ab288096 – Protein Cy5 Labeling Kit

An easy way to label proteins with Cy5 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/ab288096

Storage and Stability

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

Materials Supplied

Item	Quantity	Storage Condition
EZLabel Cy5	5 Vials	4°C
EZLabel Spin Column	10 Columns	4°C
EZLabel Elution Buffer	10 mL	4°C

Materials Required, Not Supplied

- Microcentrifuge
- DMSO/DMF
- Fresh 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. Protein Solution Preparation:
    a. 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 Cy5 conjugation. Dialyze the protein against 0.1 M sodium bicarbonate buffer (pH 8.5-9.0) just before labelling experiment is performed to remove the primary amines.*

2. Labelling reaction
    a. Each vial of EZLabel Cy5 is sufficient for labelling of 1 mg of protein.
    b. Reconstitute one vial of EZLabel Cy5 with 5-10 µl of DMSO or DMF just before use. Dissolve completely by pipetting up and down.
    c. Transfer 100 µl of the prepared protein to a 1.5 ml microcentrifuge tube.
    d. Add the reconstituted EZLabel Cy5 solution and mix well by pipetting up and down.
    e. Incubate at room temperature on rotary shaker or mixer for 1 hr. Total volume at this stage should not exceed 110 µl.

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

3. Purification of Labeled Protein
    a. During the labelling 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 110 µl of EZLabel 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.
    b. Load the labelling 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.
    c. 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.
    d. 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 aluminium 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 Cy5 per molecule of protein (degree of labelling). For that, measure the absorbance of the labelled protein at 280 nm (A280) and 650 nm (A650). If necessary, dilute the labelled protein in EZLabel Elution Buffer. Calculate the concentration of labelled protein and degree of labelling using following equations:

$$Conc.\ of\ labeled\ Protein\ (M) = \frac{A280\ -\ (A650\ \times\ 0.05)}{Protein\ Extinction\ Coefficient\ at\ 280\ nm}\ \times\ Path\ Length\ Correction\ \times Dilution\ F$$

$$\#\ of\ moles\ Cy5\ per\ mole\ Protein\ =\ \frac{A650\ \times\ Dilution\ Factor\ \times\ Path\ Length\ Correction}{250000\ \times\ Protein\ Concentration\ (M)}$$

Technical Support

For all technical or commercial enquiries please go to:

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