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Protocol

Biotin Binding Assay

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

Bayer et al. (1989)[2] reports that streptavidin may form aggregates under certain conditions. Streptavidin is highly soluble under alkaline conditions (pH > 8.5). Streptavidin is often supplied lyophilized. Under these conditions, there is a tendency for the material to aggregate if it is redissolved in water or other low ionic strength buffers at neutral or acidic pH. As a convenience to customers, Streptavidin has been lyophilized from a dilute sodium chloride solution at a mildly alkaline pH. This material is readily soluble in water. The activity of the material recovered after reconstitution under these conditions is undiminished. We recommend dissolving streptavidin in de-ionized water or, preferably, 1.0 mM sodium bicarbonate buffer (pH 9) at twice the desired final protein concentration. The protein may then be diluted with an equal volume of 2x buffer to produce a stock solution. Upon standing some turbidity may develop in certain buffers. Centrifugation will usually yield a clear solution with negligible loss of streptavidin.

Reagents Required

Product	Preparation	Item No.
0.002 M d-biotin in 0.1 M sodium phosphate, pH 7.0		
0.2 M sodium phosphate, pH 7.0	Dissolve in 0.01 M sodium hydroxide (HABA)	
Streptavidin	Dissolve at 5–10 mg/mL in de-ionized water. If the sample has a concentration outside this range, adjust the volume of the sample in the assay accordingly.	S000-01

Procedure for Cell Lysis

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

Label	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 absorbances in tubes A and B.
4. Calculations:

(106 µg/g)(A-B)MV 141(A-B) Units/mg = _____ = _____ E(Cv) C

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

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 sample (mg/mL)

E = Net molar extinction coefficient of HABA-streptavidin complex at 500 nm (34,500 M-1)

References

- Green, N. M. (1970). Spectrophotometric determination of avidin and biotin. Methods in Enzymology
- Bayer, E.A., Ben-Hur, H., Hiller, Y., & Wilchek, M. (1989). Postsecretory modifications of streptavidin. The Biochemical journal