Shrimp Alkaline Phosphatase, Recombinant

CATALOG #: 9216 (250 UN)

ALTERNATE NAMES: SAP; rSAP

SOURCE: Recombinant

PURITY: Tested for contaminating endonucleases, exonucleases, and ribonucleases.

MOL. WEIGHT: Homodimer. Monomer is 55 kDa as determined by amino acid sequence.

CONCENTRATION: 1 unit/µl

OPTIMUM PH: 10.4 in glycine buffer and pH 8.0 in Tris buffer.

OPTIMUM TEMPERATURE: 37°C

UNIT DEFINITION: One unit is the amount of enzyme which catalyzes the hydrolysis of 1 µmol of p-nitrophenyl phosphate per min in glycine buffer (pH 10.4) at 37°C.

STORAGE BUFFER: 25 mM Tris-HCl (pH 7.5), 1 mM $MgCl_2$, 50% glycerol.

STORAGE CONDITIONS: Shipped on dry ice. Store at -20°C.

ASSAY CONDITIONS: The reaction mixture contains 100 mM glycine, pH 10.4, 1 mM $MgCl_2$, 1 mM $ZnCl_2$, 10 mM p-nitrophenyl phosphate, and 0.001-0.1 units of Shrimp Alkaline Phosphatase (SAP). The change in absorbance at 405 nm is monitored.

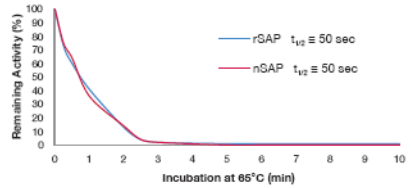
REACTION CONDITIONS: Active in NaCl, KCl. Requires Mg^{2+} for highest activity.

FUNCTIONAL TEST: Dephosphorylation of restriction enzyme digested plasmids (5 – 20 pmol of 5'-ends, 0.1 -0.5 units/pmol 5'-ends). Reduces religation to < 0.5% compared to the untreated control.

HEAT-INACTIVATION: 65°C for 15 min.

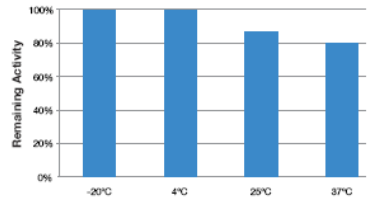
DESCRIPTION: Shrimp Alkaline Phosphatase (SAP) is a high specific activity, heat-labile alkaline phosphatase purified from a recombinant source and originally isolated from *Pandalus borealis* (arctic shrimp). SAP is useful in many molecular biology applications such as the dephosphorylation of phosphorylated ends of DNA or RNA for subsequent use in cloning or end-labeling of probes. In cloning, dephosphorylation prevents relegation of linearized plasmid DNA. SAP may also be used to treat unincorporated dNTPs in PCR reactions to prepare templates for DNA sequencing or SNP analysis. Shrimp Alkaline Phosphatase has approximately the same specific activity as Calf Intestinal Alkaline Phosphatase (CIAP), and like CIAP, is active in virtually all restriction enzyme reaction buffers. Unlike CIAP, Shrimp Alkaline Phosphatase is completely and irreversibly inactivated by heating reactions at 65°C for 15 min. Shrimp Alkaline Phosphatase is particularly useful in preparing PCR products for applications involving sequencing, SNP analysis or labeling methods. Typically, excess dNTPs remaining after PCR interfere with subsequent enzymatic reactions involving DNA synthesis. SAP dephosphorylates all of the remaining dNTPs from the PCR mixture in one easy step.

Fig. 1. Heat inactivation profile



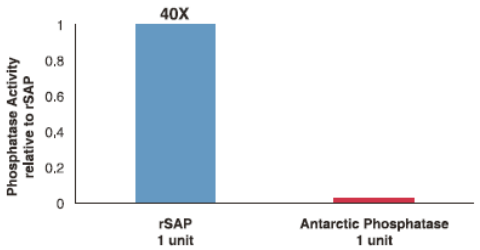
Heat inactivation curves of native and recombinant SAP at 65°C. Each phosphatase was heated at 65°C for increasing periods of time. Remaining activity was measured by standard p-nitrophenyl phosphatase assay. rSAP inactivates completely at 65°C.

Fig. 2. Improved stability of rSAP



Three month stability of rSAP at various temperatures. rSAP was stored for 3 months at the indicated temperatures and the remaining activity was measured using a standard p-nitrophenyl phosphatase activity.

Activity comparison of rSAP to Antarctic phosphatase



One unit of rSAP has about 40 times more activity than one unit of Antarctic phosphatase at pH 8.0.

APPLICATIONS:

1. Dephosphorylation of DNA prior to cloning.
2. PCR Clean-Up for sequencing and SNP applications.
3. Dephosphorylation of DNA prior to end-labelling using T4 PNK or OptiKinase.
4. Dephosphorylation of RNA.
5. Protein dephosphorylation.

FOR RESEARCH USE ONLY! Not to be used in humans.

INHIBITORS: 10 mM DTT, 0.1% β-ME

DEPHOSPHORYLATION PROTOCOL:

10X Shrimp Alkaline Phosphatase Reaction Buffer (included with the enzyme): 200 mM Tris-HCl, pH 8.0 and 100 mM $MgCl_2$.

SAP and 10X Shrimp Alkaline Phosphatase Reaction Buffer have been functionally tested in the following protocol:

Protocol for dephosphorylation of 5'-ends of DNA:

1. Resuspend 1 pmol of DNA ends (about 1 µg of a 3 kb plasmid) in nuclease-free water.
2. Prepare reaction mix in a 20 µl volume according to the following table:

DNA	> 1 µl
10X SAP Reaction Buffer	2 µl
Water, Nuclease-Free	up to 19 µl
SAP (1 unit/µl)	1 µl

3. Incubate at 37°C for 30-60 minutes.
4. Stop reaction by heating at 65°C for 15 minutes. This completely inactivates SAP.

Notes: The minimum effective amount of SAP for dephosphorylation of 1 pmol of DNA termini in 1 hour at 37°C is:
0.05 units for 5-protruding termini
0.05 units for blunt termini
0.1 units for 5'-recessed termini

Protocol for dephosphorylation of 5'-ends of DNA in restriction enzyme reaction:

1. Digest 1-5 µg of plasmid DNA in a 20 µl volume according to the following table: Note: Scale larger reaction volumes proportionally.

DNA	> 1 µl
10X Restriction Enzyme Buffer	2 µl
Water, Nuclease-Free	up to 19 µl
Restriction Endonuclease	1 µl

Note: Scale larger reaction volumes proportionally.

2. Incubate at 37°C for 60 minutes.
3. Add 1 unit of SAP for every 1 pmol of DNA ends (about 1 µg of a 3 kb plasmid) and incubate at 37°C for 30-60 minutes.
4. Stop reaction by heating at 65°C for 15 minutes. This completely inactivates SAP.

Note: Some restriction enzymes require 80°C for complete heat-inactivation. Follow manufacturers' recommendations.

RELATED PRODUCTS:

- **Exonuclease 1 (Cat. No. 9217 (500UN)**