AnaTag™ Europium Protein Labeling Kit

Revision number:1.3	*Last updated: April 2018*
Catalog #	**AS-72246**
Kit Size	1 Conjugation Reaction

- This kit is optimized to conjugate Europium to proteins (e.g., IgG).
- It provides ample materials to perform protein conjugation and purification.
- One conjugation reaction can label up to 200 µg IgG.
- The entire process only takes about half an hour.

Kit Components, Storage and Handling

Component	Function	Quantity
A. Europium isothiocyanate	Amino-reactive dye	1 vial
B. Reaction buffer	For pH adjustment of the conjugation reaction	0.6 mL
C. Solvent Buffer	For dissolving Europium	50µL
D. Spin column	To purify dye-protein conjugate	1 pre-packed column
E. Elution buffer	Buffer for eluting dye-protein conjugate	20 mL
F. Wash tube	Holds buffer for Spin column	1 tube
G. Collect tube	Collects dye-protein conjugate	1 tube

Storage and Handling

- Store all kit components at 4°C.
- Keep component A away from light and protect from moisture.
- Component A may be frozen.

Introduction

AnaSpec Europium (Eu) is an excellent tool for generating Eu-protein conjugates, and offers advantages over conventional fluorophores. Europium's long-lived fluorescence and large Stokes shift allow for no background interference during assay measurements from the sample's auto-fluorescence. The signal of Europium can be monitored at excitation/emission wavelengths = 325 nm/620 nm.

AnaSpec Europium Protein Labeling Kit provides a convenient way to label proteins by using the isothiocyanate reactive form of Europium which reacts with the free amino group on proteins to make a covalent thiourea bond (Fig 1). Europium protein conjugates can be used in biological applications such as assay development for TR-FRET (time-resolved fluorescence resonance energy transfer).

The kit has all the essential components for performing the conjugation reaction and for purifying the Eu-protein conjugates.

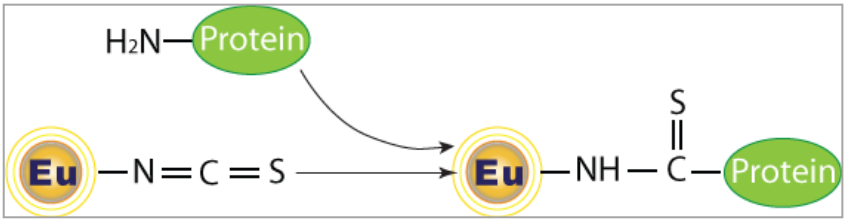

Figure 1. Labeling of an amino group (for instance, a lysine) on a biopolymer (i.e., a protein) with an isothiocyanate reactive group on Europium.

Scheme of conjugation

Protocol

1. Preparing the protein solution

1.1 Add reaction buffer (component B) at 1/10 (v/v) ratio to your target protein (e.g. antibody) solution (2-10 mg/mL is the recommended concentration range of protein).

Note 1: The protein can be dissolved in phosphate or carbonate 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 0.1 M sodium bicarbonate, pH 9.0 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 dye conjugations.

Note 2: If protein to be labeled can be prepared in 0.1M sodium bicarbonate buffer, pH 9.0 then step 1 should be omitted.

Note 3: The conjugation efficiency is poor when the concentration of protein is less than 2 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 dye solution

Add 8 µL of solvent buffer (Component C) to one vial of Europium (Component A). Completely dissolve all of the Europium contents by vortexing.

3. Performing the conjugation reaction

Note: The procedure given here is optimized for IgG (MW ~ 150,000) labeling with Europium.

3.1 Add 8μl of Europium solution to 200ug of the solution of IgG from Step 1, or amount specified in Table 1 below.
Note: The molecular weight of IgG is 150 kDa.

Table 1. The volume of dye solution needed for different amounts of IgG.

Ig G	Europium solution
100 μg	4 μL
150 μg	6 μL
200 μg	8 μL

3.2 Keep the reaction mixture away from light and shake for about half an hour at room temperature on a rotator or a shaker.

4. Purify dye-protein conjugates

4.1 Resuspend the gel in the spin column (Component D) 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 1,000 x g 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 1,000 x g for 1 min. Discard the eluent. Repeat 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 1,000 x g for 4 min.

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

4.6 The degree of substitution (DOS) of the conjugate should be determined according to the Appendix.

Appendix. Characterizing Europium-Protein Conjugate

1. Read absorbance at 280 nm (A_{280}) and 325 nm (A_{325})

For most spectrophotometers, dilute a small portion of conjugate solution in phosphate buffered saline so that the absorbance readings are in the 0.1 to 0.9 range. The maximal absorption of protein is at 280 nm (A_{280}). The maximal absorption of Europium is at 325nm (A_{325}).

2. Calculating the DOS using the following equations for IgG labeling

Molar concentration of Europium:

[Europium] = (A_{325} x dilution factor) / $\varepsilon_{Europium}$ ε_{Eu} = 21,600 $cm^{-1}M^{-1}$

ε is the extinction coefficient.

Molar concentration of protein:

[Protein]= ((A_{280} – 0.42 x A_{325}) x dilution factor) / $\varepsilon_{protein}$ ε_{IgG} = 203,000 $cm^{-1}M$

0.42 = correction factor for the fluorophore's contribution to A_{280}

DOS = [Europium]/[Protein]

Protein concentration in mg/mL for IgG:

Ig G (mg/mL)=[Ig G] x 150,000 MW_{IgG}=150,000

Storage of Europium- Protein Conjugates

The Europium-protein conjugate should be stored at 4°C in a sealed container and protected from light. We recommend adding preservative (e.g. 0.01% sodium azide). For extended storage, it can be aliquoted or lyophilized and stored at -20°C in the dark.

References

1. Hermanson GT (1996). *Bioconjugate Techniques*, Academic Press, New York.
3. Glickman,FJ. et al, *J Biomol Screen* **7**, 1 (2002)