HABA Biotin Quantitation Kit
Colorimetric

Catalog #	72096-500
Unit Size	1 Kit
Kit Size	100 Cuvette Assays or 500 Microplate Assays

This kit is optimized to quantitate the biotin to protein molar ratio in biotinylated proteins. Ample materials are provided to perform 100 assays in cuvette format or 500 assays in a 96-well format. The kit has the following features:

- ***Convenient Format:*** All essential assay components are included.
- ***Optimized Performance:*** Optimal conditions for the quantitation of biotin.
- ***Enhanced Value:*** Less expensive than the sum of individual components.
- ***High Speed:*** Minimal hands-on time.
- ***Assured Reliability:*** Detailed protocol and references are provided.

USA and Canada Ordering Information

Deleted: -

AnaSpec Corporate Headquarter
2149 O'Toole Ave.
San Jose, CA 95131
Toll-Free: 800-452-5530
Tel: 408-452-5055
Fax: 408-452-5059
Email: service@anaspec.com
Website: www.anaspec.com

International Ordering Information

A list of international distributors is available at www.anaspec.com.

Version 1.1

INTRODUCTION

Biotin and avidin (or streptavidin) bind non-covalently with a higher binding affinity than most antigen-antibody interactions. This very tight binding makes labeling proteins with biotin a useful tool for applications such as affinity chromatography and immunoanalytical methods. Reaction conditions for biotinylation are chosen such that the target molecule (e.g. an antibody) is labeled with enough biotin residues to purify or detect the molecule, but not so much that the biotin interferes with the function of the molecule.

The HABA Biotin Quantitation Kit provides a convenient method to estimate the molar ratio of biotin to protein on biotinylated conjugates. It also can be used to quantitate the biotin concentration in a solution. The assay utilizes the observation that HABA (4'-hydroxyazobenzene-2-carboxylic acid) shows dramatic spectral changes when it binds to avidin. Free HABA has an absorption peak at 348 nm, while the HABA/avidin complex has strong absorption at 500 nm. Since the affinity between HABA and avidin is relatively weak (K_d=5.8 x 10^{-6} M) compared to the affinity between biotin and avidin (K_d=1 x 10^{-15} M), biotin can easily replace HABA from the HABA/avidin complex, resulting in a decrease of absorption at 500 nm.

Avidin and HABA at an optimal ratio are included in the assay kit. The kit has a linear range 2 to 16 μM. Assay can be performed in cuvette or microplate format.

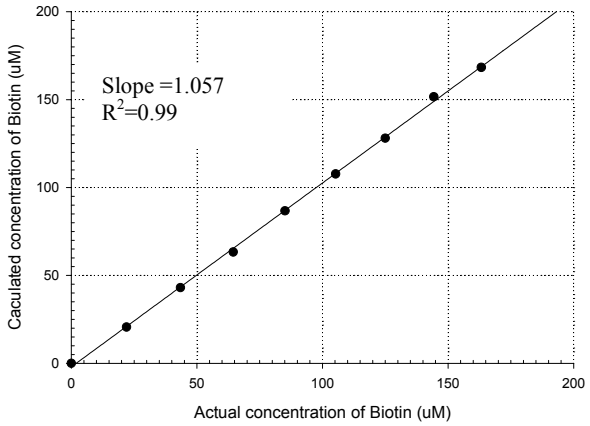

Figure 1. The calculated concentration of biotin is consistent with the actual concentration of biotin.

KIT COMPONENTS, STORAGE AND HANDLING

Note: Store Component A at -20℃ and store the rest of components at 4℃.

Component A: Avidin (4 bottles)

Component B: HABA assay buffer (110 mL)

Component C: d-biotin (100 μM, 1 mL)

OTHER MATERIALS REQUIRED (BUT NOT PROVIDED)

96-well microplate or cuvettes: Clear microplate or cuvettes.

Absorbance microplate reader or spectrophotometer: Capable of detecting absorbance at 500 nm.

PROTOCOL

Protocol A Quantitating Biotin in a Microplate Format

Note 1: It is necessary to test the biotin-containing sample at several dilutions to ensure that the concentration of biotin is within the assay linear range, 2-16 μM of biotin. You can roughly estimate the biotin concentration using the protein concentration of your conjugate solution. For example, biotinylated IgG at concentration 1 mg/mL contains 6.6 μM biotin if the labeling ratio is 1 biotin per 1 IgG molecule. If the conjugate is too diluted, you will need to concentrate the solution in order to accurately measure biotin concentration. Protein solution can be concentrated by using a speed vacuum or a centrifugal filter (Millipore, Cat# 42407).

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 dialysis or gel filtration.

1. **Prepare HABA/Avidin assay mixture.**
   - Add 25 mL of HABA assay buffer (Component B) into one bottle of avidin (Component A). Mix the reagent completely.
     Note: The unused portion of HABA/Avidin assay mixture may be stored at 4℃ up to one week.
2. **Biotin Assay:**
   - Add 180 μL of HABA/Avidin assay mixture per well in a 96-well plate. Besides the wells for test samples, prepare extra wells for negative and positive controls.
   - Add 20 μL of biotin-containing sample into each well.
   - Add 20 μL of deionized water or the same buffer used to dissolve biotin-containing sample into the negative control wells.
   - Add 20 μL of d-biotin (Component C) into the positive control wells.
   - Mix the reagents well by shaking on a plate shaker at 100-200 rpm for 5 min. Avoid creating bubbles during pipetting.
   - Read absorbance at 500 nm.
3. **Data analysis:** Refer to the Data Analysis Section.

Protocol B Quantitating Biotin in a Cuvette Format

Note 1: It is necessary to test the biotin-containing sample at several dilutions to ensure that the concentration of biotin is within the assay linear range, 2-16 μM of biotin. You can roughly estimate the biotin concentration through the protein concentration of your conjugate solution. For example, 1 mg/mL biotinylated IgG contains 6.6 μM biotin if the labeling ratio is 1 biotin per IgG molecule. If the conjugate is too diluted, you will need to concentrate the solution in order to accurately measure biotin concentration. Protein solution can be concentrated by using a speed vacuum or a centrifugal filter (Millipore, Cat# 42407).

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 dialysis or gel filtration.

1. **Prepare HABA/Avidin assay mixture.**
   - Add 25 mL of HABA assay buffer (Component B) into one bottle of avidin (Component A). Mix the reagent completely.
     Note: The unused portion of HABA/Avidin assay mixture may be stored at 4℃ up to one week.
2. **Biotin Assay:**
   - Add 900 μL of HABA/Avidin assay mixture into a cuvette. Read absorbance at 500 nm. Record this reading as $A_{500nm\ of\ HABA/Avidin}$.

- Add 100 µL of biotin-containing sample into the cuvette. Mix the reagents well and avoid creating bubbles. Read absorbance at 500 nm. Record the reading as $A_{500\ nm\ of\ HABA/Avidin/Biotin\ sample}$
- **Positive control**: Add 900 µL of HABA/Avidin assay mixture into a cuvette. Read absorbance at 500 nm. Record this reading as $A_{500nm\ of\ HABA/Avidin}$ for the positive control. Add 100 µL of d-biotin (Component C) into the cuvette. Mix the reagents thoroughly and avoid creating bubbles. Read absorbance at 500 nm. Record the reading as $A_{500\ nm\ of\ HABA/Avidin/Biotin\ sample}$ for the positive control.

3. **Data analysis:** Refer to the Data Analysis Section.

Data Analysis

Calculate data from a microplate format

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

   Note: $\varepsilon_{HABA/Biotin}$=34,500 $M^{-1}cm^{-1}$, Light path= 0.5 cm
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)

Calculate data from a cuvette format

1. $\Delta A_{500nm} = (0.9 \times A_{500\ nm\ of\ HABA/Avidin}) - A_{500\ nm\ of\ HABA/Avidin/Biotin\ sample}$
2. Biotin concentration (M) = $(\Delta A_{500nm} / 34{,}500) \times 10 \times$ dilution factor

   Note: $\varepsilon_{HABA/biotin}$=34,500 $M^{-1}cm^{-1}$, Light path= 1 cm
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)

Positive control

1. Calculate the biotin concentration of the positive control according to the above formulas and then compare it to its actual concentration of 100 µM. The calculated concentration should be very close to the actual concentration if all the procedures and calculations have been correctly performed.