Amplite® Rapid Colorimetric Biotin Quantitation Kit *Optimized to Use with Nanodrop*

Catalog number: 5537
Unit size: 100 tests

Component	Storage	Amount (Cat No. 5537)
Component A: Avidin	Freeze (< -15 °C), Minimize light exposure	1 Vial
Component B: HABA Assay Buffer	Freeze (< -15 °C), Minimize light exposure	2 Vials (1mL/Vial)
Component C: d-Biotin	Freeze (< -15 °C), Minimize light exposure	1 Vial (200 µL, 100 µM)

OVERVIEW

Amplite™ Colorimetric Biotin Quantitation Kit is designed to quantify the number of biotins attached to a protein or other macromolecule with a Nanodrop Spectrometer. The kit uses HABA (4'-hydroxyazobenzene-2-carboxylic acid) reagent that demonstrates a dramatic spectral change when bound to avidin. Biotin easily displaces HABA from the HABA/avidin complex, resulting in a decrease of absorption at 500 nm. By measuring the absorbance change before and after the addition of the biotin-containing sample at 500 nm, the amount of biotin in a sample can be quantified spectrophotometrically. The kit is best used to determine biotin concentration in the range from 2 to 16 µM. It is optimized to use with Nanodrop spectrometer. Biotin is a relatively small molecule that is routinely conjugated to antibodies and proteins with minimal interference of their biological activity. The avidin/streptavidin-biotin interaction is the strongest known binding pair between a protein and its ligand. The biotin-avidin interaction has been extensively explored for a variety of biological applications.

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

100X Avidin Stock Solution

1. To prepare a 100X Avidin stock solution, add 40 µL of ddH2O into the vial of Avidin (Component A), and mix thoroughly.

   Note: Any unused 100X Avidin stock solution should be divided into single-use aliquots and stored at -20°C.

PREPARATION OF WORKING SOLUTION

HABA/Avidin Working Solution

1. To prepare a HABA/avidin working solution, add 2 µL of the 100X Avidin stock solution into 200 µL of the HABA assay buffer (Component B), and mix thoroughly.

   Note: Any unused HABA/avidin working solution can be store for up to one week at 4°C.

SAMPLE EXPERIMENTAL PROTOCOL

Quantitate Amount Biotin with a Nanodrop

1. Prepare the following controls and samples as outlined in the table below:

Positive Control	Negative Control	Test Sample*
d-Biotin (Component C): 2 µL	ddH2O: 2 µL	2 µL
HABA/Avidin working solution: 18 µL	HABA/Avidin working solution: 18 µL	HABA/Avidin working solution: 18 µL

   Note: To ensure the concentration of biotin falls within the assay's linear range (2-16 µM of biotin final concentration), it is necessary to test the biotin-containing samples at several dilutions.

   Note: To ensure assay accuracy, avoid using buffers containing potassium, as it causes precipitation.

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

2. Incubate the reaction mixture at room temperature for 5 minutes, protected from light. Avoid creating bubbles during pipetting.

3. Monitor absorbance at 500 nm using a Nanodrop Spectrometer, with a sample measurement of 2~2.5 µL. Utilize the protein label function for readings.

DATA ANALYSIS - CALCULATIONS

1. Calculate the change of absorbance at 500 nm:

ΔA_{500} = A_{500} of negative control - A_{500} of Biotin sample or positive control

2. Calculate the biotin concentration (M):

$$M = [\Delta A_{500} \div \varepsilon_{HABA/Biotin}] \times DF$$

Where:

- $\varepsilon_{HABA/Biotin}$ = 34,500 $M^{-1}cm^{-1}$
- DF = Dilution Factor

3. Calculate protein concentration (P)

P = protein concentration (mg/mL) ÷ molecular weight of protein

4. Calculate molar ratio of biotin to protein (MR)

$$MR = M/P$$

Where:

- M = Biotin Concentration
- P = Protein Concentration

DISCLAIMER

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