



Product Information

Streptavidin—Peroxidase From *Streptomyces avidinii*

Product Number **S 5512**
Storage Temperature -0 °C

Product Description

Streptavidin is coupled to horseradish peroxidase (HRP) using citrate buffer, pH 6.0, and a modified published procedure.¹ The HRP used for coupling is Product No. P 8375, Type VI. This HRP contains predominantly the C isozyme (about 75%), but includes all others as well, except for the most acidic isozymes. The details of the method of coupling are proprietary, but maleimide activation is involved. (The HRP is first activated and then coupled to the streptavidin). S-acetylthioglycolic acid N-hydroxysuccinimide ester (Product No. A 9043) is used to convert the amino groups to sulfhydryl groups in order to perform this coupling reaction. Once coupled, the mass of peroxidase is determined by its A_{403} , which leads to its calculated A_{280} . This allows calculation of the mass % of HRP, based on a molecular weight of approximately 44 kDa.² The mass % of streptavidin is then the calculation of the masses of streptavidin and HRP divided by molecular weight of streptavidin to give the estimated molar ratio. (Streptavidin has a molecular weight of approximately 60 kDa.³) The actual molecular weight is controlled by the amount of cross-linking agent used, and is typically is a 1:1 adduct. Unconjugated material is removed by chromatography, and over-sized complexes are removed as well since they will not enter the pores of the resin. A method of preparing an enzyme-antibody (or binding protein) conjugate (covalent compound) is described using m-Maleimidobenzoic acid N-Hydroxysuccinimide ester (MBS) (Product Nos. M 8759 or M 2786).¹

This product is used with the Biotinylated SDS Molecular Weight Standards and Kit, Product Code MW-SDS-100B as described in Technical Bulletin No. MWS-877B. This product can also be used as a secondary reagent for detection of biotinylated antibodies in standard immunoblotting, ELISA, and immunocytochemistry procedures.

Precautions and Disclaimer

For Laboratory Use Only. Not for drug, household or other uses.

Preparation Instructions

This product is soluble in water (1 mg/ml). However, buffered solutions (PBS) are more stable and are preferred for long term storage. Further dilutions can be made with buffers containing 0.1% BSA as a stabilizer.

Storage/Stability

Solutions in PBS, pH 7, can be stored at 2-8 °C for up to 2 weeks. For long term storage, solutions can be frozen in working aliquots. Repeated freeze-thaw cycles should be avoided.

Procedure

For ELISA applications, it will be necessary to establish a titer for this product. The product should be reconstituted at a concentration of 1 mg/ml in either PBS or water. Aliquots can be frozen for later usage. Stock solutions can then be diluted in PBS containing 0.1% BSA as needed. Starting dilutions should be in the range of 1:100-1:1000. If the frozen stocks start giving high background readings, then new stock solutions should be prepared.

References

1. O'Sullivan, M. J., et al., A Simple Method for the Preparation of Enzyme-antibody Conjugates, *FEBS Letters*, **95(2)**, 311-313 (1978).
2. Welinder, K. G., Amino acid sequence studies of horseradish peroxidase. Amino and carboxyl termini, cyanogen bromide and tryptic fragments, the complete sequence, and some structural characteristics of horseradish peroxidase. *Eur. J. Biochem*, **96(3)**, 483-502 (1979).
3. Haeuptle, M. T., et al., Binding sites for lactogenic and somatogenic hormones from rabbit mammary gland and liver. *J. Biol. Chem.*, **258(1)**, 305-314 (1983).

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