ab288100 – Antibody FITC Labeling Kit

An easy way to label antibodies with 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/ab288100

Storage and Stability

- Store the kit at 4°C, protected from light.

Materials Supplied

Item	Quantity	Storage Condition
EZLabel FITC	5 Vials	4°C
EZLabel Spin Column	5 Columns	4°C
EZLabel Quenching Buffer	1 mL	4°C
EZLabel Elution Buffer	10 mL	4°C

Materials Required, Not Supplied

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

Reagent Preparation

- Read the entire protocol before performing the experiment.
- Briefly spin small vials prior to opening.
- Bring the kit components to room temperature before use.

Protocol

1. Antibody Solution Preparation:

– The volume of antibody solution should not exceed 100 µL. For best results, use 100 µL of ~5-10 mg/mL antibody.
Δ Note: *Buffers that contain primary amines (e.g., Tris or glycine) interfere with the intended FITC conjugation. Dialyze the antibody against 0.1 M sodium bicarbonate buffer (pH 8.5-9.0) just before labeling experiment is performed.*

2. Labelling reaction

– Each vial of EZLabel FITC is sufficient for labeling of 1 mg of antibody.
– 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 antibody to a 1.5 mL microcentrifuge tube. Add reconstituted EZLabel FITC solution and mix well by pipetting up and down.
– Incubate at RT on rotary shaker or mixer for 1 hr. 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 antibody is less than 1 mg, the amount of EZLabel FITC also needs to be lowered accordingly to avoid over-labeling of antibody with FITC that could result in potential fluorescence quenching of the antibody conjugate.*

3. Purification of Labeled Antibody

– 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.
– 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 antibody.

Δ Note: *For smaller antibody fragments, a second elution step might be necessary to recover the labeled antibody. 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*

– Optional: Dialyze the labeled antibody in the dark against a desired storage buffer containing 20-30% glycerol and if necessary, a carrier protein (e.g. BSA). Store the dialyzed antibody in a tube wrapped with aluminum foil at 4 °C (for short term) or -20 °C (for long term).

Calculation

In some cases, it is advantageous to determine the number of molecules of FITC per molecule of antibody (degree of labeling). For that, measure the absorbance of the labeled antibody at 280 nm (A280 nm) and 494 nm (A494 nm). If necessary, dilute the labeled antibody in EZLabel Elution Buffer. Calculate the concentration of labeled antibody and degree of labeling using following equations:

$$\textbf{Conc. of labeled Antibody } (M) = \frac{A280 - (A494 \times 0.3)}{203000} \times \textit{Path Length Correction} \times \textit{Dilution Factor}$$

$$\textbf{\# of moles FITC per mole Antibody} = \frac{A494 \times \textit{Dilution Factor} \times \textit{Path Length Correction}}{68000 \times \textit{Antibody Concentration } (M)}$$

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)

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