Shrimp Alkaline Phosphatase, recombinant (rSAP)

GE015.0001 – 1.000U (1U/µl)

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.

Quantity and Specifications

Specific Activity: 1U/µl. Purified from a recombinant *Pichia pastoris* strain harbouring the shrimp alkaline phosphatase gene from *Pandalus borealis*. Product consists of 1.000U of rSAP in 1ml of 25mM Tris-HCl, pH 7.6 (4ºC), containing 5mM $MgCl_2$ and 50% glycerol, and is free of any nuclease or other enzymatic activities.

Units

One Unit (1 U) 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.

Storage and Shelf Life

rSAP can be stored at -20ºC for up to 2 years. Moreover, it shows excellent stability at +4ºC (at least 6 months) and at room temperature (at least 3 months). Enzyme is resistant to multiple freeze-thaw cycles.

Quality Control

Each lot is tested for the absence of RNAses, exonucleases, and endonucleases.

Activity
Optimum pH range for rSAP is 7-9. 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 65ºC for 5 minutes or at 75ºC for 1 minute. No further treatment is necessary.

Applications
Dephosphorylation of dNTPs from PCR reaction mixtures prior to DNA sequencing (typically in a PCR Clean-up protocol[1] 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).

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 37ºC for 10 minutes.
2) Heat-inactivate rSAP and restriction enzyme(s) as recommended for your restriction enzyme(s) (rSAP requires only 5 minutes at 65ºC).

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 37ºC for 1 hour.
2) Heat-inactivate rSAP and restriction enzyme(s) as recommended for your restriction enzyme(s) (rSAP requires only 5 minutes at 65ºC).

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 37ºC for 15min.
2) Heat-inactivate at 80ºC (Exo I) for 15 min and use 5µl of purified PCR product directly for DNA sequencing. There is no need for further purification.

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