ProductInformation

STREPTAVIDIN-PEROXIDASE POLYMER LABELED
Product Number **S9420**
Storage Temperature <0°C

CAS #: N/A

Product Description
Appearance: Brown powder
Molecular weight: 60 kDa for streptavidin[1]
44 kDa for monomeric peroxidase[2]

Streptavidin (from Streptomyces avidinii) is used in biochemical research because of its extremely high affinity for biotin, similar to that of avidin. (See product S4762 and related references.[3] Peroxidase from horseradish (HRP) is widely used in protein conjugation because of its comparative stability and selection of substrates for a variety of applications. Making use of the avidin-biotin or streptavidin-biotin complex is often used over other detection systems due to the ease of biotinylating target proteins.

This product ("strep-poly-HRP") is expected to be useful for highly sensitive detection of biotin conjugates, demonstrating at least a 10-fold increase in sensitivity over the nonpolymerized enzyme conjugate, product S5512. Early tests showed use of S9420 at 5 micrograms/mL detected albumin-biotin (product A6043) at 24 ng/mL, compared to a positive response with S5512 at 390 ng/mL.[4] Use of peroxidase-polymer-labeled streptavidin in ELISA in detecting a biotinylated second antibody detected the allergen at 0.6 ng/mL.[5]

The product is a conjugate of streptavidin (product S4762) and a polymerized horseradish peroxidase (from product P0889). The streptavidin was activated using S-acetylthioglycolic acid N-hydroxysuccinimide ester (SATA, product A9043),[6] then reacted with maleimide-activated poly-peroxidase. The polymerization of HRP produced approximately 20 molecules per polymer, as determined by LALLS and gel flltration chromatography. The conjugate was purified by gel filtration chromatography and lyophilized from a sodium citrate buffer containing trehalose.[4]

Components
The product is approximately 50% protein (BCA assay), with the balance being primarily trehalose and sodium citrate.[4] Note that native peroxidase is approximately 75% protein, 25% carbohydrate and heme group.[2]

Preparation Instructions

The strep-poly-HRP dissolves at 0.25 mg/mL in phosphate buffered saline pH 6.0 to give a clear to light tan solution. Stock solutions should be stable at least two weeks at 2-8°C, but it is recommended to freeze aliquots at -20°C. Solutions in 50% glycerol should be stable for at least a year at -20°C.[4]

By analogy to usage of S5512, further dilutions should be made with PBS containing BSA and Tween 80 as blocking agents to reduce non-specific background.[7,8]

Storage/Stability
The product as shipped should be stored dry at -20°C, retaining at least 95% initial activity for a year.[4]

Procedure
The usage will be much like that of streptavidin-peroxidase S5512, but with greater sensitivity in detection of biotinylated molecules. Determining a specific titer will depend on the customer's application. However, for product S5512, a first dilution was suggested as 1:100 to 1:1000, followed by incubation at room temperature for an hour, then rinsing thoroughly with buffer before applying HRP substrate.[7,8] Given the 10-fold improvement in sensitivity, we suggest a stock solution of 1 mg/mL, with 5 µg/mL as an effective working concentration, i.e., 1:1000 or 1:10,000 as a first dilution to try.[4]

Product Profile
Streptavidin activity: 1 to4 units/mg protein
Unit definition: One unit will bind 1µg biotin.[9]
Peroxidase activity: 100 to 200 units/mg protein
Unit definition: One unit will form 1 mg purpurogallin in 20 sec from pyrogallol at pH 6.0 at 20°C.

References

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6. Julian, R., Anal. Biochem., 132, 68 (1983).

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