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Antibody/Protein Labeling Kit – FITC

1 Contents

Components	HY - K0701 - 5 Assays
FITC Dye	5 x 1 mg
Dye Labeling Buffer	5 tubes
Spin Desalting Columns	5 columns

2 General Information

FITC (Fluorescein isothiocyanate) is one of the most simple and commonly used reagents for protein fluorescent labeling. The isothiocyanate group of FITC reacts with amino terminal and primary amines in proteins. The excitation and emission wavelengths of the FITC-labeled proteins are 494 nm and 525 nm, respectively.

The Antibody/Protein Labeling Kit – FITC contains FTIC, Dye Labeling Buffer and Spin Desalting Columns. Spin Desalting Columns offer exceptional desalting for protein samples (>5 kDa). The spin-column method eliminates waiting for samples to emerge by gravity flow and the subsequent monitoring of fractions for protein recovery. Each kit provides all reagents needed to perform five separate labeling reactions and to purify the resulting conjugates.

3 Storage

Please store FITC at -20°C, store columns and buffer at 4°C. Stable for 1 year.

4 Preparation Before Use

1. Preparing Dye Labeling Buffer

Dissolve one vial of Dye Labeling Buffer in 20 mL dH_2O. This dissolved buffer can be stored stably at 4°C for one week.

2. Preparing Protein

Prepare a solution of protein in Dye Labeling Buffer at least 2 mg/mL.

1) For dry protein sample, dissolve 1 mg of protein in 0.5 mL Dye Labeling Buffer.

2) For proteins in PBS or Bicarbonate/Carbonate Buffer, these samples are compatible with labeling reaction.

3) The purified protein must be in a buffer free of ammonium ions or primary amines, as they will compete with the amine groups of the protein for the reactive dye. For example, if the buffer contains Tris or glycine, dialyze protein solution against PBS, pH 7.4, overnight at 0-5°C.

3. Dye Calculation

The amount of FITC to use for each reaction depends on the amount of the protein to be labeled. The degree of labeling can be controlled by optimizing the ratio of Dye Labeling Agent to the protein. The optimal molar ratio for FITC/protein is about 20. The amount of FITC can be calculated this way:

1) mmol (protein) = mg/mL (protein concentration) x mL (protein volume) / MW (protein)

2) mmol (FITC) = mmol (protein) x 20 (molar ratio)

3) µL (FITC volume) = mmol (FITC) x MW (FITC) / mg/µL (FITC concentration)

For example: For labeling 500 µL of 2 mg/mL IgG (MW = 150,000) with FITC (MW = 389.5), dissolve 1 mg FITC in 100 µL DMSO, you will need 5.22 µL FITC solution.

4. Preparing FITC Solution

Immediately before use, dissolve one vial of FITC in 100 µL anhydrous DMSO at 10 mg/mL. Pipette up and down until the agent is completely dissolved.

5 Procedure

1. Labeling Reaction

1) For each 0.5 mL of protein solution, add the calculated volume of the freshly prepared FITC solution. Gently invert a few times to fully mix them. Violent agitation of the protein solution can result in protein denaturation.

2) Incubate the reaction in the dark for 60 minutes at room temperature. Every 10–15 minutes, gently invert the vial several times in order to mix the two reactants and increase the labeling efficiency.

2. Purifying the Labeled Protein

1) Place a mark on the side of the column. Place column in centrifuge with the mark facing outward in all subsequent centrifugation steps.

2) Twist off the column's bottom closure and loosen cap. Place column in a collection tube. Centrifuge column at 1,000×g for 2 minutes to remove storage solution.

Note: Resin will appear compacted after centrifugation.

3) Add 5 mL Dye Labeling Buffer (or other appropriate buffer) into to the column. Centrifuge the column at 1,000×g for 2 minutes to remove the buffer.

4) Repeat step 3) two more times, ensuring the buffer is discarded after each centrifugation.

5) Place the column in a new collection tube and remove the cap. Slowly, apply the labeled protein solution to the center of the Spin Desalting Column.

6) Centrifuge the column at 1,000 ×g for 2 minutes to collect the purified labeled protein.

7) Discard the used column and store the purified labeled protein at -80°C.

6 Precautions

1. Use freshly prepared FITC solution for conjugation reaction.

2. The presence of low concentrations of sodium azide (≤3 mM / 0.02%) or thimerosal (≤1 mM / 0.01%) would not interfere with the conjugation reaction. But 20-50% glycerol will reduce the conjugation reaction.

3. This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices