

Shrimp Alkaline Phosphatase (rSAP)



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M0371S 002151017101

M0371S



500 units **1,000 U/ml** **Lot: 0021510**
Store at -20°C **Exp: 10/17**

Description: Shrimp Alkaline Phosphatase (rSAP) is a heat labile alkaline phosphatase purified from a recombinant source. rSAP is identical to the native enzyme and contains no affinity tags or other modifications. rSAP nonspecifically catalyzes the dephosphorylation of 5' and 3' ends of DNA and RNA phosphomonoesters. Also, rSAP hydrolyses ribo-, as well as deoxyribonucleoside triphosphates (NTPs and dNTPs). rSAP is useful in many molecular biology applications such as

Heat Inactivated In 5 Minutes at 65°C

the removal of phosphorylated ends of DNA and RNA for subsequent use in cloning or end-labeling of probes. In cloning, dephosphorylation prevents religation of linearized plasmid DNA. The enzyme acts on 5' protruding, 5' recessed and blunt ends. rSAP may also be used to degrade unincorporated dNTPs in PCR reactions to prepare templates for DNA sequencing or SNP analysis. rSAP is completely and irreversibly inactivated by heating at 65°C for 5 minutes, thereby making removal of rSAP prior to ligation or end-labeling unnecessary.

Source: A *Pichia pastoris* clone that carries the shrimp alkaline phosphatase gene from Northern shrimp *Pandalus borealis* (1,2).

Specific Activity: ~ 3,000 units/mg

Molecular Weight: rSAP is a homodimer. The molecular weight of the monomer is 54 kDa.

Applications:

- Dephosphorylation of cloning vector DNA to prevent recircularization during ligation.
- Dephosphorylation of DNA prior to end-labeling using T4 Polynucleotide Kinase.
- Treatment of dNTPs in PCR reactions prior to sequencing or SNP analysis.

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- Dephosphorylation of DNA and RNA.

Supplied in: 25 mM Tris-HCl (pH 7.5), 1 mM MgCl₂ and 50% glycerol.

Reagents Supplied with Enzyme:

10X CutSmart® Buffer

Reaction Conditions: 1X CutSmart Buffer.
Incubate at 37°C.

1X CutSmart Buffer:

50 mM Potassium acetate
20 mM Tris-acetate
10 mM Magnesium acetate
100 µg/ml BSA
pH 7.9 @ 25°C

Unit Definition: One unit is defined as the amount of enzyme that hydrolyzes 1 µmol of *p*-Nitrophenyl Phosphate, PNPP (NEB #P0757) in a total reaction volume of 1 ml in 1 minute at 37°C.

Unit Assay Conditions: 1 M diethanolamine-HCl (pH 9.8), 0.5 mM MgCl₂, 50 mM *p*-Nitrophenyl Phosphate. These conditions are only used for quantitating enzyme activity.

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Heat Inactivation: 65°C for 5 minutes

Functional Assay: Dephosphorylation with rSAP of a restriction enzyme-digested vector DNA with 5' recessed ends, the least favorable type for dephosphorylation, reduces re-ligation to < 0.5% compared to untreated control as measured by transformation into *E. coli*. rSAP has been functionally tested in the following protocol:

Protocol for Dephosphorylation of 5'-ends of DNA using rSAP

1. Prepare a 20 µl reaction as follows:

DNA	1 pmol of DNA ends*
CutSmart Buffer (10X)	2 µl
rSAP	1 unit
H ₂ O, purified	to 20 µl**

2. Incubate at 37°C for 30 minutes.
3. Stop reaction by heat-inactivation at 65°C for 5 minutes.

* Note: 1 pmol of DNA ends is about 1 µg of a 3 kb plasmid.

** Scale larger reaction volumes proportionally.

(see other side)

CERTIFICATE OF ANALYSIS

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* Note: 1 pmol of DNA ends is about 1 µg of a 3 kb plasmid.

** Scale larger reaction volumes proportionally.

(see other side)

CERTIFICATE OF ANALYSIS

Protocol for Dephosphorylation of 5'-ends of DNA using rSAP in Restriction Enzyme Reaction

1. Digest 1–5 µg of plasmid DNA in a 20 µl reaction as follows:

DNA	≥ 1 µl
Restriction Enzyme Buffer (10X)	2 µl
Restriction Endonuclease	1 µl
H ₂ O, purified	to 20 µl

Note: Scale larger reaction volumes proportionally.

2. Incubate at 37°C for 60 minutes or follow manufacturer's recommendations.
3. Add 1 unit of rSAP 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 heat-inactivation of rSAP and restriction enzyme (follow manufacturer's recommendations).

Note: If restriction enzyme cannot be heat-inactivated, DNA purification is required before ligation.

Page 2 (M0371)

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Usage Notes:

rSAP, as are most alkaline phosphatases, is a Zn²⁺ and Mg²⁺-dependent enzyme. Our formulation has tightly bound zinc atoms in the active center and does not require supplemental zinc or other additives.

rSAP is also active in 1X NEBuffers 1.1, 2.1, 3.1 as well as NEBuffers 1, 2, 3, 4 and NEBuffer for EcoRI.

rSAP activity is enhanced in the presence of monovalent salts.

rSAP is inhibited by metal chelators (e.g. EDTA), inorganic phosphate and phosphate analogs.

The rSAP activity is decreased in the presence of reducing agents (DTT, β-mercaptoethanol).

Quality Control Assays

Exonuclease Activity: Incubation of a 50 µl reaction containing 10 units of rSAP with 1 µg of a mixture of single and double-stranded [³H] *E. coli* DNA for 4 hours at 37°C released < 0.1% of the total radioactivity.

Endonuclease Activity: Incubation of a 50 µl reaction containing 5 units of rSAP with 1 µg of

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Endonuclease Activity: Incubation of a 50 µl reaction containing 5 units of rSAP with 1 µg of

φX174 RF I DNA for 4 hours at 37°C resulted in < 10% conversion to RF II as determined by agarose gel electrophoresis.

RNase Activity (Extended Digestion): A 10 µl reaction in CutSmart Buffer containing 40 ng of fluorescein labeled RNA transcript and 1 unit of rSAP is incubated at 37°C. After incubation for 16 hours, > 90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.

Physical Purity: Purified to > 95% homogeneity as determined by SDS-PAGE analysis using Coomassie Blue staining detection.

References:

1. Olsen, R. L. et al. (1991) *Comp. Biochem. Physiol.* 99B(4): 755–761.
2. Nilsen, I.W. et al. (2001) *Comp. Biochem. Physiol.* 129B(4): 853–861.

Companion Products Sold Separately:

CutSmart Buffer
#B7204S 5 ml

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CutSmart Buffer
#B7204S 5 ml

T4 DNA Ligase
#M0202S 20,000 units
#M0202L 100,000 units
#M0202T 20,000 units
#M0202M 100,000 units

Quick Ligation™ Kit
#M2200S 30 rxns
#M2200L 150 rxns

Instant Sticky-end Ligase Master Mix
#M0370S 50 rxns
#M0370L 250 rxns

Blunt/TA Ligase Master Mix
#M0367S 50 rxns
#M0367L 250 rxns



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rSAP is sourced from ArcticZymes AS; Quality assured, packaged and distributed by New England Biolabs, Inc.

T4 DNA Ligase
#M0202S 20,000 units
#M0202L 100,000 units
#M0202T 20,000 units
#M0202M 100,000 units

Quick Ligation™ Kit
#M2200S 30 rxns
#M2200L 150 rxns

Instant Sticky-end Ligase Master Mix
#M0370S 50 rxns
#M0370L 250 rxns

Blunt/TA Ligase Master Mix
#M0367S 50 rxns
#M0367L 250 rxns



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