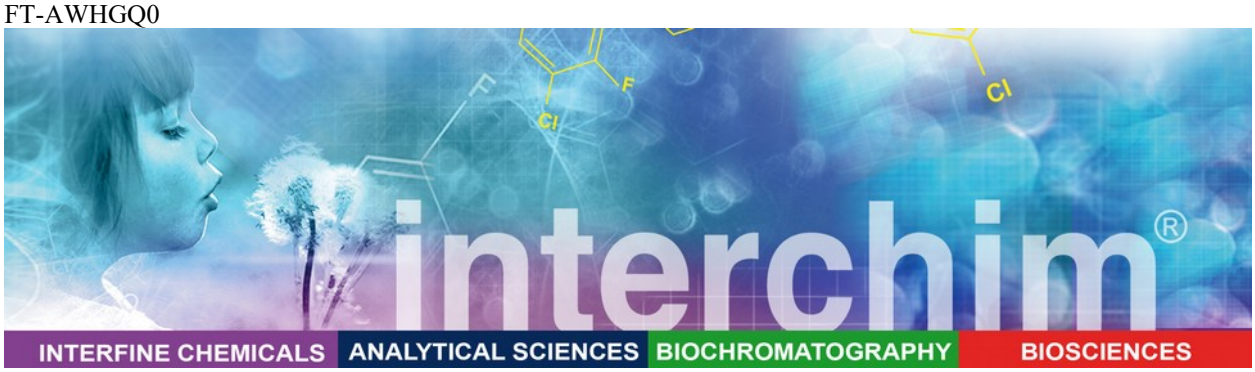
FluoProbes®

FT-AWHGQ0



Sulfo-CY$_{anine}$ Antibody Labeling Kit

Product Description

Name :	Sulfo-CY$_{anine}$3 Antibody Labeling Kit	FP-AWHGN0, 10 tests	
	Sulfo-CY$_{anine}$5 Antibody Labeling Kit	FP-AWHGQ0, 10 tests	
	Sulfo-CY$_{anine}$5.5 Antibody Labeling Kit	FP-AYQMCA, 10 tests	
	Sulfo-CY$_{anine}$7 Antibody Labeling Kit	FP-AYQMEA, 10 tests	
	Sulfo-CY$_{anine}$7.5 Antibody Labeling Kit	FP-AYQMFA, 10 tests	
Components :	Lyophilized dye NHS ester, in sealed bag with desiccant		10 ea
	Anydrous DMSO		1 mL
	Sodium azide solution (3%, 100x)		0.5 mL
	Instant PBS (for the preparation of 100 mL of 1x solution)		1 tablet
	Desalting spin column with receptacle and waste vials		10 ea
Storage:	Store at temperature below 25°C. Do not freeze! Shelf life 1 year.		

Introduction

The kit is used for the preparation of labeled antibodies having 2.5-3 labels per antibody. The kit contains all reagents and materials for ten labeling reactions, each for 100 ug of the antibody. The dye is an NHS ester, taken in controlled excess. It reacts with the amine groups of lysines the antibody at mild alkaline pH. The purification of the antibody is achieved by gel filtration using spin columns. These kits contain sulfonated, water soluble dyes, that are most suitable for the labeling of sensitive proteins, including antibodies.

Directions for use

1. Antibody preparation.

Antibody should be dissolved in 100 uL of PBS buffer (pH 7.4) or Tris-HCl buffer (pH 7.4-8.0) prior to the labeling reaction. Sodium azide is compatible with the labeling. If concentration of antibody is below 1 mg/mL, it should be concentrated.

2. Setting up the reaction.

2.1. Add a solution of antibodies in PBS (100 ug in 100 uL*), vortex, and incubate for 30 min at room temperature.

* if smaller amount of antibody is to be labeled, dissolve lyophilized dye in 10 uL of anhydrous DMSO, and take 1 uL of the solution per 10 ug of antibody. The solution of dye in DMSO should be consumed immediately.

3. Purification of the labeled antibody.

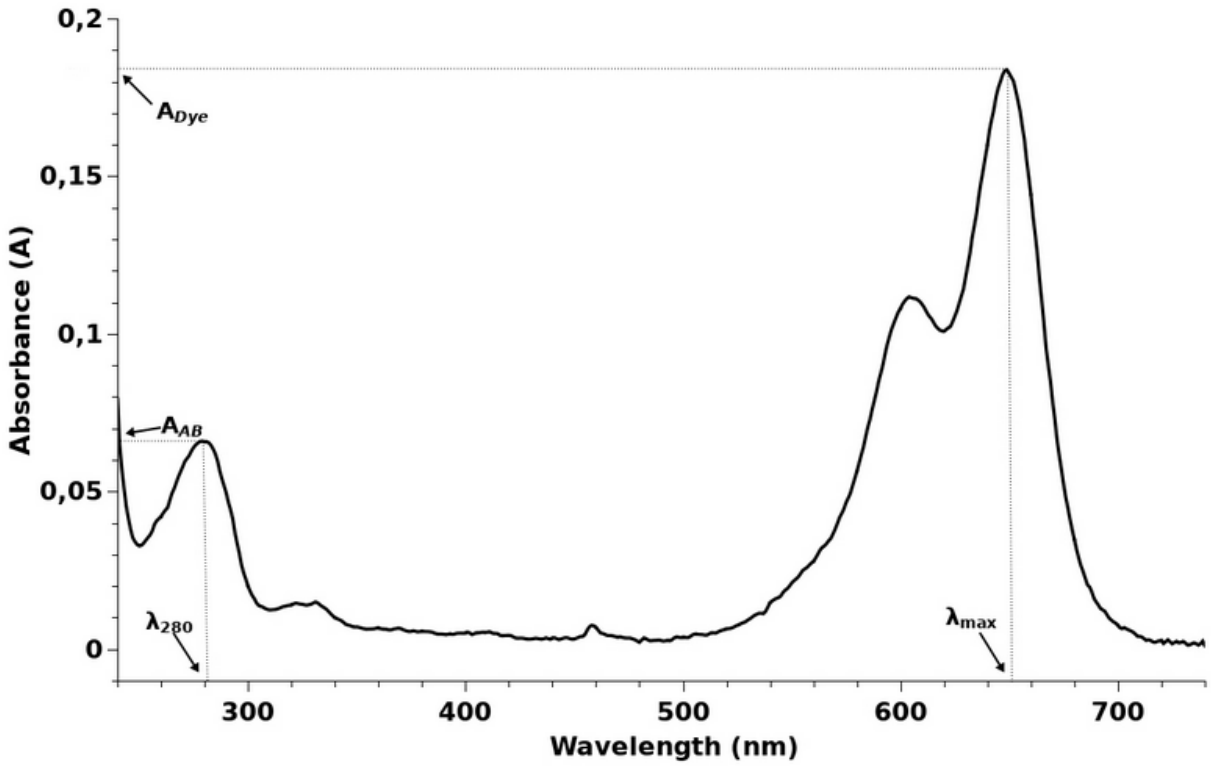
3.1. Prepare the column. The column should be at room temperature prior to use. The gel should be resuspended using vortex. Put the column into a waste receptacle (without cap), and centrifuge for 2 min at 1,000 g (speed must be carefully controlled - for standard 6 cm rotor, 1000 g = 3800 rpm). The small bulge of the column should be oriented outwards.

3.2. Apply 400 uL of 1x PBS buffer, centrifuge for 2 min at 1,000 g.

3.3. Remove the column from waste receptacle. Put it into collection tube (with cap). Apply 100 uL of the reaction mixture onto the column, incubate for 1 min, and elute the antibody by centrifugation for 2 min at 1,000 g. Add 1/100 volume of 100x sodium azide. Antibodies can be aliquoted to improve their shelf life. Current aliquot can be stored at +4°C the rest at -20°C.

4. Measurement of dye to antibody ratio.

To calculate dye to antibody ratio, measure absorption spectrum of the conjugate, or absorption at 280 nm (A_{AB}), and at dye absorption maximum (A_{Dye}). A typical absorption spectrum of a dye labeled antibody is shown below. Depending on the dye, wavelength of the maximum may vary.



An absorption spectrum of a labeled antibody contains dye peak (with longer wavelength), and antibody peak (at around 280 nm). Dye to antibody ratio is calculated using the following formula:

$$\frac{Dye}{AB} = \frac{A_{Dye} \times \varepsilon_{AB}}{(A_{AB} - A_{Dye} \times CF) \times \varepsilon_{Dye}}$$

with **Dye/AB** - average number of fluorophores per antibody molecule, $\mathbf{A_{dye}}$ - optical density of the sample at dye absorption maximum, $\mathbf{A_{AB}}$ - sample optical density at 280 nm, $\boldsymbol{\varepsilon_{AB}}$ - molar extinction coefficient of antibody at 280 nm (210,000 for IgG), $\boldsymbol{\varepsilon_{Dye}}$ - molar extinction coefficient of dye at absorption maximum (see table below), $\mathbf{CF_{280}}$ - correction factor for dye (see table below).

Dye	λ_{max}, nm	ε	CF_{280}
Sulfo-CY$_{anine}$3	548	162,000	0.06
Sulfo-CY$_{anine}$5	646	271,000	0.04
Sulfo-CY$_{anine}$5.5	673	195,000	0.11
Sulfo-CY$_{anine}$7	750	240,600	0.04
Sulfo-CY$_{anine}$7.5	778	222,000	0.09

Example of calculation

After labeling of IgG antibody with sulfo-Cyanine5 and purification, a solution has been obtained with absorption spectrum above. Determine dye to protein ratio.

Using absorption spectrum, the following values can be found: $\mathbf{A_{Dye}}$ = 0.184 at dye absorption maximum (646 nm, see the table), $\mathbf{A_{AB}}$ = 0.066 (at 280 nm), $\boldsymbol{\varepsilon_{AB}}$ = 210000 for IgG; $\boldsymbol{\varepsilon_{Dye}}$ = 271,000, and $\mathbf{CF_{280}}$ = 0.04.

$$\frac{Dye}{AB}=\frac{A_{Dye}\times\varepsilon_{AB}}{(A_{AB}-A_{Dye}\times CF)\times\varepsilon_{Dye}}=\frac{0.184\times 210\,000}{(0.066-0.184\times 0.04)\times 271\,000}=2.43$$

Dye/AB - is 2.43 fluorophore molecules per antibody.

Interpretation of results, and antibody storage

In most cases, optimal dye to antibody ratio is 2-3 dye molecules per antibody. Increase of dye loading above this value does not improve fluorescent signal because of the concentrational quenching of the fluorescence. When too few fluorophores are attached during the reaction, decrease antibody loading per reaction. Use of expired kit can also lead to this result.

We recommend to test binding of labeled antibodies with their antigens. The labeled antibodies can be stored at -20°C. An aliquot that is currently in use, should be stored at +4°C to avoid freeze-thaw cycles. The stability of the conjugate is determined by antibody itself rather than fluorophore or the chemical bond. Labeled antibodies should not be exposed to direct sunlight for extended time, but tolerate ambient lighting well.

Ordering information

Catalog size quantities and prices may be found at http://www.interchim.com.
Please inquire for higher quantities (availability, shipment conditions).

For any information, please ask : FluoProbes® / Interchim; Hotline : +33(0)4 70 03 73 06