Shrimp Alkaline Phosphatase (rSAP)

M0371S

500 units 1,000 U/ml Lot: 0021504
Store at -20°C Exp: 4/17

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 catalyzes the dephosphorylation of 5’ and 3’ ends or other modifications. rSAP nonspecifically hydrolyses ribo-, as well as deoxyribonucleoside triphosphates (NTPs and dNTPs). rSAP is useful in many molecular biology applications such as

- Dephosphorylation of cloning vector DNA to prevent recirculation 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.

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.
- Dephosphorylation of DNA prior to end-labeling using T4 Polynucleotide Kinase.
- Treatment of dNTPs in PCR reactions prior to sequencing or SNP analysis.

Molecular Weight of the Monomer: 54 kDa
Specific Activity: ~ 3,000 units/mg

Heat Inactivation: 5 min at 65°C

Clinical Applications:
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4. Stop reaction by heat-inactivation of rSAP

3. Add 1 unit of rSAP for every 1 pmol of DNA ends

2. Incubate at 37°C for 60 minutes or follow manufacturer’s recommendations.

Note: Scale larger reaction volumes proportionally.

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

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA</td>
<td>≥ 1 μl</td>
</tr>
<tr>
<td>Restriction Enzyme Buffer (10X)</td>
<td>2 μl</td>
</tr>
<tr>
<td>Restriction Endonuclease</td>
<td>1 μl</td>
</tr>
<tr>
<td>H₂O, purified</td>
<td>to 20 μl</td>
</tr>
</tbody>
</table>

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.

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 [³²P] 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 DNA, 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, 2, 3, 4 as well as NEBuffers 1, 2, 3, 4 and NEBuffer for EcoRI.

rSAP activity is enhanced in the presence of mono-ovalent 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).

Usage Notes:

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References:


Companion Products Sold Separately:

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

Endonuclease Activity: Incubation of a 50 μl reaction containing 10 units of rSAP with 1 μg of DNA for 4 hours at 37°C released < 0.1% 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: