ab185441

Biotin Quantitation Kit (Colorimetric)

Instructions for Use

For the rapid, sensitive and accurate measurement of free biotin or the substitution degree of Biotinylation (biotin labels) of proteins and antibodies.

View kit datasheet: www.abcam.com/ab185441
(use www.abcam.cn/ab185441 for China, or www.abcam.co.jp/ab185441 for Japan)

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Version 3a, Last Updated 22 March 2024

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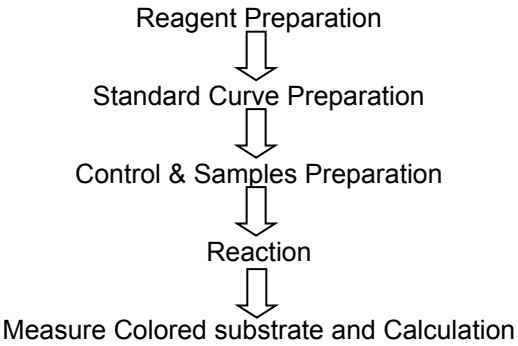
1. Overview

Biotin (Vitamin H) is an important biomolecule that has been widely used in Biotinylation reactions to label antibodies or other proteins of interest. Due to its low molecular weight, biotin usually does not cause any significant changes in protein conformation and biological activity. Biotinylated proteins/antibodies are widely isolated and assayed with streptavidin pull-down assay, affinity chromatography, ELISA and Western blotting etc. It is often desired to determine the degree of substitution of such biotinylated biomolecules.

Abcam's Biotin Quantitation Kit (Colorimetric) (ab185441) is based on the differential binding of Streptavidin to Biotin and a dye, 2-(4-hydroxyazobenzene) benzoic acid (HABA). When HABA is bound to Streptavidin, addition of free biotin or biotinylated biomolecules results in its displacement in the reaction. These changes are manifested in terms of decrease in the overall absorption (OD 500 nm) and can be quantitatively correlated to the amount of biotin present in solution. The kit also provides Biotin as a Standard and Biotinylated BSA as a positive control. This kit can be used to estimate as low as 60 pmol or 15 ng of biotin in solution.

2. Protocol Summary



3. Kits Components

Item	Quantity
Biotin Assay Buffer	25 mL
Biotin Assay Reagent A	8 mL
Biotin Assay Reagent B	1 mL
Biotin Standard/Biotin Standard Solution (10 mM in DMSO)	100 µL
Biotinylated BSA/Biotinylated BSA (2 mg/ml)	200 µL

4. Storage and Stability

Upon arrival, store the kit at-20°C and protect from light. Warm all reagents to room temperature before use. Please read the entire protocol before performing the assay. Avoid repeated freeze/thaw cycles.

Briefly centrifuge all small vials prior to opening.

5. Materials Required, Not Supplied

- Trypsin
- Trypsin Inhibitor
- Distilled water (dH_2O) or MilliQ
- 96-well white plate with flat bottom
- Multi-well spectrophotometer (ELISA reader)
- Centrifuge with cooling option

6. Assay Protocol

1. Sample Preparation

Use 3-5 different amounts of the target protein/antibody samples directly in the assay. Adjust the volume to 30 µL with Biotin Assay Buffer. As a positive control, use 5-25 µL (10-50 µg) of Biotinylated BSA (8-10 biotin/BSA). Adjust the volume to 30 µL with Biotin Assay Buffer.

NOTE:

- *For proteins with >5 biotin/protein, >10 biotin/antibody or highly biotinylated samples, 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 and assay the samples.*

2. Standard Curve Preparation

Prepare Biotin Standards as suggested in the table below by diluting Biotin Standard/Biotin Standard Solution (10 mM) with Biotin Assay Buffer. Other similar dilutions can also be used within the assay range of 10-300 µM. One tube of each of the following Biotin Standards is sufficient to run a Biotin Standard Assay in triplicates.

Tube	Volume of Biotin Solution (µL)	Volume of Biotin Assay Buffer (µL)	Final Biotin Concentration (µM)	Final Biotin Amount (nmol)*
1	10 of standard solution	90	1000	30
2	30 of tube 1	70	300	9
3	20 of tube 1	80	200	6
4	30 of tube 1	270	100	3
5	80 of tube 4	20	80	2.4
6	60 of tube 4	40	60	1.8
7	40 of tube 4	60	40	1.2
8	20 of tube 4	80	20	0.6
9	0	100	0	0

*Amounts of biotin in nmol are calculated based on amount of biotin solutions (30 µL) used in the assay

3. Add 30 µL of Control (tube 9), Biotin Standards (tubes 2-8) and target biotinylated protein sample into microtiter plate wells

4. Reaction Mix:

Prepare enough Reaction Mix for the number of assays to be performed. For each well, prepare 300 µL Mix containing:

	Reaction Mix
Biotin Assay Reagent A	75 µL
Biotin Assay Reagent B	10 µL
Biotin Assay Buffer	215 µL

Add 300 µL of the reaction mix to each well. Mix well.

5. Measurement

Cover the plate and incubate at room temperature for 15 min. Measure absorbance (OD 500 nm).

NOTE:

- *The unused Reaction Mix may be stored at 4°C for up to a week. The color of Reaction Mix should be clear orange and A_{500} of the Blank Sample (tube 9) should be ~0.9. If it is much lower, make fresh Reaction Mix.*
- *A_{500} for biotinylated samples should be between ~0.9-0.25. If the absorbance is < 0.25, dilute the sample and then perform the assay.*

- *If the graph of ΔA_{500} vs increasing amount of biotinylated protein is not linear or the solution turns cloudy, digest the biotinylated protein with an appropriate protease (see sample preparation).*

7. Data Analysis

Calculations:

Subtract all readings from 0 Standard (tube 9). Plot the Biotin Standard Curve. Using the Biotin Standard Curve, calculate the amount of biotin in nmol for samples. For degree of biotinylation substitution, plot the amount of biotin (nmol) vs amount of biotinylated protein (µg) used in the assay. If the graph is linear, calculate degree of Biotinylation (X) as:

$$No.\ of\ Biotin\ per\ molecule\ of\ protein\ (X) = \frac{a\ x\ M}{b\ x\ 1000}$$

Where:

a is the average nmol of biotin (use only the points in the linear range)

M is the molecular weight of the protein (g/mol)

b is the average amount of protein used (use only the points in the linear range)

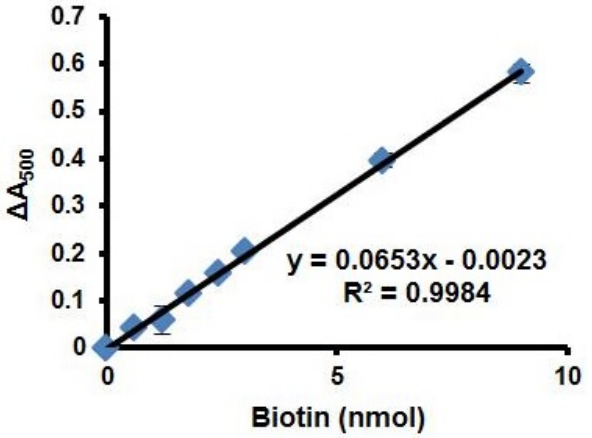


Figure 1. Biotin Standard Curve.

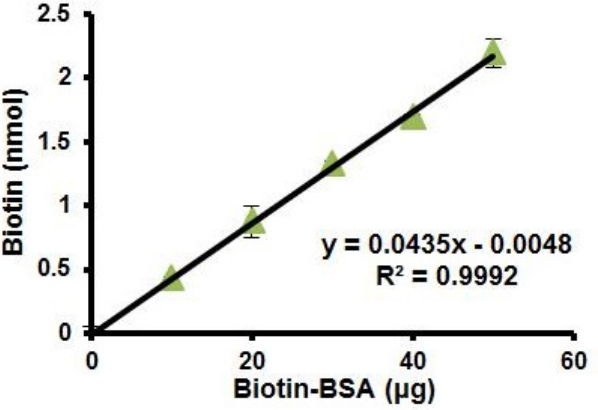


Figure 2. Linear plot obtained for the amount of biotin (nmol) vs amount of biotinylated BSA. Assays were performed according to the kit protocol.

8. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Problem	Reason	Solution
Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Problem	Reason	Solution
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)