Amplite™ Colorimetric Biotin Quantitation Kit

Catalog number: 5522
Unit size: 200 Tests

Component	Storage	Amount
Component A: Avidin	Freeze (< -15 °C), Minimize light exposure	1 vial
Component B: HABA Assay Buffer	Freeze (< -15 °C), Minimize light exposure	1 bottle (40 mL)
Component C: d-biotin	Freeze (< -15 °C), Minimize light exposure	1 vial (200 µL, 100 µM)

OVERVIEW

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. Both avidin and streptavidin have essentially irreversible biotin-binding properties since bound biotin can only be released by denaturing the subunits of the proteins. The tight and specific binding of biotin and its derivatives to various avidins has been extensively explored for a number of biological applications. 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 employs 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 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.

AT A GLANCE

Protocol summary

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

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

KEY PARAMETERS

Absorbance microplate reader

Absorbance	500 nm
Recommended plate	Clear bottom

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

Avidin stock solution(100X)
Add 400 µL ddH_2O 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.

PREPARATION OF WORKING SOLUTION

HABA/Avidin assay solution
Add 200 µL of Avidin stock solution (100X) into 20 mL of HABA Assay Buffer (Component B), and mix them well.

Note The unused portion of HABA/Avidin assay mixture might be stored at 4 °C up to one week.

SAMPLE EXPERIMENTAL PROTOCOL

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

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

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

Table 2. Reagent composition for each well

Positive Control	Negative Control	Test Sample
Compound C: 20 µL	ddH_2O: 20 µL	20 µL

Note 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 Avoid buffers containing potassium, as it will cause precipitation in the assay.

Note Free biotin must be separated from the biotinylated protein by gel filtration or dialysis .

1. Add 20 µL each of biotin-containing samples, negative control (ddH_2O or the same buffer used to dissolve biotin-containing sample), and positive Control (Component C) into a 96-well clear bottom microplate as described in Tables 1 and 2.

2. Add 180 µL of HABA/Avidin assay solution into each well of the biotin-containing samples, negative control, and positive control 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. 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.

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

EXAMPLE DATA ANALYSIS AND FIGURES

1. $\Delta A_{500nm} = A_{500\ nm}$ of negative control - $A_{500\ nm}$ of Biotin sample or positive control
2. Biotin concentration (M) = $[\Delta A_{500nm} / (34{,}500 \times 0.5)] \times$ dilution factor X 10

Note ε_{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)

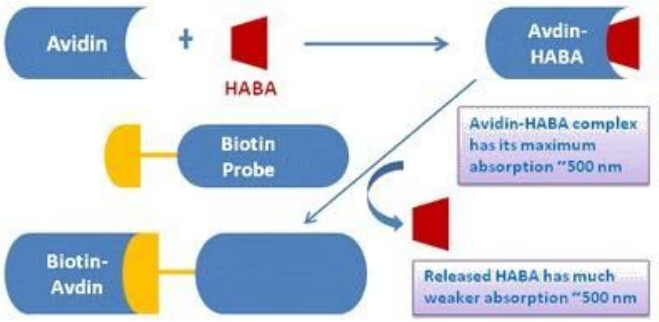

Figure 1.
HABA Assay principle for quantifying biotinylation degree.

DISCLAIMER

AAT Bioquest provides high-quality reagents and materials for research use only. For proper handling of potentially hazardous chemicals, please consult the Safety Data Sheet (SDS) provided for the product. Chemical analysis and/or reverse engineering of any kit or its components is strictly prohibited without written permission from AAT Bioquest. Please call 408-733-1055 or email info@aatbio.com if you have any questions.