Shrimp Alkaline Phosphatase (rSAP)

1-800-632-7799
info@neb.com
www.neb.com

M0371S 001140116011

M0371S

RR 37° 65°

500 units **1,000 U/ml** **Lot: 0011401**

Store at –20°C **Exp: 6/15**

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 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.
- Dephosphorylation of DNA and RNA.

Supplied in: 25 mM Tris-HCl (pH 7.5), 1 mM $MgCl_2$ and 50% glycerol.

Reagents Supplied with Enzyme:
10X CutSmart Reaction Buffer

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

1X CutSmart Reaction 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 $MgCl_2$, 50 mM *p*-Nitrophenyl Phosphate. These conditions are only used for quantitating enzyme activity.

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_2O, 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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H_2O, 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

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_2O, 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.

Usage Notes:

1. rSAP, as are most alkaline phosphatases, is a Zn^{2+} and Mg^{2+}-dependent enzyme. Our formulation has tightly bound zinc atoms in the active center and does not require supplemental zinc or other additives.
2. 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.
3. rSAP activity is enhanced in the presence of monovalent salts.
4. rSAP is inhibited by metal chelators (e.g. EDTA), inorganic phosphate and phosphate analogs.
5. The rSAP activity is decreased in the presence of reducing agents (DTT, β-mercaptoethanol).

Quality Controls 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 [^{3}H] *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 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: Incubation of a 10 µl reaction containing 5 units of rSAP with 40 ng of fluorescein labeled RNA transcript for 4 hours at 37°C resulted in < 10% degradation of the RNA as determined by gel electrophoresis using fluorescence detection.

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

Heat Inactivation: 65°C for 5 minutes

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:

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

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

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

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DNA	≥ 1 µl
Restriction Enzyme Buffer (10X)	2 µl
Restriction Endonuclease	1 µl
H_2O, purified	to 20 µl

Note: Scale larger reaction volumes proportionally.

2. Incubate at 37°C for 60 minutes or follow manufacturer's recommendations.
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Note: If restriction enzyme cannot be heat-inactivated, DNA purification is required before ligation.

Page 2 (M0371)

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