

Product Information

Streptavidin from *Streptomyces avidinii*

Catalog Number **S4762**

Storage Temperature $-20\text{ }^{\circ}\text{C}$

CAS RN 9013-20-1

Product Description

$E^{1\%} = 34$ at 280 nm (solvent: 0.1 M ammonium perchlorate, pH 8.0)

Structure: References have cited the molecular mass for the native protein as 60,000; it contains four subunits of 15,000.^{1,2,3} The molecular mass of affinity-purified streptavidin was reported more recently as 75,000; under extreme denaturing conditions, giving a major band at about 18,000.⁴ However, the structure of "truncated streptavidin" is also discussed by this author.⁴

Isoelectric point: 6.4⁵, 5-6⁶

Additional analytic methods and data have been reported.^{7,8}

Streptavidin is a protein produced by the bacterium *Streptomyces avidinii*. It has four binding sites for biotin, as does avidin. Both have been used extensively as probes in immunochemical systems, conjugated to antibodies, enzymes, or fluorochromes. However, avidin is highly basic (pI ~ 10.5), compared to streptavidin (pI $\sim 5-6$). Avidin has a slightly higher binding capacity for biotin than streptavidin does, but it has glycosylated side chains which give higher backgrounds in blotting techniques than does streptavidin, which is not a glycoprotein.⁴ Streptavidin is more resistant than avidin to dissociation into subunits by guanidinium chloride.⁹

In blotting techniques, blocking agents are frequently used to reduce non-specific binding. Gelatin, albumin, and casein (as nonfat dried milk) are commonly used for this purpose. However, it is advised that the use of nonfat dried milk be avoided if possible; Hoffmann et al. report nonfat dried milk contains an "inhibitor of the biotin-streptavidin interaction and it is recommended that milk be dialyzed or used at lower concentrations when it is employed as a diluent of streptavidin."¹⁰

This protein is isolated by a proprietary method developed by Sigma. The affinity purification step uses iminobiotin-agarose, Catalog Number I4507, binding at pH 9 and eluting at pH 2. Extensive dialysis against distilled water makes this product "essentially salt-free."¹¹ A similar affinity preparation has been reported.⁴ Due to affinity purification, the product is active, but appears to be heterogeneous on an SDS PAGE gel, giving a number of bands from 12,000 to 18,000.¹²

This product is assayed for biotin binding by a modification of Green's method.¹³ (The procedure is given below.)

Precautions and Disclaimer

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

Storage/Stability

The dry solid is stable at least three years stored frozen.

Procedure

Biotin Binding Assay

This assay is modified from Green's spectrophotometric assay for the determination of avidin.¹³

Reagents

- 0.1 M sodium phosphate buffer, pH 7.0
- 2 mM Biotin (Dissolve 4.9 mg of Catalog Number B4501 in 10 mL buffer A.)
- 10 mM 2-(4'-Hydroxyazobenzene)-benzoic acid [HABA] (Dissolve 24.2 mg of Catalog Number H5126 in 10.0 mL buffer A.)
- Streptavidin (Dissolve ~ 0.1 mg per mL buffer A.)
- Avidin control. (Dissolve Avidin, Catalog Number A9275, at 0.1 mg per mL buffer A.)

Assay Procedure

1. To a 3.0 mL cuvette (1.0 cm light path), add 2.0 mL of streptavidin solution or avidin control.
2. Zero the spectrophotometer using this solution at 500 nm at 25 °C.
3. Add 0.05 mL of HABA solution (Reagent C). Mix and record A_{500} .
4. Add 0.05 mL of Biotin solution (Reagent B). Mix and record A_{500} .
5. Calculate ΔA_{500} .
Note: Total volume in cuvette = 2.10 mL.

Results

Calculations

Streptavidin

$$\begin{aligned} \text{(mg/mL of original solution)} &= \frac{2.1 \times 15 \times \Delta A_{500}}{2 \times 35} \\ &= 0.45 \times \Delta A_{500} \end{aligned}$$

where:

2.1 = total volume in cuvette

2 = sample volume

15 = milliequivalent weight of streptavidin for biotin binding

35 = millimolar extinction coefficient of streptavidin-HABA complex at pH 7.0

Note: This is a protocol used to determine biotin binding, although this is not in a standardized format.

Extravidin[®] is also offered. It is a modified avidin that has the advantage of binding specificity of avidin along with the reduced background seen with streptavidin. View our website, <http://www.sigmaaldrich.com>, for a large number of conjugate products prepared from Extravidin.

References

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