Protein Cy5 Labeling Kit (#BN01056)

(Store at 4°C)

Cat. No. BN01056, contains sufficient reagents to label and purify 5 x 1 mg of protein

I. Introduction:

Assay Genie's Protein Cy5 Labeling Kit provides an easy way to label proteins with Cy5 in a user-friendly spin column format. Cy5 is an ideal fluorescent dye for labeling of proteins with its intense signal in the red region of the spectrum. Each pair of spin columns provided in the kit can be used to purify up to 1 mg of the labeled target protein. The kit provides all of the reagents necessary to perform five labeling reactions using up to 1 mg of protein per reaction. The Cy5-labeled protein has excitation and emission wavelengths at 650 nm and 670 nm respectively, and can be directly used for downstream applications including ELISA, western blot, Immunohistochemistry, Immunofluorescence, and FACS, etc.

II. Applications:

- Cy5 Labeled proteins can be used for ELISA, western blot, Immunohistochemistry, Immunofluorescence, FACS, etc.

III. Kit Contents:

Components	BN01056	Cap Code	Part Number
Cy5	5 vials	Red	BN01056-1
Spin Column	10 columns	-	BN01056-2
Elution Buffer	10 ml	NM	BN01056-3

IV. User Supplied Reagents and Equipment:

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

V. Reagent Preparation and Storage Conditions:

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 before use.

VI. Protein Cy5 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 Cy5 conjugation. Dialyze the protein using **Assay Genie's Dialyzer tubes** against 0.1 M sodium bicarbonate buffer (pH 8.5-9.0) just before labeling experiment is performed to remove the primary amines.

B. Labeling Reaction: Each vial of Cy5 is sufficient for labeling of 1 mg of protein. Reconstitute one vial of Cy5 with 5-10 µl of 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 Cy5 solution and mix well by pipetting up and down. Incubate at room temperature on rotary shaker or mixer for 1 hr. Total volume at this stage should not exceed 110 µl.

Note: If the amount of protein is less than 1 mg, the amount of Cy5 also needs to be lowered accordingly to avoid over-labeling of protein.

C. Purification of Labeled Protein:

1. During the labeling reaction, snap off the bottom closure of an 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 110 µl of Elution Buffer. Close the cap and centrifuge at 1500 x *g* for 1 min. Discard the flow through. Repeat this washing step for at least a total of three times.
2. Load the labeling reaction mix (max. 110 µl) to the first spin column drop by drop. Centrifuge the column for 2 min. at 1500 x *g* to collect the eluant.
3. Transfer the eluant onto the second unused spin column drop by drop. Centrifuge the column for 2 min. at 1500 x *g* to collect the labeled protein.
4. Optional: Dialyze the labeled protein in the dark against a desired storage buffer containing 20-30% glycerol and if necessary, add carrier protein (e.g. BSA) after the dialysis. 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 Cy5 per molecule of protein (degree of labeling). For that, measure the absorbance of the labeled protein at 280 nm (A_{280}) and 650 nm (A_{650}). If necessary, dilute the labeled protein in Elution Buffer. Calculate the concentration of labeled protein and degree of labeling using following equations:

$$\textbf{Concentration of labeled Protein (M)} = \frac{\mathbf{A_{280} - (A_{650} \times 0.05)}}{\textbf{Protein Extinction Coefficient at 280 nm}} \times \textbf{Path Length Correction} \times \textbf{Dilution Factor}$$

$$\textbf{\# of moles Cy5 per mole Protein} = \frac{\mathbf{A_{650}} \times \textbf{Dilution Factor} \times \textbf{Path Length Correction}}{\mathbf{250000} \times \textbf{Protein Concentration (M)}}$$

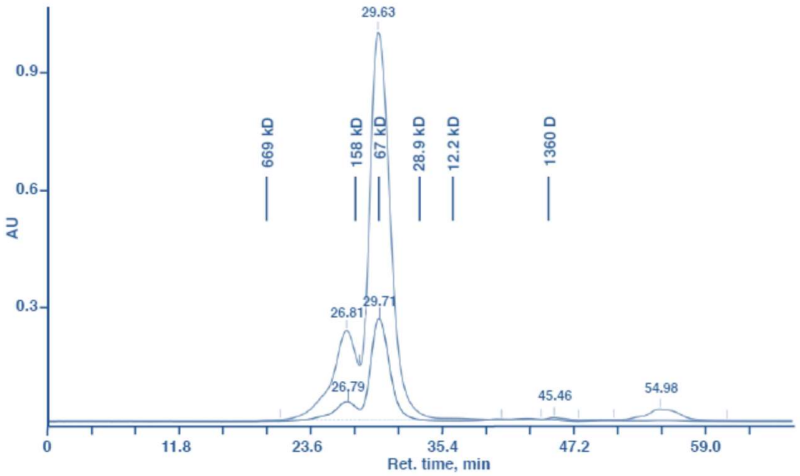

Figure: SEC chromatogram of BSA labeled with Cy5 using a Superdex 200 HR 10/30 column at 0.5 ml/min. in 50 mM Tris and 0.25 M NaCl pH 7.5. The absorbance was monitored at 280 nm (Blue line) and 650 nm (Red line). The spin column format ensured that the purification of protein was fast and there was no unreacted Cy5 left after the protein was purified according to the kit protocol.

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