Shrimp Alkaline Phosphatase

#GE015.0001 (1ml)
(FOR RESEARCH ONLY)

Product: Recombinant Shrimp Alkaline Phosphatase (rSAP) is a heat-labile multi-purpose alkaline phosphatase that catalyzes the dephosphorylation of DNA, RNA, and nucleotides. This recombinant enzyme replaces native SAP because it is much more stable at room temperature and is available at higher concentrations. For activity, rSAP requires magnesium (>1mM) and is tolerant to a wide variety of buffer conditions (salt, pH, etc.), and thus can be added directly to many molecular biology buffers, including PCR mixtures and most restriction enzyme buffers. rSAP is completely inactivated by incubation at 65ºC for 5 minutes.

Applications: Dephosphorylation of dNTPs from PCR reaction mixtures prior to DNA sequencing (typically in a PCR Clean-up protocol in combination with the use of Exonuclease I (Exo I)). Dephosphorylation of DNA prior to end-labelling (using T4 Polynucleotide Kinase). Dephosphorylation of vectors (plasmids) during cloning, in order to prevent recircularization during ligation reaction (using T4 DNA Ligase).

Content: #GE015.0001 contains 1 ml of Shrimp Alkaline Phosphatase (rSAP), purified from a recombinant strain of *Pichia pastoris* harbouring the shrimp alkaline phosphatase gene from *Pandalus borealis*, at a concentration of 1U/µl (1,000U in total), supplied in 25mM Tris-HCl pH 7.6 (4ºC), 5mM $MgCl_2$, and 50% glycerol

Properties: Free of RNases, exonucleases and endonucleases.
Specific Activity: >1U/µl. (One Unit (1U) is defined as the amount of enzyme required to catalyze the release of 1µmol/min of phosphate from 6mM 4-nitrophenyl phosphate in 0.1M glycine-NaOH pH 10.4 containing 1mM $MgCl_2$ and 1mM $ZnCl_2$ at 37ºC).

QC: Absence of ribonuclease, and endonuclease activity was confirmed by appropriate assays.

Storage: rSAP be stored for up to 3 years at -20ºC. Moreover, it shows excellent stability at +4ºC (>6 months) and room temperature (>3 months). Enzyme stability is not affected by multiple freeze-thaw cycles.

Prior to use:

Activity

Optimum pH range for rSAP is 7 to 9. Furthermore, rSAP requires magnesium (>1mM) for activity, normally present in molecular biology buffers. Additional supplements like Zinc are not required, thus rSAP can be added directly to most reaction mixtures without any adaption.

Inhibition

rSAP is inhibited by metal chelators, inorganic phosphate and phosphate analogues.

Inactivation

rSAP is completely and irreversibly inactivated by incubation at 65°C for 5 minutes or at 75°C for 1 minute. No further treatment is necessary.

Usage:

Protocol for dephosphorylation after digestion with restriction enzymes

1) Following the specific protocol for your restriction enzyme(s), simply add 5U of rSAP per µg of vector and incubate for at 37°C for 10 minutes.
2) Heat-inactivate rSAP and restriction enzyme(s) as recommended for your restriction enzyme(s) (rSAP requires only 5 minutes at 65°C).

Protocol for dephosphorylation during digestion with restriction enzymes

1) Add 1U of rSAP for every 10U of restriction enzyme and proceed conform instructions of the restriction enzyme(s) supplier. Incubate at 37°C for 1 hour.
2) Heat-inactivate rSAP and restriction enzyme(s) as recommended for your restriction enzyme(s) (rSAP requires only 5 minutes at 65°C).

Protocol for PCR Clean-up (prior to DNA sequencing)

1) Add 0.5-2.0U of rSAP and 10-20U of Exo I (**#GE014.0001**) directly to 5 µl of PCR reaction mixture and incubate at 37°C for 15min.
2) Heat-inactivate at 80°C (Exo I) for 15 min and use 5µl of purified PCR product directly for DNA sequencing. There is no need for further purification.