



AnaTag™ Biotin Protein Labeling Kit

Catalog #	71003
Unit Size	1 Kit
Kit Size	10 mg x 5 reactions

This kit is optimized to conjugate biotin-X, SE (d-Biotin-amidocaproate-N-hydroxysuccinimide ester) to proteins (e.g., IgG). It provides ample materials to perform five reactions of conjugation and purification. One reaction can label up to 10 mg IgG. The entire process only takes about half an hour.

- **Convenient Format:** Complete kit includes all the assay components.
- **Optimized Performance:** Optimal conditions for conjugation and purification.
- **Enhanced Value:** Less expensive than the sum of individual components.
- **High Speed:** Minimal hands-on time.
- **Assured Reliability:** Detailed protocol and references are provided.

USA and Canada Ordering Information

AnaSpec Corporate Headquarter

2149 O'Toole Ave.
San Jose, CA 95131
Toll-Free: 800-452-5530
Tel: 408-452-5055
Fax: 408-452-5059
E-mail: service@anaspec.com
Internet: www.anaspec.com

Technical Support

Tel: 408-452-5055
Fax: 408-434-9266
E-mail: assay@anaspec.com

International Ordering Information

A list of international distributors is available at www.anaspec.com.

Introduction

The AnaTag™ Biotin Protein Labeling Kit provides a convenient way to label proteins with biotin. Biotin-X, SE (d-Biotin-amidocaproate-N-hydroxysuccinimide ester) contains five-carbon spacer arm. The biotin-X conjugated protein has less hindrance effect when it binds to streptavidin. The succinimidyl ester (SE) reactive form of biotin-X reacts with the amine group on the protein and forms stable carboxamide bonds, which is identical to natural peptide bonds. Biotin-X-protein conjugates are very stable and can withstand treatments during immunofluorescent staining, fluorescence *in situ* hybridization, flow cytometry and other biological applications without hydrolysis.

KIT COMPONENTS, STORAGE AND HANDLING

Note: Store component A at -20 °C. Store the rest of kit components at room temperature.

Component	Function	Quantity
A. Biotin-X, SE	Amino-reactive biotin with long spacer, FW 454.5	5 vials
B. Reaction buffer	For pH adjustment of the conjugate reaction	1 mL x 2 vials
C. Purification column	Purify biotin-protein conjugates	5 columns
D. DMSO	Solvent for preparing biotin stock solution	1 mL
E. 10X Elution buffer	Buffer for eluting protein conjugate	50 mL
F. Buffer reservoir	Hold elution buffer for purification column	1

Standard Operating Protocol (SOP)

1. Preparing the protein solution

Add reaction buffer (component B) at 1/10 (v/v) ratio to your target protein (e.g. antibody) solution (1-10 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) or protein stabilizers (e.g. BSA, 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 conjugation reaction.

Note 2: The conjugation reaction is less efficient when the concentration of protein is less than 1 mg/mL. Meanwhile, the purification column included in this kit can maximally purify 2.5 mL conjugate solution. You may concentrate the protein solution using a speed vacuum or a centrifugal filter (Millipore, Cat# 42407).

2. Preparing the biotin-X, SE solution

Add 100 µL of DMSO (component D) to one vial of biotin-X, SE (component A) to get 10 mg/mL biotin-X, SE solution. Completely dissolve all the biotin-X, SE contents by vortexing.

Note: Biotin-X, SE solution must be prepared immediately before use. Extended storage of the solution may reduce biotin-X, SE activity.

3. Performing the conjugation reaction

Add the biotin-X, SE solution to the IgG solution at the molar ratio 10:1 (biotin-X: IgG). Table 1 gives a quick reference for labeling IgG. Mix the reaction mixture completely. But do not vortex. Incubate the reaction at room temperature for 15-30 minutes on a rotator or a shaker.

Table 1. The volume of biotin-X, SE solution needed for different amount of IgG.

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

Note 1: The molecular weight of IgG is 150 kDa. The molecular weight of biotin-X, SE is 454.5.

Note 2: The ratio of 10:1 (biotin-X: protein) in the reaction will give 3-6 molecules of biotin per IgG molecule on the final conjugate product. If you want different degree of substitution, you may adjust the reaction molar ratio. For proteins other than IgG, the optimal biotin-X/protein molar ratio in the reaction needs to be experimentally determined. The desired biotin-X/protein molar ratio usually should be between 2:1 and 20:1.

4. Purify biotin-X-protein conjugates

Note 1: The purification column (component C) is best suited for purifying proteins of MW>5,000. For smaller proteins, we recommend using Sephadex LH-20 or dialysis. HPLC may also be used to purify the smaller protein conjugates.

Note 2: The purification column can purify maximal 2.5 mL of sample. You may concentrate your sample if the volume is too large. Or you can dialysis your sample to get rid of unconjugated biotin-X.

- 4.1 Dilute 10X elution buffer (component E) to 1X with deionized water.
- 4.2 Hold the purification column (component C) upright. Remove the top cap of the column, and then cut its bottom tip. Place the buffer reservoir (component F) on the top of the column.
Note: The buffer reservoir is reusable.
- 4.3 Pre-equilibrate the column by adding 25 mL 1X elution buffer into the buffer reservoir and let it run through the column.
- 4.4 As soon as the liquid runs just below the top solid surface, load the column with the reaction mixture (directly from step 3).
- 4.5 As soon as the reaction mixture runs just below the top gel surface, add 10 mL 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 two methods, too.
Note: If you use O.D._{280 nm} to determine protein-containing fractions, you may observe two absorbance peaks in serial fractions. Collect the fractions of the first peak only. The second absorbance peak is caused by hydrolyzed SE byproduct.
- 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# 71161).

The Storage of Biotin-X-protein Conjugates

The biotin-X 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. 0.1% sodium azide) to the solution. The biotin-X labeled protein can be stored at 4°C for 2-3 months without significant changes. For extended storage, it should be divided into aliquots and stored at -20°C. Avoid multiple thaw-freeze cycles.