SensoLyte® Biotin Quantitation Kit
Fluorimetric

Revision Number: 1.1	*Last updated: October 2014*
Catalog #	**AS-72163**
Kit Size	200 Assays (96-well plate)

- ***Optimized Performance:*** Optimal conditions for the quantitation of biotin concentration.
- ***Enhanced Value:*** It provides enough reagents to perform 200 assays in a 96-well plate.
- ***Assured Reliability:*** Detailed protocol and references are provided.

Kit Components, Storage and Handling

Component	Description	Quantity
Component A	Reagent A	100 µL
Component B	Reagent B	20 µL
Component C	Biocytin	160 µM, 20 µL
Component D	Protease	3 vials
Component E	Assay Buffer	30 mL
Component F	Biotinylated IgG	1 mg/mL, 60 µL

Other Materials Required (but not provided)

- 96-well microplate: Black, flat-bottom, non-binding 96-well plate.
- Fluorescence microplate reader: Capable of detecting emission at 520 nm with excitation at 490 nm.

Storage and Handling

- Store all kit components at -20°C.
- Protect Components A and B from light and from moisture.
- Components E can be stored at room temperature for convenience.

Introduction

Biotin and avidin (or streptavidin) bind non-covalently with a higher binding affinity than most antigen-antibody interactions.[1, 2] This very tight binding makes labeling proteins with biotin a useful tool for applications such as affinity chromatography and immunoanalytical methods. Reaction conditions for biotinylation are chosen such that the target molecule (e.g. an antibody) is labeled with sufficient biotin residues to purify or detect the molecule, but not too much that the biotin will interfere with the function of the molecule.

The SensoLyte® Biotin Quantitation Assay Kit provides a convenient method for estimating the number of biotin on biotinylated conjugates based on FRET principle. It also can be used to quantitate biotin concentration in a solution. In the first step, Reagent A which is tagged with a quencher dye is briefly incubated with biotin samples/standards. In the second step, fluorescent biotin, Reagent B, is added to assay mixture. The assay can detect between 2 to 20 picomoles of biotin in a sample (Fig. 1). The kit also provides biotinylated IgG for use as a positive control and a protease for optional protein digestion to expose hidden biotin groups.

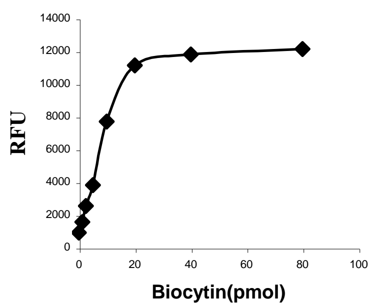

Figure 1. Biocytin standard calibration curve. Serial dilutions of Biocytin were mixed with Reagent A, followed by Reagent B. After 5 minute incubation, fluorescence was measured at Ex/Em=490/520 nm (FlexStation 384II).

Protocol

Note 1: It is necessary to test the biotin-containing sample at several dilutions to ensure that the amount of biotin is within the assay linear range, 2-20 pmol of biotin.
Note 2: If evaluating biotinylation of protein conjugates, remove free biotin from the biotinylated protein by dialysis or gel filtration.

1. Digest biotinylated protein.

1.1 Protease diluent: Prepare 1% protease solution by adding 100 µL of de-ionized water assay buffer (Component E) to one vial of protease (Component D).

1.2 Digestion of sample: The digestion of biotinylated sample with protease is necessary to expose biotin groups on protein to the detection reagents. Add the protease solution to the sample solution (IgG) at a volume ratio of about 1:25. For 200 µl of IgG solution, add 8 µl of reconstituted protease. Incubate overnight at 37°C.

1.3 Digestion of control sample: If using biotinylated IgG (Component F) as a positive control, take 6 µl of conjugate, then dilute it with 150 µl of assay buffer (Component E) and incubate overnight with 6 µl of protease from stock solution.

Note: The unused portion of protease diluents can be stored at -20°C at least 1 week.

2. Prepare working solutions.

Note 1: Warm all kit components until thawed to room temperature before starting the experiments.
Note 2: Prepare standards and reagent solutions immediately before use.

2.1 Reagent A solution: Dilute Component A 50-fold in assay buffer (Component E) according to Table 1. For each experiment, prepare fresh Reagent A solution.

Table 1. Reagent A dilution for one 96-well plate (100 assays).

Components	Volume
Reagent A (50X)	50 µL
Assay buffer (Component E)	2.45mL
Total volume	2.5 mL

2.2 Reagent B solution: Dilute the Component B 250-fold in assay buffer (Component E) according to Table 2. For each experiment, prepare fresh Reagent B solution.

Table2. Reagent B dilution for one 96-well plate (100 assays).

Components	Volume
Reagent B	10 µL
Assay buffer (Component E)	2.49 mL
Total volume	2.5 mL

2.3 Prepare dilutions of Biocytin standard: Dilute the Biocytin (Component C) 100-fold to 1.6 µM in assay buffer (Component E). Prepare 2-fold serial dilutions to get concentrations of 800, 400, 200, 100, 50, 25 nM. Include a blank control.

3. Set up the reaction.

3.1 Set up biocytin standards: Add 50 µL serially diluted biocytin reference solutions (from Step 2.3) to the wells.

3.2 Add 50 µL of test sample into microplate wells. If using the positive control (Component F) add 50 µL of the digested sample from Step 1.3.

Note: Use several 2-fold dilutions for test sample and positive control to fit in assay linear range.

4. **Run the reaction.**

4.1 Add 25 µL of Reagent A solution (from Step 2.1) into each well. Mix the reagents thoroughly. Incubate the reaction mixture for 5 minutes at room temperature. Keep plate from direct light.

4.2 Add 25 µL of Reagent B solution (from Step 2.2) into each well. Mix the reagents thoroughly. Incubate the reaction mixture for 5 minutes at room temperature. Keep plate from direct light.

4.3 Measure fluorescence signal: Measure fluorescence intensity at Ex/Em=490/520nm. Fluorescence signal is stable at room temperature for 15 minutes.

5. Data Analysis

5.1 Prepare a standard curve by plotting the average fluorescence intensity measurement for each biocytin standard on x-axis against the amount of biocytin on the y-axis. Do not subtract background.

5.2 Draw a line between two points with x values that fit the readings of the biotinylated sample. Generate a linear regression equation ($y = mx + b$). Find the amount of biotin in

picomoles (y) in each sample by extrapolating the biotin amount (y value) from the measured fluorescence (x value).

Example: Biotinylated sample shows 3000 RFU (relative fluorescence units) value. It falls within 2.5 – 5 picomoles of biocytin standards of 1523 – 3576 RFU range (Fig. 2). The equation for these two standards is y = 0.0012x + 0.6465. Solving this equation y (biotin amount) is 4.25 picomoles for biotinylated sample.

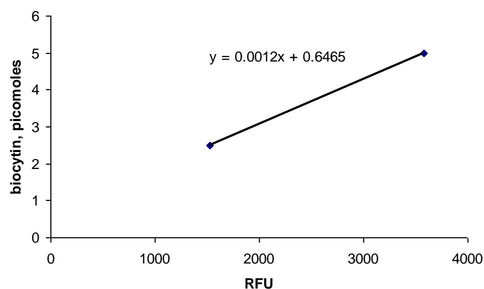

Figure 2. Example of calculation. Draw straight line between two biocytin standards that fall within the RFU of biotinylated sample. Use linear regression equation for this line to calculate amount of biotin in the sample.

5.3 The degree of substitution (DOS) is calculated by dividing the calculated picomoles of biotin by the amount of picomoles of protein in the sample.

References

1. Angerer, L. et al. *Cell.* 9, 81-90 (1976).
2. Gitlin,G. et al. *Biochem. J.* **242**,923 (1987).