Amplite™ Colorimetric Biotin Quantitation Kit

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 5522 (200 assays)	Keep at ≤ -15°C	Absorbance microplate readers

Introduction

The avidin/streptavidin-biotin interaction is the strongest known non-covalent biological interaction (Kd = 10-15 M^{-1}) between a protein and its ligand. One avidin binds four biotins. The bond formation between biotin and avidin/streptavidin is very rapid and, once formed, is unaffected by pH, organic solvents and other denaturing agents. The tight and specific binding of biotin and its derivatives to various avidins has been extensively explored for a number of biological applications. Our Amplite™ Colorimetric Biotin Quantitation Kit provides a convenient method for estimating the molar ratio of biotin to protein in biotin-protein conjugates or for quantitating biotin concentration in a solution. The assay uses HABA (4'-hydroxyazobenzene-2-carboxylic acid), a reagent that shows dramatic spectral changes when bound to avidin. Biotin easily displaces HABA from the HABA/Avidin complex, resulting in a decrease of absorption at 500 nm. The kit provides an optimal ratio of Avidin and HABA, and it is best used to determine biotin concentration in the range from 2 to 16 µM. The assay can be performed in a cuvette or microplate format.

Kit Components

Components	**Amount**
Component A: Avidin	1 vial
Component B: HABA assay buffer	40 mL
Component C: d-biotin	100 µM, 200 µL

Assay Protocol for One 96-Well Plate

Brief Summary

Prepare HABA/Avidin assay mixture (180 µL) → Add test samples (20 µL) → Incubate at room temperature for 5 minutes → Monitor absorbance decrease at 500 nm

Note: Thaw all the kit components to room temperature before starting the experiment.

1. Prepare 100X Avidin stock solution by adding 400 µL ddH2O into the vial of Avidin (Component A), Mix well.
Note: The unused 100X Avidin stock solution should be divided into single use aliquots and stored at -20°C

2. Prepare HABA/Avidin assay mixture by adding 200 µL of 100X Avidin stock solution (from Step 1) into 20 mL of HABA assay buffer (Component B) . Mix the reagent completely.
Note: The unused portion of HABA/Avidin assay mixture might be stored at 4°C up to one week.

3. Quantitate Biotin with a 96-well Microplate
3.1. Add 20 µL each of biotin-containing samples, negative control (ddH2O or the same buffer used to dissolve biotin-containing sample), and positive Control (Component C) into a 96-well white/clear bottom microplate as described in Tables 1 and 2.

Table 1 Layout of biotin-containing test samples, negative or positive controls in a white/clear bottom 96-well microplate

NC	NC	TS	TS	….	….						
PC	PC	….	….	….	….						
TS	TS										
….	….										
….	….										
….	….										
….	….										
….	….										

Note: NC= Negative Control, PC=Positive Control, TS=Test Samples.

Table 2 Reagent composition for each well

Positive Control	Negative Control	Test Sample*
Component C: 20 µL	ddH2O: 20 µL	20 µL

**Note1: It is necessary to test the biotin-containing samples at several dilutions to ensure that the concentration of biotin is within the assay linear range, 2-16 µM of biotin (final concentration).*
Note 2: Avoid buffers containing potassium, as it will cause precipitation in the assay.
Note 3: Free biotin must be separated from the biotinylated protein by gel filtration or dialysis.

3.2 Add 180 µL of HABA/Avidin assay mixture (from Step 2) into each well of the biotin-containing samples, negative control, and positive control (see Step 3.1) to make the total biotin assay volume of 200 µL/well.
Note: For a Cuvette Format, add 100 µL sample with 900 µL HABA/Avidin assay mixture.

3.3 Incubate the reaction mixture at room temperature for 5 minutes by shaking on a plate shaker at 100-200 rpm, protected from light and avoid creating bubbles during pipetting.

3.4 Monitor the absorbance decrease with an absorbance plate reader at 500 nm.

Data Analysis

1. ΔA_{500nm} = $A_{500\ nm}$ of negative control - $A_{500\ nm}$ of Biotin sample or positive control
2. Biotin concentration (M) = [ΔA_{500nm} / (34,500 x 0.5)] x dilution factor

   Note: $\varepsilon_{HABA}/Biotin=34{,}500\ M^{-1}cm^{-1}$
3. Protein concentration (M) = protein concentration (mg/mL)/molecular weight of protein
4. Molar ratio of biotin to protein = Biotin concentration (M)/Protein Concentration (M)

References

1 Zu Y, Zhang Y, Zhao X, Zhang Q, Liu Y, Jiang R. (2009) Optimization of the preparation process of vinblastine sulfate (VBLS)-loaded folate-conjugated bovine serum albumin (BSA) nanoparticles for tumor-targeted drug delivery using response surface methodology (RSM). Int J Nanomedicine, 4, 321.

2 Enomoto H, Li CP, Morizane K, Ibrahim HR, Sugimoto Y, Ohki S, Ohtomo H, Aoki T. (2008) Improvement of functional properties of bovine serum albumin through phosphorylation by dry-heating in the presence of pyrophosphate. J Food Sci, 73, C84.

3 Ledesma-Osuna AI, Ramos-Clamont G, Vazquez-Moreno L. (2008) Characterization of bovine serum albumin glycated with glucose, galactose and lactose. Acta Biochim Pol, 55, 491.

4 Wang JH, Wang HQ, Zhang HL, Li XQ, Hua XF, Cao YC, Huang ZL, Zhao YD. (2007) Purification of denatured bovine serum albumin coated CdTe quantum dots for sensitive detection of silver(I) ions. Anal Bioanal Chem, 388, 969.

5 Zheng C, Bi J, Ma G, Su Z. (2007) Polyethylene glycol improves conjugation of bovine hemoglobin and human serum albumin in a controlled ratio. Artif Cells Blood Substit Immobil Biotechnol, 35, 568.