AnaTag™ Biotin Protein Labeling Kit

Revision number: 1.3	*Last updated: June, 2017*
Catalog #	**AS-72057**
Kit Size	3 Conjugation Reactions

- This kit is optimized to conjugate Biotin, SE (*d - Biotin N - hydroxysuccinimide ester*) to proteins (e.g., IgG).
- It provides ample materials to perform three protein conjugations and purifications.
- One conjugation reaction can label up to 10 mg 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 conjugate reaction	1 mL
C. Desalting column	Purify biotin-protein conjugate	3 Pre-packed columns
D. DMSO	Solvent for preparing biotin solution	1 mL
E. 10X Elution buffer	Solution for eluting biotin-protein conjugates	30 mL

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*) 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 of protein).

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 3 mL 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 100 µL of DMSO (component D) to one vial of Biotin, SE (component A). This gives a 10 mg/mL of biotin solution (22 mM). Completely dissolve all the biotin contents by vortexing.

Note: Biotin solution should 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, SE solution to the solution of IgG or your protein at a biotin: protein molar ratio of about 10:1. Mix thoroughly. Table 1 gives a quick reference for labeling IgG.

Note: The molecular weight of IgG is 150 kDa.

Table 1. The volume of Biotin solution needed for different amount of IgG.

Ig G	Biotin solution
1 mg	3.0 μL
1.5 mg	4.5 μL
2 mg	6.1 μL
2.5 mg	7.6 μL
3 mg	9.1 μL
3.5 mg	10.6 μL
4 mg	12.1 μL
4.5 mg	13.6 μL
5 mg	15.1 μL
5.5 mg	16.7 μL
6.5 mg	18.2 μL
7 mg	19.7 μL
7.5 mg	21.2 μL
8 mg	22.7 μL
8.5 mg	24.2 μL
9 mg	25.8 μL
9.5 mg	27.3 μL
10 mg	28.8 μL
10.5 mg	30.0 μL

3.2 Incubate the reaction at room temperature for 15 minutes – 1 hour on a rotator or a shaker.

4. Purify Biotin-protein conjugates

Note: The desalting column (component C) is best suited for purifying proteins of MW>6,000. For smaller proteins, we recommend using Sephadex LH-20 or dialysis. HPLC may also be used to purify the smaller protein or peptide conjugates.

4.1 Dilute 10X elution buffer (component E) to 1X in deionized water.

4.2 Hold the desalting column (component C) upright. Remove the top cap of the column, and then cut its bottom tip. Pour off the excess buffer above the top frit.

4.3 Add 25 mL 1X elution buffer to pre-equilibrate the column.

4.4 Allow the buffer to drain to the top frit. The column will not run dry. Flow will stop when the buffer level reaches the top frit. Load the column with the reaction mixture (directly from step 3.2.).

4.5 Allow entire sample to enter the column, add 10 mL 1X elution buffer into the column.

4.6 Immediately start to collect the eluent at 0.5-1 mL per fraction.

4.7 Use Bio-rad protein assay reagent or $O.D._{280\ nm}$ to determine which fractions contain biotin-protein conjugates. Combine the protein-containing fractions. Determine the protein concentration using above-mentioned methods.

Note: If you use $O.D._{280\ nm}$ please note the first peak is protein conjugate; the second peak is hydrolyzed SE byproduct. Do not collect the second peak.

4.8 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.