

Product Information

Colorimetric Biotin Assay Kit

Catalog Number **MAK171**

Storage Temperature -20°C

TECHNICAL BULLETIN

Product Description

Biotinylation is the process of labelling a molecule with a biotin molecule to take advantage of the strong binding and high specificity of biotin and streptavidin for purification or detection applications. Biotinylation is typically used to label proteins or nucleic acid based targets for these applications. Quantitation of biotinylation can be used to optimize the labelling of a given target. Levels of biotinylation can affect detection sensitivity or functionality of a purification product.

This Colorimetric Biotin Quantitation Kit provides a convenient method for estimating the molar ratio of biotin to protein in biotin-protein conjugates or for quantitating biotin concentration in a solution. The assay utilizes HABA [2-(4-Hydroxyphenylazo)benzoic acid], a reagent that shows dramatic spectral changes when bound to avidin. Biotin easily displaces HABA from the HABA/Avidin complex, resulting in a decrease of absorption at 500 nm.

The kit provides an optimal ratio of Avidin and HABA, and it is best used to determine biotin concentration in the range from 2–16 μM . The assay can be performed in a cuvette or microplate format.

Components

The kit is sufficient for 200 assays in 96 well plates.

Avidin	1 ea
Catalog Number MAK171A	
HABA Assay Buffer	40 mL
Catalog Number MAK171B	
100 μM D-Biotin Solution	0.2 mL
Catalog Number MAK171C	

Reagents and Equipment Required but Not Provided

- 96 well flat-bottom plate – It is recommended to use clear plates for colorimetric assays.
- Spectrophotometric multiwell plate reader

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on dry ice and storage at -20°C , protected from light, is recommended.

Preparation Instructions

Allow reagents to come to room temperature and briefly centrifuge vials before opening. To maintain reagent integrity, avoid repeated freeze/thaw cycles. Use ultrapure water for the preparation of reagents.

Prepare 100 $\times$ Avidin Stock Solution by adding 400 μL of ultrapure water into the vial of Avidin (MAK171A) and mixing well.

Note: The unused 100 $\times$ Avidin Stock Solution should be divided into single use aliquots and stored at -20°C .

Prepare HABA/Avidin Assay Mixture by adding 200 μL of 100 $\times$ Avidin Stock Solution to 20 mL of HABA Assay Buffer (MAK171B). Mix the reagent completely.

Note: The unused portion of HABA/Avidin Assay Mixture may be stored at 2–8 $^{\circ}\text{C}$ for up to one week.

Procedure

All samples and standards should be run in duplicate.

Sample Preparation

Add 20 μL of each of biotin-containing sample, negative control (ultrapure water or the buffer used to dissolve biotin-containing sample), and positive Control (MAK171C) into the appropriate wells of a 96 well white/clear bottom microplate.

Notes: It is necessary to test the biotin-containing samples at several dilutions to ensure the concentration of biotin is within the assay linear range, 2–16 μM of biotin (final concentration).

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

Free biotin must be separated from the biotinylated protein sample by gel filtration or dialysis prior to analysis.

Assay Reaction

1. Add 180 μL of HABA/Avidin Assay Mixture into each well of the biotin-containing samples, negative control, and positive control to make the total biotin assay volume of 200 μL /well.

Notes: Avoid creating bubbles during pipetting.

For a cuvette assay, add 100 μL of sample with 900 μL of HABA/Avidin Assay Mixture.

2. Incubate the reaction mixture at room temperature for 5 minutes by shaking on a plate shaker at 100–200 rpm, protected from light.
3. Monitor the absorbance decrease with an absorbance plate reader at 500 nm (A_{500}).

Results

Calculations

$\Delta A_{500} = A_{500} \text{ negative control} - A_{500} \text{ biotin sample or positive control}$

Biotin Concentration (M) = $\frac{\Delta A_{500} \times \text{dilution factor}}{(34,500 \times 0.5)}$

where:

Molar Extinction Coefficient of HABA/Biotin = $34,500 \text{ M}^{-1} \text{ cm}^{-1}$

Molar ratio of = $\frac{\text{Biotin concentration (M)}}{\text{Protein Concentration (M)}}$

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