# Biotin

# Colorimetric Microplate Assay Kit

# User Manual

## Catalog # CAK1332

(Version 1.1A)

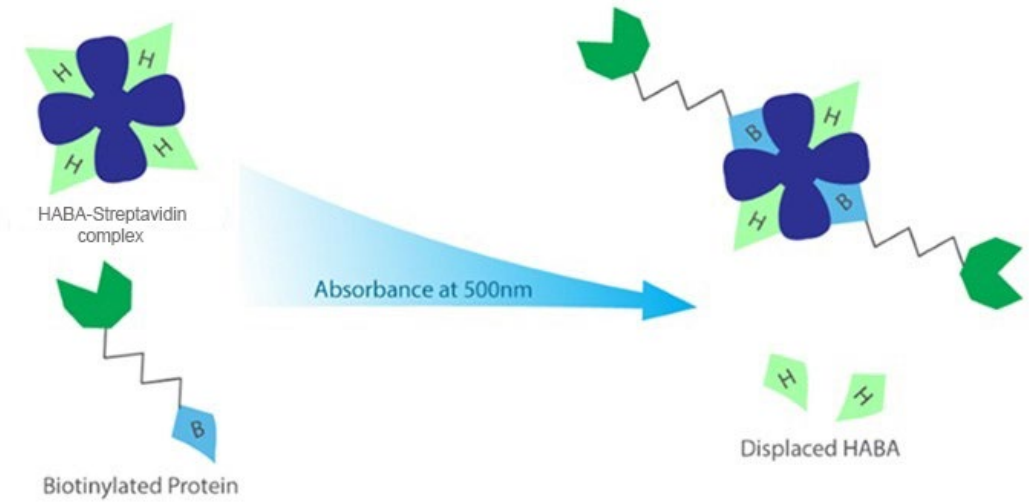
Detection and Quantification of free or bound Biotin Content in Purified Biotinylated Samples.

**For research use only. Not for diagnostic or therapeutic procedures.**

## I. INTRODUCTION

Biotin is a small-molecule vitamin that serves as a critical molecular bridge in life science research due to its exceptionally high affinity for streptavidin. Biotinylation is a versatile modification strategy involving the covalent labeling of antibodies, proteins, or peptides to facilitate target capture, isolation, and detection.

Biotin Colorimetric Microplate Assay Kit provides a convenient tool for quantitative determination of free biotin in solution and the assessment of labeling efficiency in various biotinylated products. This kit employs the HABA dye-displacement method, utilizing changes in absorbance (OD 500 nm) resulting from biotin competitively binding to streptavidin to quantitatively determine free biotin concentrations and the degree of protein biotinylation.



## II. KIT COMPONENTS

| Component | Volume | Storage |
|---|---|---|
| 96-Well Microplate | 1 plate | |
| Reaction Buffer | 30 ml x 1 | 4 °C |
| Streptavidin Solution | 1.0 ml x 2 | -20 °C |
| HABA Solution | 1.5 ml x 1 | -20 °C, keep in dark |
| Standard | Powder x 1 | 4 °C |
| Technical Manual | 1 Manual | |

Note: Avoid freeze-thaw cycles

## III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 500 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer

## IV. REAGENT PREPARATION

**Standard**: Briefly centrifuge prior to opening. Dissolve in 1 ml Reaction Buffer to generate 5 mmol/L of standard stock solution, store at 4 °C for 2-3 months or -20 °C for 6 months after reconstitution. Then dilute to 250 µmol/L standard top solution by adding 50 µl stock solution into 950 µl Reaction Buffer. Perform 2-fold serial dilutions of the top standard solution using distilled water to make the standard curve. The concentration of standard curve could be 250/125/62.5/31.25/ 15.625/15.625/7.813/3.906 µmol/L.

**HABA-Streptavidin Working Solution:** Prepare HABA-Streptavidin Working Solution by mixing Reaction Buffer, Streptavidin Solution and HABA Solution and incubate for 5-10 minutes at room temperature before the test. Store the unused solution in dark at 4°C for at most 3 days in dark. The volumes of reagent could be enlarged according to the specified numbers of well. The table below gives some examples.

| No. of wells | Reaction Buffer (µl) | Streptavidin Solution (µl) | HABA Solution (µl) |
|---|---|---|---|
| 1 | 145 | 20 | 15 |
| 5 | 725 | 100 | 75 |
| 10 | 1450 | 200 | 150 |
| 50 | 7250 | 1000 | 750 |
| 100 | 14500 | 2000 | 1500 |

**Note:** Divide into small aliquots to avoid repeated freeze-thaw cycles.

## V. SAMPLE PREPARATION

1. For detecting Free Biotin only or Protein-bound Biotin in lowly biotinylated samples (proteins with <5-10 biotin/protein), detect directly or diluent with reaction buffer.

2. For detecting Protein-bound Biotin in highly biotinylated samples (proteins with >5-10 biotin/protein), release covalently bound biotin into free biotin that can be quantified using the HABA-streptavidin assay. Digest the sample with Trypsin or other suitable protease (1-5% of the protein) overnight at room temperature. Then, deactivate Trypsin by adding a Trypsin inhibitor or heating at 95°C for several minutes. Detect directly or diluent with reaction buffer.

## VI. ASSAY PROCEDURE

Add following reagents into the microplate:

| Reagent* | Sample** | Control | Standard |
|---|---|---|---|
| HABA-Streptavidin Working Solution | 180 µl | 180 µl | 180 µl |
| Reaction Buffer | -- | 20 µl | -- |
| Sample | 20 µl | -- | -- |
| Standard | -- | -- | 20 µl |
| Mix thoroughly and incubate at room temperate for 4-5 minutes. Measured at 500 nm and record the absorbance. | | | |

**Note:**

*Reagents must be added sequentially and should not be premixed prior to addition.

** OD 500 nm for biotinylated samples should be between ~0.8-0.2. If the absorbance is < 0.2, dilute the sample and then perform the assay. If the absorbance is > 0.8, increase volume of the sample or concentrate the sample and then perform the assay.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

## VII. CALCULATION

1. Calculate the sample concentration in ASSAY PROCEDURE according to the slope of the standard curve

$$C_{Biotin} = \frac{(OD_{Control} - OD_{Sample}) - Intercept}{Slope} \times n \ (\mu mol/L)$$

Calculate the initial concentration according to sample preparation procedure.

2. According to one point of the standard OD and concentration

$$C_{Biotin} = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Control} - OD_{Sample})}{(OD_{Control} - OD_{Standard}) \times V_{Sample}} \ (\mu mol/L)$$

3. Calculate the degree of biotinylation

$$R = \frac{C_{Biotin}}{(C_{Protein}/MW_{Protein}) \times 1000}$$

Slope: the absorbance slope of standard curve

n: the dilution factor

$C_{Standard}$: the biotin concentration in standard, μmol/L

$C_{Biotin}$: the biotin concentration in detected sample, μmol/L

$V_{Standard}$: the volume of standard in assay procedure, μl

$V_{Sample}$: the volume of sample in assay procedure, μl

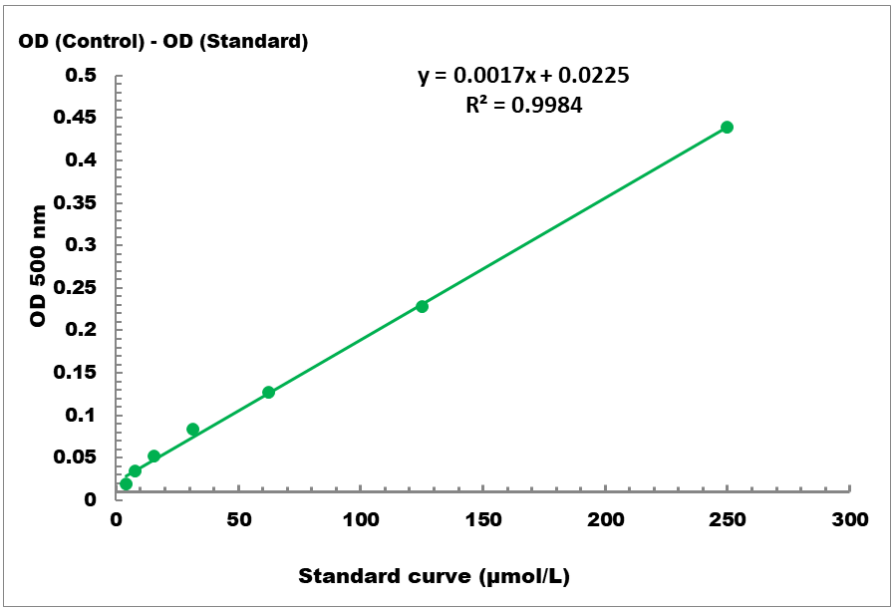
$C_{Protein}$: the sample protein concentration, mg/ml

$MW_{Protein}$: the molecular weight protein concentration, Da

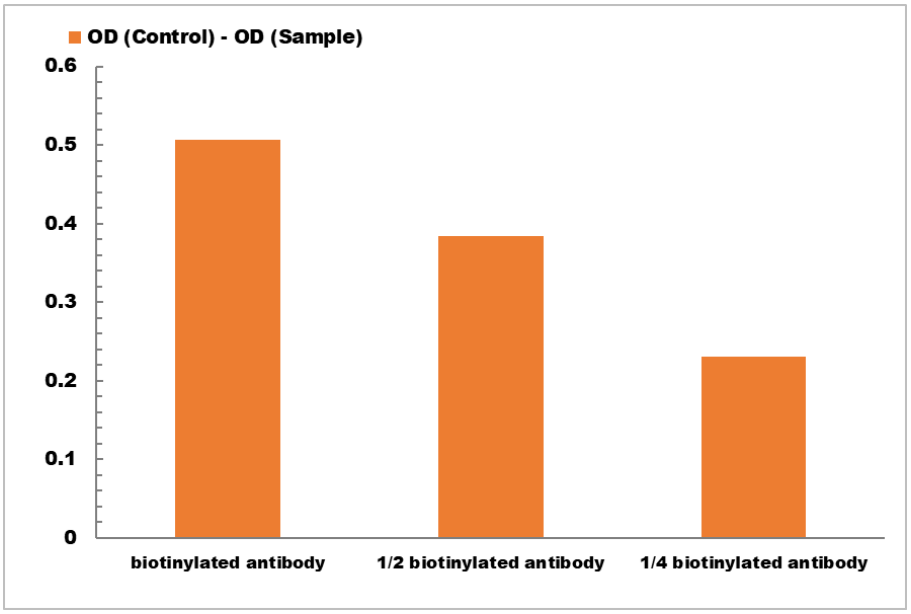
R: Molar ratio of biotin: protein

## VIII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.




Detection Range: 2.5 μmol/L - 250 μmol/L




Determination of Biotin in biotinylated antibody samples