For general laboratory use.
FOR *IN VITRO* USE ONLY.

Alkaline Phosphatase, shrimp

From northern shrimps *(P. borealis)*
Orthophosphoric-monoester phosphohydrolase (alkaline optimum), EC 3.1.3.1

Cat. No. 11 758 250 001 1000 U

Version May 2005
Store at −15 to −25°C

Special Quality for molecular biology

1. What this Product Does

Content

Alkaline Phosphatase, shrimp	• 1000 U • Storage buffer: 25 mM Tris-HCl, 1 mM $MgCl_2$, 0.1 mM $ZnCl_2$, 50% glycerol (v/v), pH 7.6 (4°C).
Dephosphorylation buffer, 10×	0.5 M Tris-HCl, 50 mM $MgCl_2$, pH 8.5 (20°C).

Storage and stability

Stable at –15 to –25°C until the expiration date printed on the label.

Application

SAP catalyzes the dephosphorylation of 5' phosphates from DNA and RNA. Unlike calf intestinal phosphatase, SAP is completely and irreversible inactivated by heat treatment for 15 min at 65°C. Thus the whole proceeding including restriction enzyme digestion, dephosphorylation, enzyme inactivation, ligation or 5' end-labeling can be performed in one single tube by just adding the appropriate reagents. SAP is as well active on either 5'-protruding, 5'-recessive and blunt ends.

2. How To Use this Product

Overview

Stage	Description
1	Digestion of vector DNA.
2	Dephosphorylation using alkaline phosphatase, shrimp (SAP).
3	Inactivation of alkaline phosphatase, shrimp (SAP).
4	Ligation reaction, performed with the Rapid DNA Ligation Kit* or T4 DNA ligase*.

Incubation procedure (4)

The reaction assay is adjusted with 1/10 volume 10× conc. dephosphorylation buffer.

- Up to 1 pmol 5'-terminal phosphorylated DNA-fragments either 5'-protruding or 5'-recessive ends are incubated with 1 U SAP at 37°C for 10 min.
- Up to 0.2 pmol 5'-terminal phosphorylated DNA-fragments blunt-ended are incubated with 1 U SAP at 37°C for 60 min.
- Up to 1 pmol 5'-terminal phosphorylated RNA fragments are incubated with 1 U SAP at 37°C for 60 min.

** available from Roche Applied Science*

Procedure

For sticky-end cloning please refer to the following table.

Step 1 • Pipette the following into a sterile microcentrifuge tube on ice:

Component	Amount
Vector DNA	0.5 µl (50 ng)
Restriction endonuclease	1 µl (3 U)
corresp. SuRE/Cut Buffer, 10×	0.7 µl
add double dist water	4.8 µl
Final volume	**7 µl**

• Mix contents.

Step 2 Incubate for 10 min at 37°C.

Step 3 Inactivate restriction endonuclease for 15 min at 65°C.

Step 4 • Pipette in the same vial:

Component	Amount
Dephosporylation Buffer, 10×	0.9 µl
Phosphatase alkaline, shrimp	1 µl (1 U)

• Incubate for 10 min at 37°C.

For dephosphorylation of blunt-ended DNA-fragments incubate for 60 min at 37°C.

Step 5 Inactivate SAP for 15 min at 65°C.

Step 6 • Pipette into the same vial:.

Component	Amount
DNA fragment	0.5 µl (150 ng)
Ligation buffer (2x)	10 µl
T4 DNA Ligase	1 µl (5 U)

• Incubate for 5 min at 15 to 25°C

Step 7 The assay is directly used after ligation in the transformation reaction without heating.

Ligation can be performed by using the Rapid DNA Ligation Kit* or T4 DNA Ligase*

3. Additional Information on this Product

Source

Shrimp alkaline phosphatase (SAP) is isolated from cold-living northern shrimps (P. borealis) (1).

Advantage of SAP

One major advantage of SAP is that it is readily inactivated by heating at 65°C for 15 min. Thus, the vector can be cut, dephosphorylated and the enzymes inactivated simply by heating after each step prior to adding insert DNA for ligation.

Volume activity

1×10^3 U/ml; Shrimp alkaline phosphatase is assayed according to (2). One unit of SAP is the enzyme activity which hydrolyzes 1 µmol of 4-nitrophenyl phosphate in 1 min at 37°C under assay conditions.

According to (2) 5 U SAP (37°C; diethanolamine buffer) correspond to 1 U SAP (25°C; glycine/NaOH buffer).

See data label for lot-specific values.

Specific activity

Approx. 2 200 U/mg according to (2) and (3).

See data label for lot-specific values.

References

1 Ragnar, L. et al. (1991) *Comp. Biochem.Physiol.* Vol. **99**, B No 4PP, 755-761.

2 Moessner, E. et al. (1980) *Z. Physiol. Chem.* **361**, 543.

3 Bradford, M.(1976) *Anal. Biochem.* **72**, 248.

4 Maxam, A.M. & Gilbert, W. (1980) *Methods Enzymol.* **65**, 499.

5 Yoneyama, H. et al, (2000) Function of the Membrane Fusion Protein, MexA, of the MexA, B-OprM Efflux Pump in Pseudomonas aeruginosa without an Anchoring Membrane *J. Biol. Chem.* **275:** 4628-4634.

6 Akula, N. et al (2002) Utility and Accuracy of Template-Directed Dye-Terminator Incorporation with Fluorescence-Polarization Detection for Genotyping Single Nucleotide Polymorphisms *BioTechniques* **32**:1072-1078

Quality Control

See data for lot-specific values.

Absence of deoxyribonucleases

1) 1 µg λDNA is incubated with SAP for 1 h at 37°C in 25 µl dephosphorylation buffer. The number of enzyme units which shows no degradation of λDNA after agarose gel electrophoresis is stated under "DNases (1)".

2) 1 µg *Eco* RI/*Hind* III fragments of λDNA is incubated with SAP for 1 h at 37°C with 25 µl dephosphorylation buffer. The number of enzyme units which shows no change in banding pattern after agarose gel electrophoresis is stated under "DNases (2)".

Absence of nicking activities

1 µg "supercoiled" pBR322-DNA is incubated with SAP for 1 h at 37°C in 25 µl dephosphorylation buffer.

The number of enzyme units which shows no relaxing of the supercoiled structure of pBR322 DNA after agarose gel electrophoresis is stated under under "Nick".

Absence of exonucleases

15 nmol of sonicated [^{3}H]-DNA (approx. 100 000 cpm/g) from calf thymus are incubated with SAP for 4 h at 37° C in 100 µl buffer (50 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM dithioerythritol, pH 7.5). The number of enzyme units that does not liberate radioactivity is stated under "Exo".

Absence of ribonucleases

5 µg MS2-RNA are incubated with SAP for 1 h at 37°C in 50 µl dephosphorylation buffer. The number of enzyme units which shows no change of MS2 RNA after agarose gel electrophoresis is stated under "RNase".

4. Supplementary Information

Symbols

In this Instruction Manual, the following symbols are used to highlight important information:

Symbol	Description
	Information Note: Additional information about the current topic or procedure.
	Important Note: Information critical to the success of the procedure or use of the product.

4.1 Ordering Information

Roche Applied Science offers a large selection of reagents and systems for life science research. For a complete overview of related products and manuals, please visit and bookmark our homepage http://www.roche-applied-science.com.

Restriction Enzymes Please refer to website or catalogue

Product	Pack size	Cat. No.
Polynucleotide kinase, T4	200 U 1000 U	10 174 645 001 10 633 542 001
T4 Polynucleotide kinase, 3'phosphatase-free	200 U 1000 U	10 709 557 001 10 838 292 001
T4 DNA Ligase	100 U 500 U (1 U/µl) 500 U	10 481 220 001 10 716 359 001 10 799 009 001
T4 RNA Ligase	500 U	11 449 478 001
Rapid DNA Ligation Kit	1 kit (40 reactions)	11 635 379 001
Agarose Gel DNA Extraction Kit	1 kit (max 100 reactions)	11 696 505 001

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