

Description

Biotin, or vitamin B7, is a water-soluble vitamin involved in metabolism, cell growth and protein synthesis. It is a cofactor to multiple carboxylases necessary for metabolising 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 or avidin's strong affinity for biotin in biochemical assays such as ELISAs. This kit is based on avidin's weak affinity for HABA and strong affinity for biotin. The avidin-HABA complex reagent has an absorbance at 500 nm; when the coloured reagent is introduced to biotin, the biotin binds to avidin, displacing the HABA and causing a decrease in absorbance that is directly proportional to the biotin in the sample.

Specifications

Detection Method	Colorimetric (OD 500 nm, 470-520 nm)
Detection Range	8 - 200 uM biotin (30 uL sample, 96-well) or 10 - 200 uM biotin (10 uL sample, 384-well)
Sample Type	Foods, cosmetic products, supplements, biotinylated proteins and antibodies
Species Reactivity	All
Assay Time	20 minutes
Kit Size	100 Assays
Equipment Required	Microplate reader
Storage	Shipped at room temperature. Store all components at -20 degrees C upon receipt.
Shelf Life	12 months after receipt
Shipping	Room Temperature

Kit Components

Component	Quantity	Storage
Reagent A	35 mL	-20 degrees C
Reagent B	1.8 mL	-20 degrees C
Standard (10 mM Biotin)	120 uL	-20 degrees C

Ordering Information

Assay Genie Cat. No.	Pack Size
BA0211	100 Assays

Assay Procedure

Note: This is a sample protocol. Protocols are batch/lot specific - always follow the instructions supplied with your kit.

1. Prepare 500 uL of 200 uM Premix by mixing 10 uL of the 10 mM Standard and 490 uL of water, then dilute the standards in 1.5-mL centrifuge tubes as described in the standard table (200, 120, 60 and 0 uM).
2. Transfer 30 uL of each standard into separate wells of a clear, flat-bottom 96-well plate and 30 uL of each sample into separate wells.
3. Prepare sufficient Working Reagent (WR) by mixing, for each well, 300 uL of Reagent A and 15 uL of Reagent B.

4. Add 300 uL of Working Reagent to the four standards and the sample wells, tap the plate to mix briefly and thoroughly, and incubate for 20 minutes at room temperature.
5. Read the optical density at 500 nm (470-520 nm).
6. For the 384-well procedure, prepare standards as above, transfer 10 uL of standards and samples into wells, add 100 uL of Working Reagent (100 uL Reagent A and 5 uL Reagent B per well), incubate 20 minutes and read at 500 nm.

Standard Dilution

No	Premix	+ dH2O	Biotin (uM)
1	100 uL	0 uL	200
2	60 uL	40 uL	120
3	30 uL	70 uL	60
4	0 uL	100 uL	0

Calculation

Subtract the standard values from the blank value (#4) and plot the delta-OD against standard concentrations. Determine the slope and calculate the biotin concentration of a sample as: $[Biotin] = [(OD_BLANK - OD_SAMPLE) / Slope (per\ uM)] \times n (uM)$, where OD_BLANK and OD_SAMPLE are the optical density readings of the blank (#4) and sample respectively, and n is the dilution factor. If the calculated biotin concentration is higher than 200 uM, dilute the sample in water and repeat the assay. Conversions: 1 uM biotin equals 24.43 ug/dL or 244.3 ppb.

Notes

- Briefly centrifuge the Standard tube before opening and equilibrate all components to room temperature prior to the assay.
- Samples should be clear, precipitate-free and less than 10 mg/mL protein. For samples with precipitates, centrifuge for 5 minutes at 14,000 rpm and use the supernatant. Dilute samples that have more than 10 mg/mL protein with water.
- Reagents are for research use only. Refer to the MSDS for detailed safety information.

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Technical specifications are based on manufacturer documentation and may be updated. Protocols shown are summaries - always follow the instructions supplied with your kit/lot.