Biotin Quantitation Kit (Colorimetric) (#BN01027)

(Catalog # BN01027; 100 assays, Store kit at -20°C)

I. Introduction:

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. Assay Genie offers a variety of ready-to-use Biotinylation reagents. It is often desired to determine the degree of substitution of such biotinylated biomolecules. Assay Genie's Biotin Assay Kit 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.

II. Applications:

Measuring free biotin or the substitution degree of Biotinylation (biotin labels) of proteins and antibodies.

III. Kit Contents:

Components	BN01027	Cap Code	Part Number
Biotin Assay Buffer	25 ml	WM	BN01027-1
Biotin Assay Reagent A	8 ml	NM	BN01027-2
Biotin Assay Reagent B	1 ml	Brown	BN01027-3
Biotin Standard Solution (10 mM in DMSO)	100 µl	Yellow	BN01027-4
Biotinylated BSA (2 mg/ml)	200 µl	Clear	BN01027-5

IV. User Supplied Reagents and Equipment:

- 96-well clear microplate with flat bottom, microplate reader, trypsin and trypsin inhibitor.

V. Storage and Handling:

Store kit at -20°C. Warm all reagents to room temperature before use. Read the entire protocol before performing the assay.

VI. Preparation of Standards & Samples:

- **Preparation of Biotin Standards:**
  Prepare Biotin Standards as suggested in the table below by diluting 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

- **Preparation of Biotinylated Protein Samples**: 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 (3 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.

VII. Biotin Assay Protocol:

1. Add 30 µl of Control (tube 9), Biotin Standards (tubes 2-8) and target biotinylated protein sample into microtiter plate wells.
2. **Reaction Mix**: Prepare enough reagents based on number of assays to be performed (Biotin Standards and protein samples). For each well, prepare 300 µl of Reaction Mix.

	Reagent Mix
Biotin Assay Reagent A	75 µl
Biotin Assay Reagent B	10 µl
Biotin Assay Buffer	215 µl

Mix well. Add 300 µl of the Reaction Mix to each well. Mix well.

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

Note:

(a) 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.

(b) 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.

(c) 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).

4. **Calculations**: Subtract all readings from the 0 Standard reading (Tube 9). Plot the Biotin Standard Curve. Using 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:

$$\textit{No. of}\ \textbf{Biotin per molecule of protein}\ (\mathbf{X}) = \frac{\boldsymbol{a} \times \boldsymbol{M}}{\boldsymbol{b} \times \mathbf{1000}}$$

Where, **a** = Average nmol of biotin (use only the points in the linear range)
M = Molecular weight of the protein (g/mol)
b = Average amount of protein used (use only the points in the linear range)

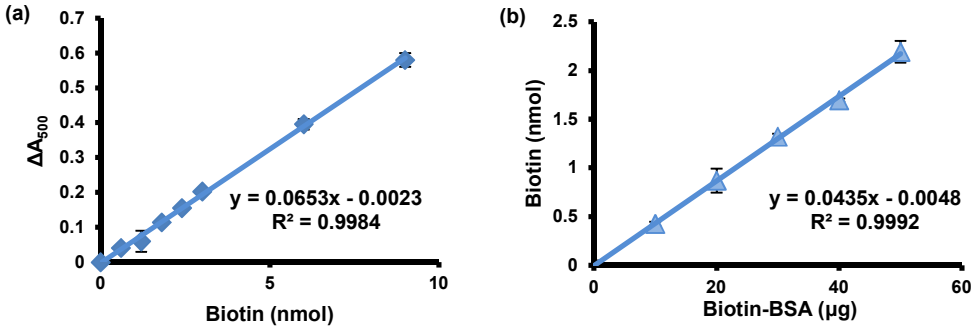

Figure: (a) Biotin Standard Curve (b) Linear plot obtained for the amount of biotin (nmol) vs amount of biotinylated BSA. Assays were performed according to the kit protocol.

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