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LIFE SCIENCES

CERTIFICATE OF ANALYSIS

Shrimp Alkaline Phosphatase (SAP)

#EF0511 500 u

Lot: **Expiry Date:**

Concentration: 1 u/µl

Supplied with: 3 x 1 ml of 10X Reaction Buffer

Store at -20°C

In total 4 vials. BSA included

ISO 9001 ISO 14001

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Description

Shrimp Alkaline Phosphatase (SAP) catalyzes the release of 5'- and 3'-phosphate groups from DNA, RNA and nucleotides. This enzyme can also remove phosphate groups from proteins.

Applications

- Dephosphorylation of cloning vector DNA to avoid recircularization.
- Degradation of dNTPs in PCR mixture prior to sequencing of PCR products (1).
- Dephosphorylation of DNA and RNA 5'-ends prior to labelling with T4 Polynucleotide Kinase.
- Dephosphorylation of DNA and RNA (2), *see* protocol on back page.
- Dephosphorylation of proteins (3).

Source

Arctic shrimp *Pandalus borealis.*

Definition of Activity Unit

One unit of the enzyme hydrolyzes 1 µmol of 4-nitrophenylphosphate in 1 min at 37°C.

Activity Assay

Enzyme activity is assayed in the following mixture: 1 M diethanolamine-HCl (pH 9.8), 0.5 mM $MgCl_2$ and 10 mM 4-nitrophenylphosphate.

Storage Buffer

The enzyme is supplied in: 25 mM Tris-HCl (pH 7.6 at 4°C), 1 mM $MgCl_2$, 0.1 mM $ZnCl_2$ and 50% (v/v) glycerol.

10X Reaction Buffer

0.1 M Tris-HCl (pH 7.5 at 37°C), 0.1 M $MgCl_2$ and 1 mg/ml BSA.

Inhibition and Inactivation

- Inhibitors: metal chelators, inorganic phosphate and phosphate analogs.
- Inactivated by heating at 65°C for 15 min.

Note

- Binding of SAP to DNA may result in a band shift in agarose gels. To avoid this, incubate the samples with 6X DNA Loadind Dye & SDS Solution (#R1151) at 65°C for 10 min and chill on ice prior to electrophoresis.
- Activity in Fermentas Buffers, % (in comparison to activity in assay buffer)

FastDigest®/ FastDigest® Green	B, G, O, R	Tango™		BamHI EcoRI, KpnI	EcI136II, SacI	*Taq* with KCl	*Taq* with $(NH_4)_2SO_4$	*Pfu*	RT
		1X	2X						
100	100	100	100	100	75-100	100	100	100	100

QUALITY CONTROL ASSAY DATA

Endodeoxyribonuclease Assay

~10% conversion of covalently closed circular DNA to nicked DNA was observed after incubation of 10 units of SAP with 1 µg of pUC19 DNA in 50 µl of buffer (25 mM Tris-HCl, 5 mM $MgCl_2$, 5 mM NaCl (pH 8.0)) for 1 hour at 37°C.

Ribonuclease Assay

≤0.5% of the total radioactivity was released into trichloroacetic acid-soluble fraction after incubation of 10 units of SAP with 1 µg of [^{3}H]-RNA in 50 µl of buffer (25 mM Tris-HCl, 5 mM $MgCl_2$, 5 mM NaCl (pH 7.2)) for 1 hour at 37°C.

Blue/White Cloning Assay

Less than 3% 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 enzymes. The digests were incubated with 1 unit of SAP for 1 hour at 37°C. Then the 5'-termini of DNA were phosphorylated using T4 Polynucleotide Kinase.

Labeled oligonucleotide (LO) Assay

Single-stranded and double-stranded oligonucleotides were incubated with 2 units of SAP for 4 hours at 37°C and then labeled by T4 Polynucleotide Kinase and [γ-^{33}P]-ATP. The reaction products were separated on a polyacrylamide gel and evaluated by phosphoroimaging. ≤2% degradation of labeled oligonucleotides was detected.

Functional Assay

No recircularization of linear plasmid DNA was determined after dephosphorylation of 5'-termini of pUC57 DNA/PstI, ligation with T4 DNA Ligase and transformation of *E.coli* cells.

Quality authorized by:  Jurgita Zilinskiene

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Protocol for Dephosphorylation of DNA 5'-termini

This protocol is suitable for removal of 3'- and 5'-phosphate groups from DNA and RNA. The protocol below is an example for dephosphorylation of ~3 kb linear vector DNA.

1. Prepare the following reaction mixture:

Linear DNA (~3 kb plasmid)	1 µg (~1 pmol termini)
10X reaction buffer	2 µl
Shrimp Alkaline Phosphatase	1 µl (1 u)
Water, nuclease-free (#R0581)	to 20 µl
Total volume	20 µl

2. Mix thoroughly, spin briefly and incubate at 37°C: 30 min for 5'-overhangs or blunt ends, 60 min for 3'-overhangs.
3. Stop reaction by heating for 15 min at 65°C.

Notes

- SAP may be diluted for immediate use in 1X reaction buffer.
- SAP is active in virtually all restriction enzyme buffers and may be added directly to digested DNA. Heat inactivation of the restriction enzyme before dephosphorylation reaction is not necessary.
- For efficient dephosphorylation plasmid DNA should be free of RNA and genomic DNA.

Reference

1. Sambrook, J., Russell, D.W., Molecular Cloning: A Laboratory Manual, the third edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 2001.
2. Werle, E., et al., Convenient single-step, one tube purification of PCR products for direct sequencing, Nucleic Acids Res., 22, 4354-4355, 1994.
3. Khosravi, R., et al., Rapid ATM-dependent phosphorylation of MDM2 precedes p53 accumulation in response to DNA damage, Proc. Natl. Acad. Sci USA, 96, 14973-14977, 1999.
4. Nilsen, I.W., etal., Thermolabile alkaline phosphatase from Northern shrimp (*Pandalus borealis*): protein and cDNA sequence analyses, Comparative Biochemistry and Physiology, B, 129, 853-861, 2001.

The purchase of this product allows the purchaser to use it for preparing amplified DNA fragments under a license from GE Healthcare of U.S. Patent Nos. 5,741,676 and 5,756,285 and other foreign patents.

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