HABA Biotin Quantitation Kit
Colorimetric

Catalog #	71161
Unit Size	1 Kit
Kit Size	100 Cuvette Assays or 500 Microplate Assays

This kit is optimized to quantitate the biotin 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

AnaSpec Corporate Headquarter
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Technical Support
Tel: 408-452-5055
Fax: 408-434-9266
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International Ordering Information

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

Version 1.1

INTRODUCTION

Because of their high affinity for each other, biotin and avidin (or streptavidin) have been widely used in a variety of immunoassays. Numerous proteins, including antibodies, have been conjugated to biotin.

The HABA Biotin Quantitation Kit provides a convenient method to estimate the mole-to-mole 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 phenomenon 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.

Pre-mixed avidin and HABA at an optimal ratio is included in this assay kit. The kit can detect biotin as low as 2 nmole in a sample and has a linear range from 20 to 160 μM. It can be performed in cuvette or microplate format.

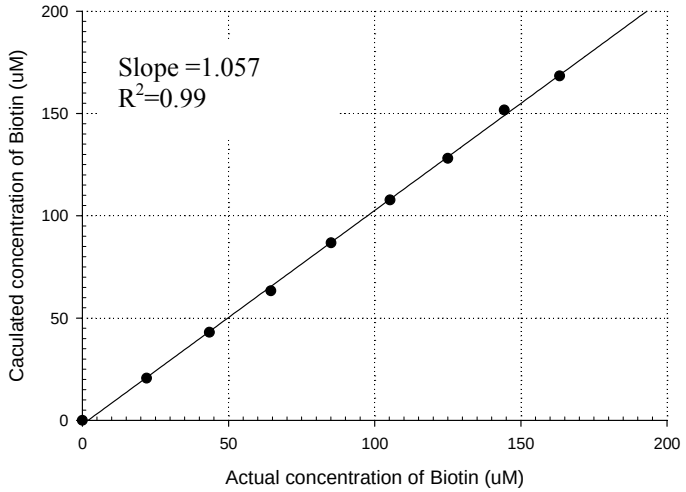

Figure 1. The calculated concentration of biotin is very comparable to the actual concentration of biotin.

KIT COMPONENTS, STORAGE AND HANDLING

Note: Store all kit components at room temperature.

Component A: HABA/Avidin mixture (10 vials)

Component B: 0.1 M sodium phosphate 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.

STANDARD OPERATION PROTOCOL

Protocol A Quantitate Biotin in a Microplate Format

1. **Prepare HABA/Avidin assay mixture.**
   - Add 9 mL of 0.1 M sodium phosphate buffer (component B) into one vial of HABA/Avidin mixture (component A). Dissolve the contents completely.
2. **Biotin Assay:**
   - Add 180 µL of HABA/Avidin assay mixture per well in a 96-well plate. In addition to wells for test samples, prepare two extra wells for negative and positive controls.
   - Add 20 µL of biotin-containing sample into each well. Also add 20 µL of deionized water or the same buffer used to dissolve biotin-contained sample into the negative control well.
   - **(Optional)** Add 20 µL of d-biotin (component C) into the positive control well.
   - Mix the reagents well by shaking on a plate shaker at 100-200 rpm for 30-60 sec.

     Note 1: It is necessary to test the biotin-containing sample at different dilutions to make sure that the concentration of biotin is within the assay linear range.

     Note 2: Avoid buffers containing potassium, as it will cause precipitation in the assay.

     Note 3: Free biotin must be removed from the biotinylated protein by dialysis or gel filtration.
   - Read absorbance at 500 nm.
3. **Data analysis:** Refer to the Data Analysis Section.

Protocol B Quantitate Biotin in a Cuvette Format

1. **Prepare HABA/Avidin assay mixture.**
   - Add 9 mL of 0.1 M sodium phosphate buffer (component B) into one vial of HABA/Avidin mixture (component A). Dissolve the contents completely.
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.
   - (Optional) Positive control: Add 100 µL of d-biotin (component C) instead of test sample into the cuvette. Mix the reagents well.

     Note 1: It is necessary to test the biotin-containing sample at different dilutions to make sure that the concentration of biotin is within the assay linear range.

     Note 2: Avoid buffers containing potassium, as it will cause precipitation in the assay.

     Note 3: Free biotin must be removed from the biotinylated protein by dialysis or gel filtration.
   - Read absorbance at 500 nm. Record this reading as $A_{500nm\ of\ HABA/Avidin/Biotin\ sample}$.
3. **Data analysis:** Refer to the Data Analysis Section.

Data Analysis

Calculate data from a microplate format

1. $\Delta A_{500nm} = A_{500\ nm\ of\ HABA/Avidin/Biotin\ sample} - A_{500\ nm\ control\ well}$
2. Biotin concentration (M) = [ΔA_{500nm} / (34,500 x 0.5)] x 10 x dilution factor
3. Protein concentration (M) = protein concentration (mg/mL) / molecular weight
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) = (ΔA_{500nm} / 34,500) x 10 x dilution factor
3. Protein concentration (M) = protein concentration (mg/mL) / molecular weight
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 above formulae 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.