Buffer by 10-fold dilution of included 10 \times CIP Buffer (Catalog Number C3225).
2. Dissolve DNA in 1 \times CIP Reaction Buffer (0.5 μg DNA/10 μl).
3. For 5' overhang DNA, add 0.1 unit/pmol; for 3' overhang or blunt end DNA add 1 unit/pmol.
4. Incubate 60 minutes at $37\text{ }^{\circ}\text{C}$.
5. Extract with phenol/chloroform² (Catalog Number P3803 or P2069) or gel purify the DNA.
Note: Phenol extraction or gel purification makes heat inactivation unnecessary.
6. Recover the DNA by alcohol precipitation.²

Heat Inactivation

Greater than 95% of the activity can be inactivated by heating to $75\text{ }^{\circ}\text{C}$ for 10 minutes in the presence of 5 mM EDTA.

References

1. Moessner, E. et al., Z. Physiol. Chem. **361**, 543.
2. Sambrook, J. et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory (1989), p 5.72, 6.22-6.47 and E.3-E.13.

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