AssayGenie

Protein Cy3 Labeling Kit (#BN01054)

(Store at 4°C)

Cat. No. BN01054, contains sufficient reagents to label and purify 5 x 1 mg of protein

I. Introduction:

Assay Genie's Protein Cy3 Labeling Kit provides an easy way to label proteins with Cy3 in a user-friendly spin column format. Cy3 is a better alternative to Alexa Fluor 555, Rhodamine Red-X, and TAMRA due to its high extinction coefficient and far red emission. 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 Cy3-labeled protein has excitation and emission wavelengths at 550 nm and 570 nm respectively, and can be directly used for multiple downstream applications including ELISA, western blot, Immunohistochemistry, Immunofluorescence, FACS, etc.

II. Applications:

- Cy3 Labeled proteins can be used for ELISA, western blot, Immunohistochemistry, Immunofluorescence, FACS, etc.

III. Kit Contents:

Components	BN01054	Cap Code	Part Number
Cy3	5 vials	Red	BN01054-1
Spin Column	10 columns	-	BN01054-2
Elution Buffer	10 ml	NM	BN01054-3

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 Cy3 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 Cy3 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 Cy3 is sufficient for labeling of 1 mg of protein. Reconstitute one vial of Cy3 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 Cy3 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 Cy3 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 Cy3 per molecule of protein (degree of labeling). For that, measure the absorbance of the labeled protein at 280 nm (A_{280}) and 550 nm (A_{550}). If necessary, dilute the labeled protein in Elution Buffer. Calculate the concentration of labeled protein and degree of labeling using following equations:

$$\textbf{Concentration of labeled Protein (M)} = \frac{\mathbf{A_{280} - (A_{550} \times 0.08)}}{\textbf{Protein Extinction Coefficient at 280 nm}} \times \textbf{Path Length Correction} \times \textbf{Dilution Factor}$$

$$\textbf{\# of moles Cy3 per mole Protein} = \frac{\mathbf{A_{550}} \times \textbf{Dilution Factor} \times \textbf{Path Length Correction}}{\mathbf{150000} \times \textbf{Protein Concentration (M)}}$$

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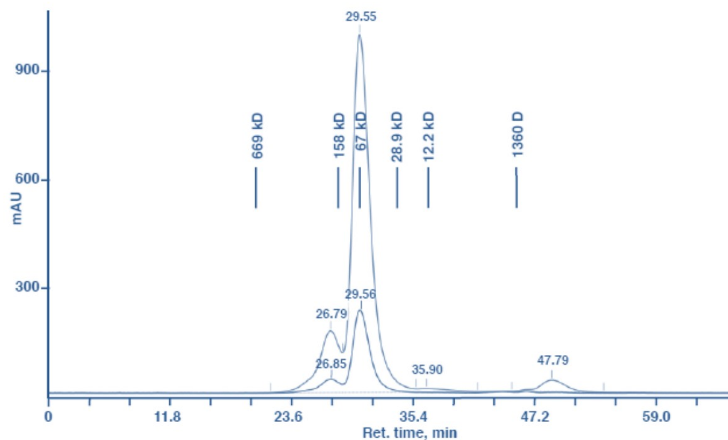

Figure: SEC chromatogram of BSA labeled with Cy3 using a Superdex 200 HR 10/30 column at 0.5 ml/min. in 50 mM Tris and 0.25 M NaCl pH 7.5. The absorbance was monitored at 280 nm (Blue line) and 550 nm (Red line). The spin column format ensured that the purification of protein was fast and there was no unreacted Cy3 left after the protein was purified according to the kit protocol.

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