Data Sheet

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Agilent Streptavidin

Specifications	
Product Code	SA10
Specific Activity	≥14.0 U/mg
Purity	Predominantly a single band by SDS-PAGE. Shipped with ice pack for next day delivery.
Storage	–20 °C
Formulation	Lyophilized (contains ~0.9 mg protein/mg lyophilizate; balance is sodium chloride).
Stability	Lyophilized streptavidin is stable at least through the expiry date printed on the label and Certificate of Analysis when stored dessicated at -20 °C. Streptavidin reconstituted in deionized water is stable for at least 6 months at either 2–8 °C or -20 °C.

Streptavidin (CAS No. 9013-20-1) (formerly ProZyme) is a biotin-binding protein found in the culture broth of the bacterium *Streptomyces avidinii*. Like its namesake avidin, streptavidin binds four moles of biotin per mole of protein with a high affinity virtually unmatched in nature (K_d~10^{-15}). Streptavidin lacks the carbohydrate side chains present on avidin, and has an isoelectric point nearer to neutrality where most useful biological interactions occur (pI of 5 to 6 versus 10 for avidin). As a result, streptavidin frequently exhibits lower levels of nonspecific binding than does avidin when the proteins are used in applications relying upon the formation of avidin/biotin complexes.[1]

In ELISA-based systems, antibodies directed against a particular antigen may be covalently attached to reporter enzymes. Antigens are then quantitated by enzymatic assay after binding to these conjugated molecules.

Unfortunately, the precise conditions for accomplishing such covalent attachments must be determined individually for each antibody/reporter combination, and often result in significant loss of either the enzymatic activity of the reporter enzyme or the binding functions of the antibodies.

Streptavidin finds utility in these systems because antibody molecules are easily modified by the covalent attachment of derivatives of biotin with little or no loss in the ability of the antibody molecules to bind their antigens. These biotinylated antibodies may be detected by their interaction with conjugates of streptavidin and the reporter enzymes.[2] The same preparation of conjugated streptavidin-reporter enzyme may be used with any number of different biotinylated antibodies, making this system a highly flexible one.

The reporter molecule may be bound to streptavidin covalently, or biotinylated and attached to streptavidin through the streptavidin-biotin interaction. Since streptavidin is multivalent (binding four molecules of biotin per tetrameric protein molecule) it may be used in combination with biotinylated antibody and biotinylated reporter enzymes to obtain amplified signals. Such amplification in ELISA is otherwise difficult to obtain and requires the introduction of additional antibody components. ELISA systems using streptavidin can readily detect sub-nanogram amounts of antigens.

Characteristics

Molecular weight

52,000 Daltons, composed of four essentially identical polypeptide chains

Composition

Streptavidin is a homotetramer with 24 to 32 lysines per tetramer. The protein contains no cysteine residues, carbohydrate side chains, or associated cofactors. Different preparations of streptavidin show considerable heterogeneity at both the amino- and carboxy-termini of each subunit polypeptide due to proteolysis during biosynthesis and secretion. Monomeric subunits of streptavidin are synthesized as 183 amino acid prepeptides. During secretion by *Streptomyces* sp., a 24-amino acid leader sequence is cleaved from these polypeptides resulting in newly secreted monomers of 159 amino acids.[3] Upon longer incubations in culture, these monomers are progressively cleaved to core subunits containing 125 to 127 amino acids.[4,5] Preparations of streptavidin are relatively stable over a wide pH range and extremely heat stable, requiring up to 20 minutes at 100 °C in 0.2% SDS to dissociate the subunits.[6] Strong chaotropic agents such as 6 M urea have been reported to dissociate the streptavidin tetramer into dimers.[7] These dimers appear to be stable in urea without the appearance of monomers.

Binding properties

Unproteolyzed and proteolyzed preparations of streptavidin appear to bind biotin with equal affinity. The most highly proteolyzed tetramers may bind over 16 µg of d-biotin per mg of protein. Bayer (1989)[4] reports that biotinylated enzymes bind most effectively to truncated streptavidin in ELISA-type assays.

The dissociation constant for biotin is approximately 10 to 15 M.[1] The formation of the streptavidin-biotin complex is stable over wide pH and temperature ranges. The complex is generally disrupted only by conditions that lead to irreversible denaturation of the protein. Analogs of biotin such as 2-imino-biotin bind reversibly to the protein with complex formation at high pH (>9.5) and dissociation at low pH (<4).[6,8]

Extinction coefficient[9]

$E_{280}^{1\%} = 32$

Homogeneity

The streptavidin gene product is subject to cleavage by endogenous proteases during fermentation. In some instances, this can result in a heterogeneous product. The crude material may be treated with exogenous proteases during purification to reduce this heterogeneity. Agilent streptavidin is a pure, homogeneous preparation, which shows predominantly a single band by SDS PAGE.

Applications

- Enzyme conjugates and complexes
- Southern blots and other methodologies related to DNA and RNA analysis
- Western blots
- Purification of proteins or other antigens with biotinylated antibodies or lectins by use of immobilized streptavidin

Origin: USA

Assay

The biotin-binding activity of streptavidin is determined using a modification of the dye-binding assay of Green (1970).[10] One unit will bind 1 µg of d-biotin at pH 7.0.

Reagents

- 10 mM 2-(4'-hydroxyazobenzene) benzoic acid (Sigma) dissolved in 10 mM sodium hydroxide (HABA)
- 0.2 M sodium phosphate, pH 7.0
- 2 mM d-biotin in 0.1 M sodium phosphate, pH 7.0
- Streptavidin sample: dissolve at 5 to 10 mg/mL in de-ionized water

Procedure

1. Adjust the spectrophotometer to read at 500 nm.
2. To two tubes labeled A and B, add as follows:

	A (mL)	B (mL)
Streptavidin Sample	0.05	0.05
Phosphate Buffer	0.5	0.5
HABA Stock	0.1	0.1
Biotin Stock	–	0.25
H_2O	0.35	0.1
Total Volume	1.0	1.0

3. After mixing, zero the spectrophotometer with water, and read the absorbance values in tubes A and B.

Calculation

$$\text{Units/mg} = \frac{(10^6\mu g/g)(A-B)MV}{\varepsilon Cv} = \frac{141(A-B)}{C}$$

where:

M = Formula weight of d-biotin (244 g/mol)

V = Volume of assay in liters (0.001 L)

v = Volume of streptavidin sample in milliliters (0.05 mL as written)

C = Concentration of streptavidin in the sample (mg/mL)

ε = Net molar extinction coefficient of HABA-streptavidin complex at 500 nm (34,500 M^{-1})

The measured specific activity of a given lot depends on the assay method used, as well as the method used to quantitate the streptavidin (gravimetric versus spectrophotometric). For comparison purposes, Agilent also reports the results of a biotin titration assay.

Biotin-binding capacity

1 Streptavidin + 4 biotin → Streptavidin·$(biotin)_4$

The formation of the complex is measured at 233 nm

Reagents

- Ammonium carbonate buffer: 200 mM $(NH_4)_2CO_3$, pH 8.9
- 1 mg/mL solution of d-biotin (dissolved in ammonium carbonate buffer)
- 10 mg/mL streptavidin sample (dissolved in de-ionized water)

Procedure

1. Adjust the spectrophotometer to read at 233 nm.
2. Dilute the dissolved streptavidin sample to 0.1 mg/mL in ammonium carbonate buffer. Prepare at least 1 mL.
3. Dilute the 1 mg/mL biotin solution to ~0.1 mg/mL in ammonium carbonate buffer. Prepare at least 1 mL.
4. Add 1 mL of 0.1 mg/mL streptavidin sample to a cuvette. Take an initial reading of unbound streptavidin.
5. Add diluted biotin (0.1 mg/mL) in increments of 3 µL. Mix carefully after each addition and take absorbance readings.
6. Continue adding biotin until absorbance readings plateau or decline repeatedly.
7. Graph a titration curve as absorbance (ordinate) versus volume of biotin added (abscissa). Note the volume (µL) of biotin added at the inflection point of the curve.
8. The amount of biotin at the inflection point is divided by the concentration of the streptavidin sample to yield the specific activity.

Calculation

$$\text{Units/mg} = \frac{V_1 x C_1}{V_2 x C_2}$$

where:

V_1 = Volume of d-biotin at inflection point

C_1 = Concentration of d-biotin solution

V_2 = Volume of streptavidin sample

C_2 = Concentration of streptavidin sample

Suggestions for use

Measurement

Because the lyophilizate is ~10% NaCl by weight, we suggest that the customer dissolve it and measure it using its extinction coefficient $E_{280}(1\%) = 32$ rather than weighing it.

Aliquots may be stored at -20 °C for long periods. SA10 reconstituted in deionized water is stable for at least 6 months at 2–8°C or -20 °C. If storage for longer periods is desired, an antimicrobial agent such as 0.05% sodium azide should be added to retard growth and subsequent proteolysis from exogenous enzymes.

Solubility

Streptavidin is readily soluble in water or salt-containing buffers, up to 50 mg/mL or more.

Bayer (1989)[4] reports that streptavidin may form aggregates under certain conditions. There is a tendency for lyophilized streptavidin to aggregate when it is redissolved in water or other low ionic strength buffers at neutral or acidic pH.

Agilent streptavidin has been lyophilized from a dilute sodium chloride solution at mildly alkaline pH to minimize aggregate formation.

However, in rare cases streptavidin may contain a small amount of insoluble material when dissolved in de-ionized water or low ionic strength buffers, either at the time it is dissolved or after freezing and rethawing. This effect is generally not seen in the presence of salt-containing buffers such as PBS.

If undissolved material is observed, it can be removed by centrifugation and does not constitute a significant fraction of the total protein. The activity of the material recovered after reconstitution under these conditions is undiminished.

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