User Manual

FITC Labeling Kit

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Store at 2-8 °C.

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures. Not for Human or Animal Consumption.

Product Overview

The Calbiochem® FITC Labeling Kit provides a means of quickly and simply labeling antibodies or other proteins with fluorescein. FITC-antibody conjugates are useful in many applications, such as flow cytometry and immunocytochemistry. Other proteins such as steroids may also be labeled with FITC. Direct labeling of the primary antibody eliminates the need for a secondary antibody, and therefore results in a lower background and an overall higher signal to noise ratio.

Materials Provided

This kit contains sufficient reagents to perform 5 conjugations of proteins with 0.1-2.5 mg each.

- FITC (Component No. KP4801): 5 vials, 0.85 mg each
- Solvent Reagent (Component No. KP4802): 1 vial, 5.0 mL
- Carbonate Buffer Concentrate (100X) (Component No. KP4803): 1 bottle, 100 mL
- PBS Buffer Concentration (10X; with 1% Sodium azide) (Component No. KP4804): 1 bottle, 100 mL

Preparation

Antibody/Protein Preparation: Add 10 mL of carbonate buffer concentrate to 990 mL of distilled water and dialyze antibody in buffer overnight.

Protocol

1. Remove the antibody or other protein from dialysis and dilute or concentrate to a final concentration of 2 mg/mL. Place in container suitable for vortexing.
2. Add 850 µL of Solvent Reagent to one vial of FITC. Mix thoroughly. The FITC should now be at a concentration of 1 mg/mL.
3. If the protein being conjugated is not IgG, reserve a sufficient amount of the protein so that a 1 mg/mL solution can be measured in a cuvette of 1.0 cm path length in order to calculate the approximate concentration of the final conjugate (see step 3 of Determining the FITC/Protein Molar Ratio). This reserved protein will be used to calculate $E^{0.1\%}$.
4. For 2.5 mg of antibody or other protein, pipette 500 µL of FITC mixture into the antibody solution and vortex immediately. This provides a conjugation ratio of 200 µg FITC to 1 mg antibody/protein. Mix end-over-end for 2 hours at room temperature, or transfer to a flask and mix with a stir bar and magnetic stirrer. If possible, seal container with aluminum foil while stirring to block out ambient light.

5. Dilute PBS buffer concentrate 1:10 in distilled water (final azide concentration is 0.1%) for dialysis. Reserve sufficient diluted PBS buffer to prepare a spectrophotometric blank for determining the FITC to protein molar ratio. Transfer the conjugate to dialysis tubing and dialyze overnight at room temperature in the diluted PBS.
6. Remove from dialysis tubing. Keep refrigerated (short term) or frozen (long term) and protect from light.

Calculations

1. Read the absorbance of the FITC-antibody conjugate at two wavelengths: 280 nm and 495 nm. Use the 1X PBS/0.1% NaN_3 as the spectrophotometric blank. Optimal absorbance readings of the conjugated protein will be within the range of 0.2-1.2 ABS.
2. Using the two absorbance readings, calculate the F/P molar ratio of the FITC/Protein conjugate using the following equation:

   Figure 1: F/P Molar Ration

   Molar F/P = Mole FITC/Moles Protein

   $$\text{Molar F/P} = \frac{2.77 \times A_{495}}{A_{280} - (0.32 \times A_{495})}$$

3. There are 2 equations used for the determination of the concentration of the FITC-antibody conjugate. If the protein used for conjugation was IgG, use the following equation to estimate the concentration of the labeled antibody:

   Figure 2: Concentration of FITC-IgG

   $$\text{Antibody (mg/mL)} = \frac{A_{280} - (0.32 \times A_{495})}{1.4}$$

   If the protein conjugated was not IgG, use the following equation to estimate the concentration of conjugated protein:

   Figure 3: Concentration of FITC-Protein

   $$\text{Protein X (mg/mL)} = \frac{A_{280} - (0.32 \times A_{495})}{E^{0.1\%}}$$

   Where $E^{0.1\%}$ is the A_{280} reading of a 1 mg/mL solution of the unconjugated protein, as measured in a cuvette of 1 cm path length.

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Document Template 20769660 Ver 4.0

23212731 Ver 1.0, Rev 17Apr2025, UD