ab288089 – EZLabel Protein FITC Labeling Kit

For labeling proteins with Fluorescein Isothiocyanate (FITC) in a user-friendly spin column format.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab288089

Storage and Stability

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

Materials Supplied

Item	Quantity	Storage Condition
EZLabel Elution Buffer	10 ml	+4°C
EZLabel FITC	5 vials	+4°C
EZLabel Quenching Buffer	1 ml	+4°C
EZLabel Spin Column	5 units	+4°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

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

Protein FITC Labeling Protocol

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.
  Δ 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.

Labeling Reaction:

1. 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.
2. Dissolve completely by pipetting up and down.
3. Transfer 100 µl of the prepared protein to a 1.5 ml microcentrifuge tube.
4. Add reconstituted EZLabel FITC solution and mix well by pipetting up and down. Incubate at RT on rotary shaker or mixer for 1 hr.
5. After incubation, add 20 µl EZLabel Quenching Buffer to quench the reaction & incubate again at RT for 30 min. Total volume at this stage should not exceed 130 µl.
   Δ 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.

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.
2. 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.
3. 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.
4. 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.
   Δ 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.
5. **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).

Calculation (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} nm) and 494 nm (A_{494} nm).
- If necessary, dilute the labeled protein in EZLabel Elution Buffer. Calculate the concentration of labeled protein and degree of labeling using following equations:

$$\textbf{Concentration of Labeled Protein } (M) =$$

$$\frac{A_{280} - (A_{494} \times 0.3)}{Protein\ Extinction\ Coefficient\ at\ 280\ nm} \times Path\ Length\ Correction \times Dilution\ Factor$$

$$\textbf{\# of moles FITC per mole Protein} = \frac{A_{494} \times Dilution\ Factor \times Path\ Length\ Correction}{6800 \times Protein\ Concentration\ (M)}$$

Technical Support

For all technical or commercial enquiries please go to:
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