INSTRUCTIONS

# Pierce FITC Antibody Labeling Kit

## 53027

2078.3

| Number | Description |
|---|---|
| **53027** | **Pierce FITC Antibody Labeling Kit,** contains sufficient reagents to label and purify 3 × 1mg (2mg/mL) of IgG or similar amounts of other proteins |

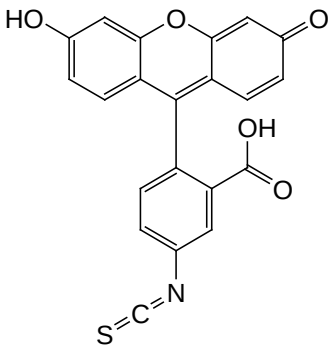
**Kit Contents:**

**FITC (fluorescein isothiocyanate),** 3 × 50µg vials

Molecular mass: 389.2

Extinction coefficient: 70,000 $M^{-1}$ $cm^{-1}$
(at 494nm in aqueous buffer, pH 8)

Ex/Em wavelength : 494/517nm




**Borate Buffer (0.67M),** 1mL

**Purification Resin,** 5mL

**Spin Columns,** 6 each

**Microcentrifuge Collection Tubes,** 12 each

**Storage:** Upon receipt store FITC at -20°C. Store all other components at 4°C. The kit is shipped with an ice pack.

## Introduction

The Thermo Scientific™ Pierce™ FITC Antibody Labeling Kit contains all the necessary components for three protein labeling reactions and subsequent excess dye removal. Fluorescein isothiocyanate (FITC) crosslinks to amino, sulfhydryl, imidazoyl, tyrosyl and carbonyl groups on proteins; however, only derivatives of primary and secondary amines yield stable products. Reactions are most efficient at pH 8-9 and must be performed in an amine-free buffer such as borate or carbonate/ bicarbonate. Generally, the protein is reacted with a 15- to 20-fold molar excess of FITC, which results in several FITC molecules conjugated to each protein molecule.

The purification resin and spin columns eliminate equilibration steps and the need to collect and monitor gravity-flow fractions. This system enables efficient removal of excess FITC and, therefore, accurate determination of the dye-to-protein ratio and exceptional protein recovery.

## Important Product Information

- FITC is moisture-sensitive. Prepare the FITC Labeling Reagent immediately before use and discard any unused reconstituted reagent. Do not store reconstituted labeling reagent.
- For fluorescent imagers, use a spectral line of a green (526) laser.
- Low concentrations of sodium azide (≤ 3mM or 0.02%) or thimerosal (≤ 0.02mM or 0.01%) will not significantly interfere with protein labeling; however, 20-50% glycerol will reduce labeling efficiency.
- Avoid primary amine-containing buffers (e.g., Tris, glycine) because they compete with the labeling reaction.

Pierce Biotechnology 3747 N. Meridian Road | PO Box 117 Rockford, IL 61105 USA | (815) 968-0747 (815) 968-7316 fax | www.thermoscientific.com/pierce

## Additional Materials Required

- Variable-speed centrifuge
- Phosphate-buffered Saline (PBS; for measuring the FITC-to-protein ratio)

## Procedure for Antibody Labeling

### A. Protein Preparation

**Note:** If the Borate Buffer has precipitated during storage, solubilize it by warming and vortexing the vial.

1. The optimal labeling buffer is 50mM sodium borate, pH 8.5. For best results use 1mg of protein at ~2mg/mL. Prepare the protein as follows:
   - **Proteins lyophilized in PBS:** Just before use, prepare the labeling buffer by diluting the Borate Buffer (0.67M) to 0.05M in PBS or ultrapure water. Prepare only enough labeling buffer required for the reaction (for example, to prepare 1mL, add 75µL of Borate Buffer (0.67M) to 925µL of ultrapure water or PBS). Reconstitute 1mg of protein with 0.5mL of labeling buffer.
   - **Proteins in PBS:** Add 40 µl of the Borate Buffer (0.67M) to 0.5mL of 2mg/mL protein in PBS. If the protein is > 2mg/mL, adjust the concentration to 2mg/mL with labeling buffer (e.g., 0.05 M sodium borate – see the above bullet point: Proteins lyophilized in PBS).
   - **Proteins in other buffers:** Protein must be in a buffer free of ammonium ions or primary amines (e.g., Tris or glycine). If necessary, replace buffer with 50mM sodium borate (Product No. 28384), pH 8.5 by dialysis or buffer exchange.

### B. Protein Labeling

1. Bring all the reagents to room temperature.
2. Add 0.5mL of the prepared protein to the vial of FITC Reagent and pipette up-and-down 10 times until all the dye is dissolved. Vortex briefly if required.

   **Note:** The reagent must be completely dissolved for effective labeling.
3. Briefly centrifuge the vial to collect the sample in the bottom of the tube.
4. Incubate the reaction mixture for 60 minutes at room temperature protected from light.

### C. Protein Purification

1. Place two spin columns in separate microcentrifuge collection tubes.
2. Mix the Purification Resin to ensure uniform suspension and add 400µL of the suspension into both spin columns. Centrifuge for 30-45 seconds at ~1,000 × *g* to remove the storage solution. Discard the used collection tubes and place the columns in new collection tubes.
3. Add 250-270µL of the labeling reaction to each spin column and mix the sample with the resin by pipetting up and down or briefly vortexing.
4. Centrifuge columns for 30-45 seconds at ~1,000 × *g* to collect the purified proteins. Combine the samples from both columns (~0.5mL total). Discard the used columns.
5. Store the labeled protein protected from light at 4°C for up to one month. Alternatively, store labeled protein in single-use aliquots at -20°C. Avoid repeated freeze/thaw cycles. If the final concentration of conjugate is < 1mg/mL, add a stabilizing agent, such as bovine serum albumin at 1-10mg/mL.

### D. FITC-to-Protein Ratio Estimation

1. Dilute a small amount of labeled purified protein in PBS.
2. Use a 1cm path length cuvette to measure absorbance at 280nm and 495nm (i.e., the $A_{max}$ of FITC).

3. Calculate protein concentration as follows:

$$\text{Protein concentration (M)} = \frac{A_{280} - (A_{max} \times CF)}{\varepsilon_{protein}} \times \text{dilution factor}$$

- $\varepsilon_{protein}$ = protein molar extinction coefficient (e.g., the molar extinction coefficient of IgG is ~210,000 $M^{-1}$ $cm^{-1}$)
- CF = Correction factor $= \frac{A_{280}}{A_{max}} = 0.3$

4. Calculate the degree of labeling as follows:

$$\text{Moles FITC per mole protein} = \frac{A_{max} \text{ of the labeled protein}}{\varepsilon_{fluor} \times \text{protein concentration (M)}} \times \text{dilution factor}$$

- $\varepsilon_{fluor}$ = 70,000 (FITC molar extinction coefficient)

## Troubleshooting

| Problem | Possible Cause | Solution |
|---|---|---|
| Protein was not labeled | Protein buffer contained amines that interfered with labeling | Perform buffer exchange via dialysis or other method into 50mM sodium borate |
| | FITC is hydrolyzed and non-reactive | Prepare labeling reagent immediately before use – do not store reagent in aqueous solution |
| The downstream application was unsuccessful | Protein was not labeled | Determine if the protein was labeled by calculating the FITC-to-protein ratio |
| Sample or buffer does not flow through resin | Centrifugation problem | Ensure that centrifuge is in proper working condition |
| Low yield | Improper centrifugation | Make sure to use the indicated centrifugation speed |
| | | Add 40µL of suitable buffer to the top of the resin and repeat centrifugation step |
| | Unstable protein | Equilibrate the column with PBS or other suitable buffer before adding the labeled protein |

## Additional Information

**Please visit our website for additional information including the following items:**

- Tech Tip #43: Protein Stability and Storage
- Tech Tip #6: Extinction Coefficients Guide
- Tech Tip #31: Calculate Dye:Protein (F/P) Molar Ratios
- Tech Tip #40: Convert Between Times Gravity (× *g*) and Centrifuge Rotor Speed (RPM)

## Related Thermo Scientific Products

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|---|---|
| **46424** | **FITC,** 1g |
| **46425** | **FITC,** 100mg |
| **22858** | **Fluorescent Dye Removal Columns** |
| **46402** | **DyLight 488 NHS Ester,** 1mg |
| **46403** | **DyLight 488 NHS Ester,** 5 × 50µg |
| **53024** | **DyLight 488 Antibody Labeling Kit** |
| **53025** | **DyLight 488 Microscale Antibody Labeling Kit** |