



## Biotin Binding Assay Protocol

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.

### I. Reagents Required

Reagent	Preparation
0.01 M 2-(4'-Hydroxyazobenzene) Benzoic Acid	Dissolve in 0.01 M sodium hydroxide (HABA).
0.2 M Sodium Phosphate, pH 7.0	N/A
0.002 M d-biotin in M Sodium Phosphate, pH 7.0	N/A
Streptavidin	Dissolve at 5-10 mg/mL in deionized water. If the sample has a concentration outside this range, adjust the volume of sample in the assay accordingly.

### II. Procedure

1. Adjust spectrophotometer to read at 500 nm.
2. Label two tubes A and B 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 <sub>2</sub> O	0.35	0.1
<b>Total Volume</b>	<b>1.0</b>	<b>1.0</b>

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

$$(106 \text{ mg/g})(A-B)MV 141(A-B) \text{ Units/mg} = \frac{\text{Absorbance}}{E} = \frac{E(Cv)C}{E}$$

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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<sup>-1</sup>)

### III. Suggestions for Use

Bayer (1989) 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 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 deionized water or, preferably, 1.0 mM sodium bicarbonate buffer (pH 9) at twice the desired final protein concentration. Then protein may 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.

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