AnaTag™ 5-TAMRA Protein Labeling Kit

Catalog #	71002
Unit Size	1 Kit
Kit Size	5 Conjugation reactions

This kit is optimized to conjugate 5-TAMRA (5-Carboxytetramethylrhodamine) to proteins (e.g., IgG). It provides sufficient materials to perform five protein conjugations and purifications. One conjugation reaction can label up to 5 mg protein. The entire process takes only half an hour.

- ***Convenient Format:*** Complete kit including 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.

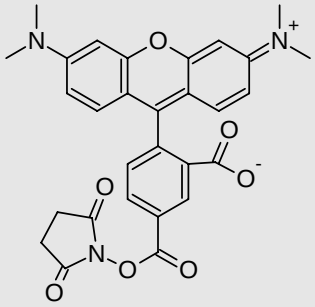
USA and Canada Ordering Information

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

International Ordering Information

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

Introduction



Physical and Spectral Properties of 5-TAMRA, SE:

Fluorescent color: Orange

Molecular weight: 527.53

Maximum absorption: 555 nm

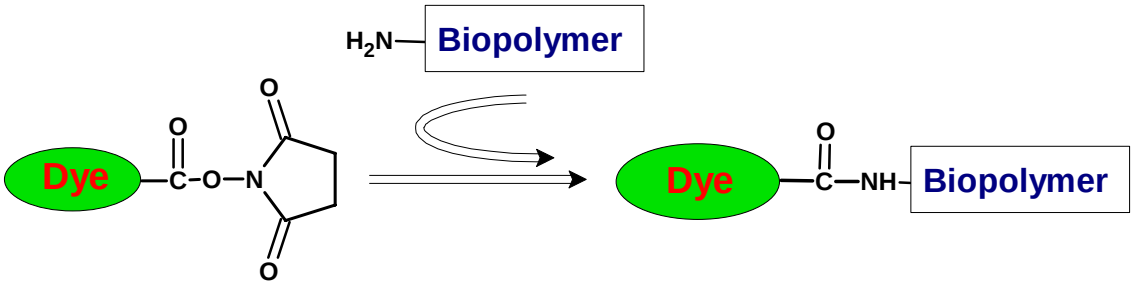
Maximum emission: 581 nm

Reactive form: Succinimidyl esters (amino-reactive)

5-TAMRA is one of the most popular orange fluorophores used to label proteins. 5-TAMRA is the purified single isomer of 5-(and 6)-TAMRA that provides reproducibility for critical biological applications and avoids the inconsistency brought by minor positional difference between 5-TAMRA and 6-TAMRA.

The AnaTag™ 5-TAMRA Protein Labeling Kit provides a convenient way to label proteins by using the succinimidyl ester (SE) reactive form of 5-TAMRA. Succinimidyl ester shows good reactivity and selectivity with aliphatic amines of the protein and forms a carboxamide bond, which is identical to, and is as stable as the natural peptide bond (Scheme 1). The 5-TAMRA-protein conjugates are very stable and suitable for immunofluorescent staining, fluorescence *in situ* hybridization, flow cytometry and other biological applications.

The kit has all the essential components for performing the conjugation reaction and for purifying the 5-TAMRA--protein conjugates.



Scheme 1: The succinimidyl ester group of fluorophore reacts with amine groups on the protein to form a stable carboxamide bond.

KIT COMPONENTS, STORAGE AND HANDLING

Note: Store component A at -20℃ with desiccant. Store all the rest kit components at room temperature.

Component	Function	Quantity
A. 5-TAMRA, SE	Amino-reactive dye	5 vials
B. Reaction buffer	For pH adjustment of the conjugate reaction	0.5 mL
C. Desalting column	Purify dye-protein conjugate	5 Pre-packed columns
D. DMSO	Solvent for preparing dye solution	1 mL
E. 10X Elution buffer	Solution for eluting dye-protein conjugates	30 mL
F. Buffer reservoir	Hold buffer for desalting column	1

Standard Operating 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 (2-10 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) and protein stabilizers (e.g. BSA). 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 dye conjugations.
Note 2: The conjugation efficiency is poor when the concentration of protein is less than 2 mg/mL.

2. Preparing the dye solution

Add 20 µL of DMSO (component D) to one vial of 5-TAMRA SE (component A). The concentration of 5-TAMRA is 10 mg/mL. Completely dissolve all the dye contents by vortexing.

Note: Prepare fresh dye solution for each conjugation reaction. Extended storage of the dye solution may reduce the dye activity. Dye solution should be kept from light.

3. Performing the conjugation reaction

3.1 Add the dye solution to the Ig G solution at dye: protein molar ratio 5:1. Table 1 gives a quick reference for labeling Ig G. Mix the reaction mixture completely.

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

Ig G	Dye solution	Ig G	Dye solution
0.5 mg	0.9 µL	3 mg	5.3 µL
1 mg	1.8 µL	3.5 mg	6.2 µL
1.5 mg	2.6 µL	4 mg	7.0 µL
2 mg	3.5 µL	4.5 mg	7.9 µL
2.5 mg	4.4 µL	5 mg	8.8 µL

Note 1: The molecular weight of Ig G is 150 kDa.

Note 2: The molar ration of 5:1 is optimized for labeling IgG with 5-TAMRA SE. For proteins other than IgGs, the optimal dye/protein molar ratio needs to be determined. The desired dye/protein molar ratio is between 5:1 and 20:1.

3.2 Keep the reaction mixture from light and shake for 15 minutes at room temperature on a rotator or a shaker.

4. Purify dye-protein conjugates

Note: Desalting 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.

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. Place the buffer reservoir (component F) on the top of the column.

4.3 Add 25 mL 1X elution buffer into the buffer reservoir to pre-equilibrate 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 3.2.).

4.5 As soon as the reaction mixture runs just below the top gel surface, add 10 mL 1X elution buffer into the column.

4.6 When the reaction mixture runs down the column, you should see it separated into two bands. The faster-running band (lower band) contains the desired dye-labeled protein, while the slower-running band (upper band) contains the free dye.

4.7 Collect the faster-running band only. Avoid the slower-running band, which will contaminate your conjugate.

4.8 The degree of substitution (DOS) of the conjugate can be determined according to Appendix I.

Appendix I. Characterizing the Dye-Protein Conjugate

The Degree of substitution (DOS) is the most important factor for characterizing dye-labeled protein. Proteins of lower DOS usually have weaker fluorescence intensity, but proteins of higher DOS (e.g. DOS>6) tend to have reduced fluorescence as well. For TAMRA, the optimal DOS is around 2-4. We used the following method to determine the DOS of 5-TAMRA labeled proteins.

1. Read absorbance at 280 nm (A_{280}) and A_{max} of 5-TAMRA

For most spectrophotometers, dilute a small portion of conjugate elution in phosphate buffered saline so that the absorbance readings are in the 0.1 to 0.9 range. The absorbance at 280 nm (A_{280}) is the maximal absorption of protein. The maximal absorption of 5-TAMRA amide (A_{max}) is approximately at 547 nm (Figure 1 and 2).

2. Calculating DOS using the following equations for IgG labeling

The molar concentration of dye:

$[dye]=A_{max}/\varepsilon_{5\text{-TAMRA}}$ X dilution factor

$\varepsilon_{5\text{-TAMRA}}$= 65,000 $cm^{-1}M^{-1}$

ε is the extinction coefficient.

The molar concentration of protein:

$[protein]=(A_{280} - 0.36 \text{ X } A_{max})/\varepsilon_{protein}$ X dilution factor

$\varepsilon_{IgG=}$ 203,000 $cm^{-1}M^{-1}$

DOS = [dye]/[protein]

Protein concentration (mg/mL):

Ig G (mg/mL)=[Ig G] X 150,000

$MW_{Ig\ G}$=150,000

For effective labeling, the degree of substitution should fall within 2-4 moles of 5-TAMRA to one mole of protein.

Storage of protein conjugates

The dye 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), and the addition of preservatives (e.g. 0.1% sodium azide) is highly recommended. The labeled protein can be stored at 4°C for two months without significant change if kept from light. For extended storage, dye labeled protein should be divided into aliquots or lyophilized and stored at -20°C in the dark.

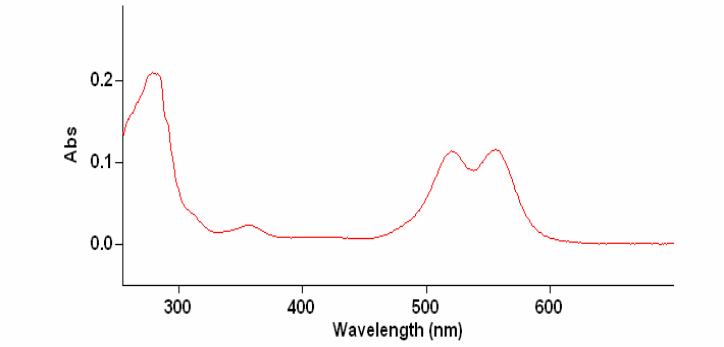


Figure 1. The absorption spectrum of 5-TAMRA-Ig G conjugate.

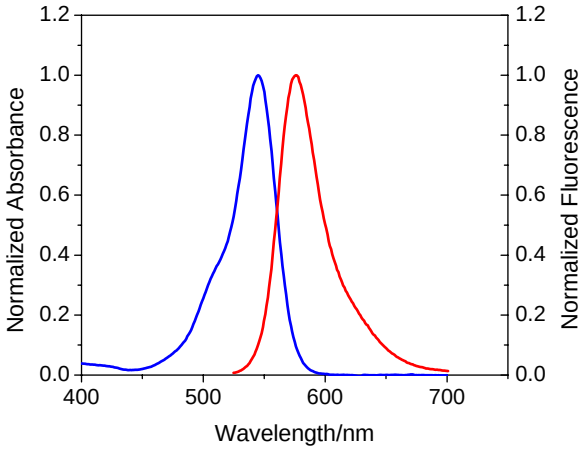


Figure 2: The spectral properties of 5-TAMRA (left curve: absorption; right curve: emission).

Reference

1. Hermanson GT (1996). *Biocojugate Techniques*, Academic Press, New York.
2. Haugland RP (1995). Coupling of monoclonal antibodies with fluorophores. *Methods Mol Biol* **45,** 205-21.
3. Brinkley M (1992). A brief survey of methods for preparing protein conjugates with dyes, haptens, and cross-linking reagents. *Bioconjug Chem* **3,** 2-13.