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EZLabel™ Protein FITC Labeling Kit

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(Store at 4°C)

Cat. No. K832-5, contains sufficient reagents to label and purify 5 x 1 mg of protein

I. Introduction:

BioVision's EZLabel™™ Protein FITC Labeling Kit provides an easy way to label proteins with Fluorescein Isothiocyanate (FITC) in a user-friendly spin column format. FITC is an ideal dye for fluorescent labeling of proteins among the most commonly used fluorescent dyes for labeling proteins. Each spin column provided in the kit can be used to label up to 1 mg of the target protein. The kit provides all of the reagents necessary to perform five labeling reactions using up to 1 mg of protein per reaction. FITC-labeled protein has an excitation and emission wavelengths at 494 nm and 520 nm respectively, and can be directly used for downstream applications including ELISA, western blot, Immunohistochemistry, Immunofluorescence, and FACS analysis, etc.

II. Applications:

- Label proteins can be used for ELISA, western blot, Immunohistochemistry, Immunofluorescence, and FACS analysis

III. Kit Contents:

Components	K832-5	Cap Code	Part Number
EZLabel™ FITC	5 vials	Red	K832-5-1
EZLabel™ Spin Column	5 columns	-	K832-5-2
EZLabel™ Quenching Buffer	1ml	Clear	K832-5-3
EZLabel™ Elution Buffer	10 ml	NM	K832-5-4

IV. User Supplied Reagents and Equipment:

- Microcentrifuge, ethanol/DMSO/DMF, and 0.1 M Sodium Bicarbonate buffer (pH 8.5-9.0).

V. Reagent Preparation and Storage Conditions:

Store kit at 4°C, protected from light. Read entire protocol before performing the experiment. Briefly spin small vials prior to opening. Bring kit components to room temperature before use.

VI. Protein FITC Labeling Protocol:

A. Protein Solution Preparation: The volume of protein solution should not exceed 100 µl. For best results, use 100 µl of ~5-10 mg/ml protein.

Note: Buffers that contain primary amines (e.g. Tris or glycine) interfere with the intended FITC conjugation. Dialyze the protein against 0.1 M sodium bicarbonate buffer (pH 8.5-9.0) just before labeling experiment is performed to remove primary amines.

B. Labeling Reaction: Each vial of EZLabel™ FITC is sufficient for labeling of 1 mg of protein. Reconstitute one vial of EZLabel™ FITC with 5-10 µl of ethanol, DMSO, or DMF just before use. Dissolve completely by pipetting up and down. Transfer 100 µl of the prepared protein to a 1.5 ml microcentrifuge tube. Add reconstituted EZLabel™ FITC solution and mix well by pipetting up and down. Incubate at room temperature on rotary shaker or mixer for 1 hr. After incubation, add 20 µl EZLabel™ Quenching Buffer to quench the reaction & incubate again at room temperature for 30 min. Total volume at this stage should not exceed 130 µl.

Note: If the amount of protein is less than 1 mg, the amount of EZLabel™ FITC also needs to be lowered accordingly to avoid over-labeling of protein with FITC that could result in potential fluorescence quenching of the protein conjugate.

C. Purification of Labeled Protein:

1. During the labeling reaction, snap off the bottom closure of an EZLabel™ Spin Column and place in a fresh microcentrifuge tube. Centrifuge at ~1500 x *g* for 1 min. to remove the residual storage buffer. Discard the flow through and wash the resin with 130 µl of EZLabel™ Elution Buffer. Close the cap and centrifuge at 1500 x *g* for 1 min. Discard the flow through. Repeat this step at least for total of three times.
2. Load the labeling reaction mix (max. 130 µl) to the spin column drop by drop. Centrifuge the column for 2 min. at 1500 x *g* to collect the labeled protein.

   Note: For smaller protein, a second elution step might be necessary to recover the labeled protein. However care must be taken to avoid eluting unconjugated FITC. In such cases, the fractions may be combined and transferred to a Centricon ultracentrifuge column or new EZLabel™ Spin Column, followed by washing with EZLabel™ Elution Buffer or other suitable storage buffer of choice.
3. Optional: Dialyze the labeled protein in the dark against a desired storage buffer containing 20-30% glycerol and if necessary, a carrier protein (e.g. BSA). Store the dialyzed protein in a tube wrapped with aluminum foil at 4°C (for short term) or -20°C (for long term).

D. Calculations (Optional): In some cases, it is advantageous to determine the number of molecules of FITC per molecule of protein (degree of labeling). For that, measure the absorbance of the labeled protein at 280 nm (A_{280}) and 494 nm (A_{494}). If necessary, dilute the labeled protein in EZLabel™ Elution Buffer. Calculate the concentration of labeled protein and degree of labeling using following equations:

$$\textbf{Concentration of labeled Protein (M)} = \frac{\mathbf{A_{280} - (A_{494} \times 0.3)}}{\textbf{Protein Extinction Coefficient at 280 nm}} * \textbf{Path Length Correction} \times \textbf{Dilution Factor}$$

$$\#\ \textbf{of moles FITC per mole Protein} = \frac{\mathbf{A_{494}} \times \textbf{Dilution Factor} \times \textbf{Path Length Correction}}{\mathbf{68000} \times \textbf{Protein Concentration (M)}}$$

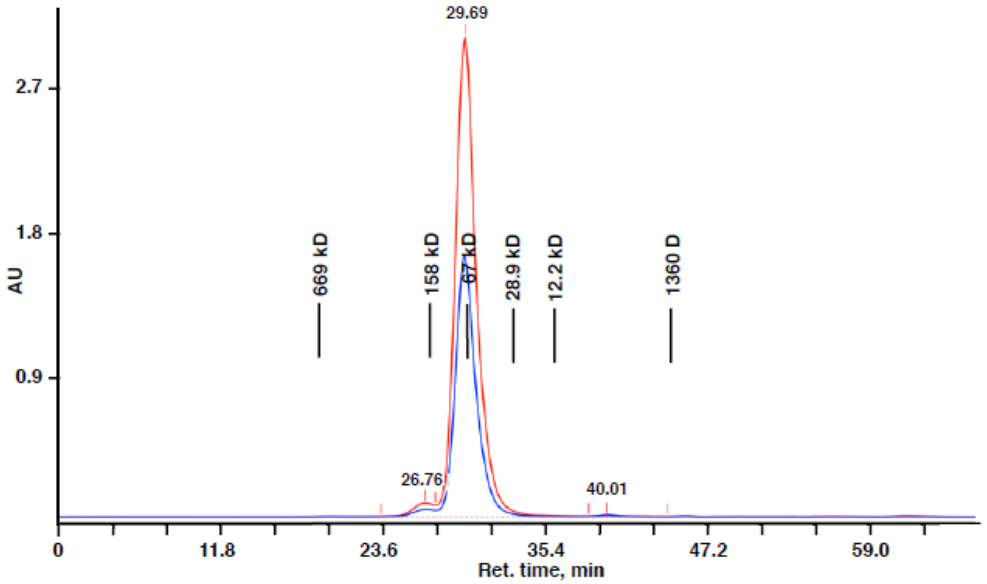

Figure: SEC chromatogram of a BSA labeled with FITC using a Superdex 200 HR 10/30 column at 0.5 ml/min. in 50 mM Tris and 0.25 M NaCl pH 7.5. The absorbance was monitored at 280 nm (Blue line) and 494 nm (Red line). The overlapping peaks of 280 nm and 494 nm indicate successful labeling of the protein with FITC. In addition, the spin column format ensured that the purification of protein was fast and there was no unreacted FITC left after the protein was purified according to the kit protocol.

VII. RELATED PRODUCTS:

EZLabel™ Antibody-FITC Labeling Kit (K831)
Annexin V-FITC Apoptosis Kit (K101)
Annexin V-FITC Reagent (1001)
BSA (2119)
Albumin, Human Plasma (7546)
Annexin V-Biotin Apoptosis Kit (K109)
Annexin V- Cy5 Apoptosis Kit (K103)
Annexin V- PE Apoptosis Detection Kit (K128)
Red Fluorescent Protein-R-PE (R-Phycoerythrin) (6005)
CD11b FITC Monoclonal Antibody (Clone ICRF44) (6955)
CD3 FITC Monoclonal Antibody (Clone OKT3) (6952)
CD8 FITC Monoclonal Antibody (Clone OKT-8) (6954)
Ez-Desalt™ Spin Desalting Columns (6564)
Hi-Bind™ Protein A-Agarose (6520)
Hi-Bind™ Albumin-IgG Depletion Beads (7933)
Biotinylated AGE-BSA (7929)
Hi-Bind Ni QR Agarose Beads (6562)
Annexin V-Cy3 Apoptosis Kit (K102)
Annexin V-EGFP Apoptosis Kit (K104)
Annexin V-PE-Cy5 Apoptosis Detection Kit (K129)
Red Fluorescent Protein Monoclonal Antibody (3984)
CD11c FITC Monoclonal Antibody (Clone 3.9) (6956)
CD4 FITC Monoclonal Antibody (Clone RPA-T4) (6953)
Goat Anti-Human IgG (H&L) FITC (6915)

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