



STREPTAVIDIN-PLUS™

SPECIFICATIONS

Product Code: SA20
Specific activity: ≥14.0 U/mg
Contains ~0.9 mg protein/mg lyophilizate (balance is sodium chloride).
Shipped with ice pack for next day delivery. Store at -20°C.
Stability: Streptavidin-*plus* is stable as a lyophilizate for at least one year when stored desiccated at -20°C.

ProZyme Streptavidin-*plus* is manufactured by a proprietary process and has somewhat different properties from ProZyme's SA10 Streptavidin. In particular, passive adsorption to polystyrene is enhanced, and thermal stability of the protein may be improved. Streptavidin-*plus* retains the high specific activity of ProZyme's SA10 Streptavidin.

Extinction Coefficient: $E_{280}^{1\%} = 32$ (estimate)

Origin: USA

ASSAY

The biotin-binding activity of streptavidin is determined using a modification of the dye-binding assay of Green (1970). One unit will bind one microgram 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 5-10 mg/ml in de-ionized water.

Procedure

Adjust spectrophotometer to read at 500 nm.

To two tubes labeled A and B add as follows:

	A	B
Streptavidin- <i>plus</i> sample	0.05 ml	0.05 ml
Phosphate Buffer	0.5 ml	0.5 ml
HABA stock	0.1 ml	0.1 ml
Biotin stock	--	0.25 ml
H ₂ O	0.35 ml	0.1 ml
Total Volume	1.0 ml	1.0 ml

After mixing, zero the spectrophotometer with water and read the absorbances in tubes A and B.

Calculation

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

where:

M = formula weight of d-biotin (244 g/mole)

V = volume of assay in liters (.001 liters)

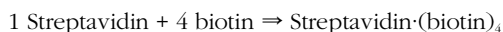
v = volume of streptavidin sample in milliliters (.05 ml as written)

C = concentration of streptavidin in 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 *vs* spectrophotometric). For comparison purposes, ProZyme also reports the results of a biotin titration assay.

Biotin-binding capacity:



The formation of the complex is measured at 233 nm.

Reagents

Ammonium Carbonate Buffer:

200 mM $(\text{NH}_4)_2\text{CO}_3$, 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

Adjust spectrophotometer to read at 233 nm.

Dilute the dissolved streptavidin sample to 0.1 mg/ml in Ammonium Carbonate Buffer. Prepare at least 1 ml.

Dilute the 1 mg/ml biotin solution to ~0.1 mg/ml in Ammonium Carbonate Buffer. Prepare at least 1 ml.

Add 1 ml of 0.1 mg/ml Streptavidin sample to cuvette. Take initial reading of unbound streptavidin.

Add diluted biotin (0.1 mg/ml) in increments of 3 μl . Mix carefully after each addition and take absorbance readings.

Continue adding biotin until absorbance readings plateau or decline repeatedly.

Graph titration curve as absorbance (ordinate) *vs.* volume of biotin added (abscissa). Note the volume (μl) of biotin added at the inflection point of the curve.

The amount of biotin at the inflection point is divided by the concentration of the streptavidin sample to yield the specific activity.

Calculation

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

where:

V_1 = vol 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, 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. If kept refrigerated, an antimicrobial agent such as 0.05% sodium azide should be added to retard growth and subsequent proteolysis from exogenous enzymes.

Solubility

Bayer (1989) 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.

ProZyme Streptavidin-*plus* has been lyophilized from a dilute sodium chloride solution at mildly alkaline pH in order to minimize aggregate formation. This material is readily soluble in water or salt-containing buffers, up to 50 mg/ml or more.

However, in rare cases Streptavidin-*plus* 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 re-thawing. 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.

REFERENCES

- Bayer, E. A., Ben-Hur, H., Hiller, Y. and M. Wilchek. Postsecretory Modifications of Streptavidin. **Biochem J** **259**: 369–376 (1989).
- Green, N. M. Spectrophotometric Determination of Avidin and Biotin, in **Meth Enzymol 18** (McCormick, D. B. and L. D. Wright, eds.) Academic Press, New York, p. 418 (1970).



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