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ProductInformation

Streptavidin-Alkaline Phosphatase
from *Streptomyces avidinii*

Catalog Number **S2890**
Storage Temperature –20 °C

Synonym: Streptavidin-AP

Product Description

Both avidin (Catalog Number A9275) and streptavidin (Catalog Number S4762) have been used in biochemical research because of their extremely high affinity for biotin. Avidin from egg white is a glycoprotein; the carbohydrates often stain nonspecifically, giving higher background. Streptavidin, a homotetramer purified from *Streptomyces avidinii*, is not a glycoprotein, and gives lower background in staining procedures. (See Product Information Sheets for each product).

Streptavidin has been conjugated with either peroxidase (Catalog Number S5512) or alkaline phosphatase. These enzymes have been used as protein conjugates because of their comparative stability and a wide range of suitable substrates for different applications. The researcher's choice of enzyme conjugate and substrate depends on specific application.

Streptavidin-AP has been used in many ways, including:

In an ELISA assay to detect herpes simplex virus: pretitrated solution, dilution 1:500, 100 µL per well, 30 minute incubation at 37 °C, and detection using *p*-nitrophenyl phosphate substrate (available in bulk or tablets).[2]

As a label of nucleic acid probes, using nitroblue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate (NBT/BCIP) as substrate.[3]

To measure indirectly the amount of biologically available peptide on the surface of polymer beads, using NBT/BCIP as substrate[4]

On a Western blot with two different substrates: Naphthol AS-GR phosphate and Fast Blue BN gave water-insoluble blue reaction products. Naphthol AS-TR phosphate and Fast Red TR gave water-insoluble red reaction products.[5]

In an ELISA assay to detect antibody fragments, using phenolphthalein monophosphate as the AP substrate, and 1:1,000 dilution of stock solution equivalent to 1,000 units/mL.[6]

Sigma offers a Biotinylated Molecular Weight Marker Kit (Catalog Number B2787), which is compatible with the use of streptavidin-alkaline phosphatase,[7] although the kit uses streptavidin-peroxidase to detect the biotinylated markers. Please consult the datasheet for B2787 for solutions and techniques that might be useful.

This product is supplied as a lyophilized powder (the balance primarily trehalose with EPPS, phosphate, and traces of $MgCl_2$ and $ZnCl_2$.)[1]

The conjugate is ~2:1 streptavidin to alkaline phosphatase. It is coupled by heterobifunctional cross-linking reagents maleimidohexanoic NHS ester and S-acetylthioacetic NHS ester (SATA) which incorporate spacer arms. Bonding is by amide bond to the protein; spacer arms are coupled through the stable thioether. The biotin-binding sites were protected during the activation.[1,7] The final product is subjected to ultrafiltration to remove unconjugated enzyme and streptavidin.

Unit Definition: One unit will hydrolyze 1.0 µmole of *p*-nitrophenyl phosphate per minute at pH 9.8 at 37 °C.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions
Although Streptavidin-Ap is soluble in water(1mg/ml), the alkaline phosphatase is more stable and active in buffer containing 1 mM $MgCl_2$, for example, Tris-buffered saline at pH 8.[1] Do **not** use phosphate buffer.

Solutions should be stable at 2–8 °C for about two weeks, but if stored as aliquots at –20 °C, the product should be stable much longer, at least several months. Freeze-thaw cycles should be avoided.[1]

Storage/Stability
Store the product at –20 °C.

Product Profile
An initial stock solution of 1 mg/mL in Tris buffered saline (pH 9.2) is generally used. Subsequent dilutions can be made with buffer containing blocking agents such as 0.05% (w/v) TWEEN® 20 or 1% (w/v) nonfat dry milk.[5] The stock must be titered by serial dilution to determine an optimal working concentration. Typically, 1:500 is a suitable initial dilution for ELISA assays or 1:50 for histochemical detection. In one case, 1:5,000 was satisfactory for Western blotting.[5]

References

1. Sigma production or quality control.
2. Nerurkar, L.S., Meth. Enzymol., **184**, 541 (1990).
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4. Shin, J.S. et al., Anal. Biochem., **236**, 9-13 (1996).
5. Fransen, M. et al., Anal. Biochem., **242**, 26-30 (1996).
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7. Duncan, R.J.S. et al., Anal. Biochem., **132**, 68 (1983).
8. Billingsley, M. et al., Biotechniques, **5**, 31 (1987).

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