AnaTag™ Biotin Microscale Protein Labeling Kit

Revision number: 1.2	*Last updated: June, 2017*
Catalog #	**AS-72058**
Kit Size	3 Conjugation Reactions

- This kit is optimized to conjugate Biotin, SE (*d - Biotin N - hydroxysuccinimide ester; Biotin, NHS ester*) to proteins (e.g., IgG).
- It provides ample materials to perform three protein conjugations and purifications.
- One conjugation reaction can label up to 200 µg protein.
- The entire process only takes about half an hour.

Kit Components, Storage and Handling

Component	Function	Quantity
A. Biotin. SE	Amino-reactive biotin with long spacer, FW 341.4	3 vials
B. Reaction buffer	For pH adjustment of the conjugation reaction	0.5 mL
C. Spin column	Purify biotin labeled protein	3 pre-packed columns
D. DMSO	Solvent for preparing biotin stock solution	150 µL
E. Elution buffer	Buffer for eluting biotin labeled protein	20 mL
F. Wash tube	Holds buffer for Spin column	3 tubes
G. Collect tube	Collects biotin labeled protein	3 tubes

Storage and Handling

- Store all kit components at 4°C.
- Component A may be frozen.

Introduction

The AnaTag™ Biotin Protein Labeling Kit provides a convenient way to label proteins with biotin. Biotin, SE (*d - Biotin N - hydroxysuccinimide ester; Biotin, NHS ester*) contains five-carbon spacer arm. The Biotin conjugated protein has less steric hindrance effect when it binds to streptavidin. The succinimidyl ester (SE) reactive form of Biotin reacts with the amine group on the protein and forms stable carboxamide bonds, which is identical to natural peptide bonds.

Biotin-protein conjugates are very stable and can withstand treatments during immunofluorescent staining, fluorescence *in situ* hybridization, flow cytometry and other biological applications without hydrolysis. The kit has all the essential components for performing the conjugation reaction and for purifying the conjugate.

Protocol

1. Preparing the protein solution

Add reaction buffer (component B) at 1/10 (v/v) ratio to your target protein (e.g. antibody) solution (3-10 mg/mL is the recommended concentration range).

Note 1: The protein can be dissolved in phosphate, carbonate, borate, triethanolamine or MOPS buffer, pH 7.2-7.5, without reducing reagents (e.g. DTT), protein stabilizers (e.g. BSA) or sodium azide. If the protein is dissolved in Tris or glycine buffer, it should be dialyzed against 0.01 M phosphate buffer saline, pH 7.2-7.4 to get rid of free amines. Ammonium salts (such as ammonium sulfate and ammonium acetate) that are widely used for protein precipitation must also be removed before performing the conjugations.
Note 2: The conjugation efficiency is poor when the concentration of protein is less than 3 mg/mL. Meanwhile, the purification column included in this kit can maximally purify 100 µl conjugate solution. You may concentrate the protein solution using a speed vacuum or a centrifugal filter (Millipore, Cat# MRCPRT010).

2. Preparing the biotin solution

Add 10 µL of DMSO (component D) to one vial of Biotin, SE (component A). This gives a 2mM of biotin solution. Completely dissolve all the biotin contents by vortexing.

Note: Biotin, SE solution must be prepared fresh for each conjugation reaction. Extended storage of the solution may reduce Biotin, SE activity.

3. Performing the conjugation reaction

Note: The procedure given here is optimized for IgG (MW ~ 150,000) labeling with Biotin, SE. The Biotin: protein molar ratio is 10:1. For proteins other than IgG, the optimal biotin/protein molar ratio may need to be determined. It will normally be between 2:1 and 20:1.

3.1 Add the Biotin solution to the solution of IgG or your protein at a Biotin to protein molar ratio of 10:1. For 200 µg IgG, add 6.7 µl of 2 mM Biotin solution.

Note: The molecular weight of IgG is 150 kDa.

3.2 Incubate the reaction mixture for 15-30 min at room temperature on a rotator or a shaker.

4. Purify biotin-protein conjugates

4.1 Resuspend the gel in the spin column (component C) by inverting sharply several times. Avoid bubbles.

4.2 Remove the top cap of the column, and then cut its bottom tip. Place the column into a wash tube (component F) and centrifuge at 1,000xg for 2 min. Discard the eluted buffer.

4.3 Exchange the gel-packing buffer by adding 500 μL of elution buffer (component E) to the spin column and centrifuge at 1,000xg for 1 min. Discard the eluent. Repeat the above step three times.

4.4 Place the spin column into a clean collection tube (component G). Apply the reaction mixture from Step 3 to the center of gel bed surface. Centrifuge the column at 1,000xg for 4 min.

4.5 The Biotin-protein conjugate is in the collection tube.

4.6 The amount of biotin molecules conjugated to protein molecule (the degree of substitution, DOS) can be determined by HABA biotin quantitation kit (AnaSpec Cat#AS-72096).

Storage of Biotin-Protein Conjugates

The Biotin labeled protein should be stored at > 0.5 mg/mL or in the presence of a carrier protein (e.g., 0.1% Bovine Serum Albumin). We recommend adding preservative (e.g. 0.01% sodium azide). The biotin labeled protein can be stored at 4°C for two months without significant changes. For extended storage, it can be aliquoted or lyophilized and stored at -20°C.