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Product Information

Streptavidin-Peroxidase from *Streptomyces avidinii*

Catalog Number **\$5512** Storage Temperature –20 °C

Product Description

Steptavidin is coupled to horseradish peroxidase (HRP) using citrate buffer, pH 6.0, and a modified published procedure. The HRP used for coupling is Catalog Number P8375, Type VI. This HRP contains predominantly the C isozyme (~75%), but includes all others as well, except for the most acidic isozymes.

The details of the conjugate preparation are proprietary, but maleimide activation is involved. The HRP is first activated and then coupled to the streptavidin. The molecular mass of the conjugate is controlled by the amount of crosslinking reagent used and typically results in a 1:1 conjugate. HRP has a molecular mass of ~44 kDa and steptavidin has a molecular mass of ~60 kDa. ^{2,3} Unconjugated proteins are removed by gel filtration chromatography and oversized 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 3-maleimidobenzoic acid *N*-hydroxysuccinimide ester (MBS) (Catalog Numbers M8759 or M2786).¹

This product can be used as a secondary reagent for detection of biotinylated antibodies in standard ELISA, immunoblotting, and immunocytochemistry procedures.

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

The product should be reconstituted at a concentration of 1 mg/ml in either PBS or water. Buffered solutions (PBS) are more stable and are preferred for long term storage. The 1 mg/ml Stock Solution can be frozen in aliquots for later usage. If the frozen Stock Solutions start giving high background readings, then prepare new Stock Solutions.

Stock Solutions can be diluted in buffers containing 0.1% BSA as needed.

Storage/Stability

Store the product at -20 °C.

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.

Product Profile

Direct ELISA: ≥1:50,000 dilution of 1 mg/ml Stock Solution

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 C. 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).

KAA, CMH, RXR, MAM 12/08-1