

CERTIFICATE OF ANALYSIS

Calf Intestine Alkaline Phosphatase

#EF0341 200u

Lot: Quality guaranteed:

Concentration: 1u/ μ l

Supplied with: 1ml of 10X Reaction Buffer

Store at -20°C

2

In total 2 vials.

Description

Calf Intestine Alkaline Phosphatase (CIAP) catalyzes the release of 5'- and 3'-phosphate groups from DNA, RNA, deoxyribonucleoside and ribonucleoside mono-, di- and triphosphates. Also this enzyme can remove phosphate groups from proteins.

Source

Calf intestine.

Unit Definition

One unit of the enzyme hydrolyzes 1 micromole of 4-nitro-phenylphosphate in 1min at 37°C .

Activity Assay

1M diethanolamine-HCl (pH 9.8), 0.5mM MgCl_2 and 10mM 4-nitrophenylphosphate.

Storage Buffer

20mM Tris-HCl (pH 8.0), 1mM MgCl_2 , 50mM KCl, 0.1mM ZnCl_2 and 50% glycerol.

10X Reaction Buffer

0.1M Tris-HCl (pH 7.5 at 37°C), 0.1M MgCl_2 .

Applications

- Dephosphorylation of DNA and RNA (1), see protocol on back page.
- Dephosphorylation of proteins (2).

Inactivation

By heating at 85°C for 15min or by phenol/chloroform extraction.

Note

Activity in Fermentas REase Buffers, %
(in comparison to activity in assay buffer)

B	G	O	R	Tango™		BamHI	Ecl136II, Sacl	EcoRI	KpnI
				1X	2X				
100	100	100	100	100	100	100	25-50	100	100

QUALITY CONTROL ASSAY DATA

Endodeoxyribonuclease Assay

No detectable conversion of covalently closed circular DNA to nicked DNA was observed after incubation of 15 units of Calf Intestine Alkaline Phosphatase with 1µg of pBR322 DNA in 50µl of reaction buffer for 1 hour at 37°C.

Exodeoxyribonuclease Assay

0% of the total radioactivity was released into trichloroacetic acid-soluble fraction after incubation of 50 units of Calf Intestine Alkaline Phosphatase with 1µg of sonicated *E.coli* [³H]-DNA in 50µl of reaction buffer for 1 hour at 37°C.

Ribonuclease Assay

0% of the total radioactivity was released into trichloroacetic acid-soluble fraction after incubation of 50 units of Calf Intestine Alkaline Phosphatase with 1µg of [³H]-RNA in 50µl of reaction buffer for 1 hour at 37°C.

Blue/White Cloning Assay

Less than 2% white colonies were detected after transformation of *E.coli* XL1-Blue cells with ligated pUC57 DNA. Before ligation pUC57 DNA was digested with HindIII, PstI and SmaI restriction endonucleases. The digests were incubated with 10 units of Calf Intestine Alkaline Phosphatase for 1 hour at 37°C. Then the 5'-termini of DNA were phosphorylated using T4 Polynucleotide Kinase.

Functional Assay

Calf Intestine Alkaline Phosphatase was tested for dephosphorylation 5'-termini of DNA.

Quality authorized by:



Jurgita Zilinskiene

(continued on back page)

Protocol for Dephosphorylation of DNA

5'-termini

- ❶ Dissolve DNA (1-20 picomoles of DNA termini) in 10-40µl water, nuclease-free.
- ❷ Prepare the following reaction mixture:

DNA solution	10-40µl
10X reaction buffer	5µl
water, nuclease-free	to 49µl
Calf Intestine Alkaline Phosphatase	1u (1µl)
- ❸ Incubate at 37°C for 30 minutes.
- ❹ Stop reaction by heating at 85°C for 15min or extract DNA with phenol/chloroform and then precipitate with ethanol.

Note

- Dephosphorylation can be performed by adding Calf Intestine Alkaline Phosphatase directly in mixture after DNA cleavage with a restriction endonuclease.
- We recommend using 0.05 units Calf Intestine Alkaline phosphatase for dephosphorylation of 1 picomole of DNA termini. The enzyme can be diluted with 1X reaction buffer.

References

1. Ausubel, F.M. et al., Current Protocols in Molecular Biology, vol. 1, John Wiley & Sons, Inc., Brooklyn, New York, 3.10.1-3.10.2, 1994-2004.
2. Ahmad, Z., Huang, K.P., Dephosphorylation off rabbit skeletal muscle glycogen synthase (phosphorylated by cyclic AMP-independent synthase kinase 1) by phosphatases, J. Biol.Chem., 256, 757-760, 1981.

Related Products

- T4 Polynucleotide Kinase #EK0031
#EK0032
- Exonuclease I, *E.coli* #EN0581
#EN0582
- Water, nuclease-free #R0581
#R0582

PRODUCT USE LIMITATION.

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

Please refer to www.fermentas.com for Material Safety Data Sheet of the product.

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