QuantiChrom™ Biotin Assay Kit (DBIO-100)

Quantitative Colorimetric Determination of Biotin

DESCRIPTION

BIOTIN, or Vitamin B_7, is a water soluble vitamin involved in metabolism, cell growth, and protein synthesis. It is a cofactor to multiple carboxylases necessary for metabolizing fatty acids, glucose, and amino acids. Biotin is found in a wide range of foods and is often taken as a dietary supplement. In the biotechnology industry, biotin is commonly conjugated to proteins in a process called biotinylation. These biotinylated proteins can then be specifically selected using streptavidin and/or avidin's strong affinity for biotin in biochemical assays such as ELISAs.

BioAssay Systems' Biotin Assay Kit is based on avidin's weak affinity for 4'-hydroxyazobenzene-2-carboxylic acid (HABA) and strong affinity for biotin. The avidin-HABA complex reagent has an absorbance at 500nm. When the colored avidin-HABA reagent is introduced to biotin, the biotin binds to avidin, displacing the HABA and causing a decrease in absorbance. The decrease in absorbance at 500nm is directly proportional to the biotin in the sample.

KEY FEATURES

Fast and sensitive. Linear detection range: 8 to 200 µM biotin with 30 µL sample (96-well) or 10 to 200 µM biotin with 10 µL sample (384-well).

Convenient. The procedure involves adding a single working reagent, and reading the absorbance after 20 minutes.

High-throughput. "Add-mix-read" type assay. Can be readily automated as a high-throughput 96-well or 384-well plate assay for thousands of samples per day.

APPLICATIONS

Direct Assays: Biotin in foods, cosmetic products, supplements, and biotinylated proteins or antibodies.

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Reagent A:	35 mL	**Standard:**	120 µL (10 mM Biotin)
Reagent B:	1.8 mL		

Storage conditions. The kit is shipped at RT. Store all components at -20°C upon receiving. Shelf life: 12 months after receipt.

Precautions: Reagents are for research use only. Briefly centrifuge Standard tube before opening. Equilibrate all components to room temperature prior assay. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

Samples: Samples should be clear, precipitate free, and < 10 mg/mL protein. For samples with precipitates, centrifuge 5 min at 14,000 rpm and use supernatant for assay. Dilute samples that have > 10 mg/mL protein with water.

PROCEDURES

Procedure using 96-well plate (Sufficient for 100 tests)

1. *Standards*. Prepare 500 µL of 200 µM Premix by mixing 10 µL of the 10 mM Standard and 490 µL of the dH_2O. Dilute standards in 1.5-mL centrifuge tubes as described in the Table.

No	Premix + dH_2O	Biotin (µM)
1	100 µL + 0 µL	200
2	60 µL + 40 µL	120
3	30 µL + 70 µL	60
4	0 µL + 100 µL	0

2. Transfer 30 µL of standards into separate wells of a clear, flat-bottom 96-well plate. Transfer 30 µL of each sample into separate wells.
3. Prepare sufficient Working Reagent (WR) by mixing, for each well: 300 µL Reagent A and 15 µL Reagent B.
4. Add 300 µL Working Reagent to the *four Standards* and the *Sample Wells.* Tap plate to mix briefly and thoroughly. Incubate 20 min at room temperature.
5. Read optical density at 500 nm (470-520 nm).

Procedure using 384-well plate (Sufficient for 320 tests)

1. *Standards*. Prepare standards as described in step #1 of the 96-well plate procedure.
2. Transfer 10 µL of standards into separate wells of a clear, flat-bottom 384-well plate. Transfer 10 µL of each sample into separate wells.
3. Prepare sufficient Working Reagent (WR) by mixing, for each well: 100 µL Reagent A and 5 µL Reagent B.
4. Add 100 µL Working Reagent to the *four Standards* and the *Sample Wells.* Tap plate to mix briefly and thoroughly. Incubate 20 min at room temperature.
5. Read optical density at 500 nm (470-520 nm).

CALCULATION

Subtract the standard values from the blank value (#4) and plot the ΔOD against standard concentrations. Determine the slope and calculate the biotin concentration of Sample as follows:

$$[\text{Biotin}] = \frac{OD_{BLANK} - OD_{SAMPLE}}{\text{Slope } (\mu M^{-1})} \times n \quad (\mu M)$$

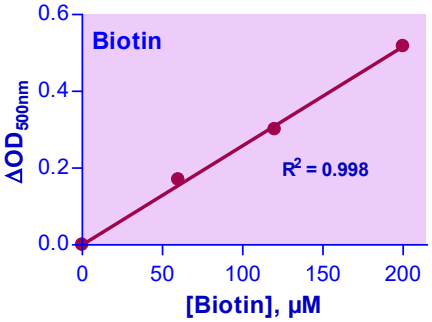
OD_{BLANK} and OD_{SAMPLE} are optical density readings of the Blank (#4) and Sample, respectively.

Note: If the calculated biotin concentration of a sample is higher than 200 µM, dilute sample in water and repeat the assay. Multiply result by the dilution factor *n*.

Conversions: 1 µM Biotin equals 24.43 µg/dL, or 244.3 ppb.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices, centrifuge tubes, clear flat-bottom 96-well or 384-well plates (e.g. VWR cat# 82050-760 or VWR cat # 82051-298), and plate reader.



Biotin Standard Curve in dH_2O (96-well plate)

LITERATURE

1. Yang, W., et al (2009). Targeting cancer cells with biotin–dendrimer conjugates. Eur. J. Med. Chem. 44(2): 862-868.
2. Tong, L. (2013). Structure and function of biotin-dependent carboxylases. Cell. Mol. Life Sci. 70(5): 863-891.
3. Lesch, H.P., et al (2010). Avidin-biotin technology in targeted therapy. Expert Opin. Drug Deliv. 7(5): 551-564.