Antibody FITC Labeling Kit (#BN01048)

(Store at 4°C)

Cat. No. BN01048 contains sufficient reagents to label and purify 5 x 1 mg of antibody

I. Introduction:

Assay Genie's Antibody FITC Labeling Kit provides an easy way to label antibodies with Fluorescein Isothiocyanate (FITC) in a user-friendly spin column format. FITC is an ideal dye for fluorescent labeling of antibodies among the most commonly used fluorescent dyes for labeling antibodies. Each spin column provided in the kit can be used to label up to 1 mg of the target antibody. The kit provides all of the reagents necessary to perform five labeling reactions using up to 1 mg of antibody per reaction. FITC-labeled antibody 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:

- Labeled antibodies can be used for ELISA, western blot, Immunohistochemistry, Immunofluorescence, and FACS analysis

III. Kit Contents:

Components	BN01048	Cap Code	Part Number
FITC	5 vials	Red	BN01048-1
Spin Column	5 columns	-	BN01048-2
Quenching Buffer	1 ml	Clear	BN01048-3
Elution Buffer	10 ml	NM	BN01048-4

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. Antibody FITC Labeling Protocol:

A. 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.

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.

B. Labeling Reaction: Each vial of FITC is sufficient for labeling of 1 mg of antibody. Reconstitute one vial of 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 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 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 antibody is less than 1 mg, the amount of 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.

C. Purification of Labeled Antibody:

1. During the labeling reaction, snap off the bottom closure of a 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 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 antibody.

   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 Spin Column, followed by washing with Elution Buffer or other suitable storage buffer of choice.
3. 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).

D. Calculations (Optional): 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 (A_{280}) and 494 nm (A_{494}). If necessary, dilute the labeled antibody in Elution Buffer. Calculate the concentration of labeled antibody and degree of labeling using following equations:

$$\textbf{Concentration of labeled Antibody (M)} = \frac{\mathbf{A_{280} - (A_{494} \times 0.3)}}{\mathbf{203000}} * \textbf{Path Length Correction} \times \textbf{Dilution Factor}$$

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

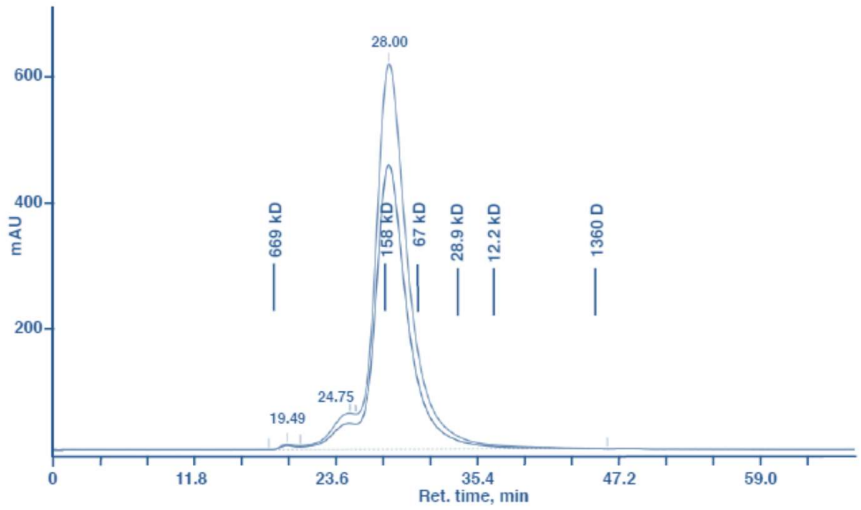

Figure: SEC chromatogram of an Anti-BSA IgG labeled with FITC using a Superdex 200 HR 10/30 column at 0.5 ml/min. in 50 mM Tris and 0.25 M NaCl pH 7.5. The absorbance was monitored at 280 nm (Blue line) and 494 nm (Red line). The overlapping peaks of 280 nm and 494 nm indicate successful labeling of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

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