ab288093 – Protein Biotin Labeling Kit

Easy to use Kit to label up to 5 mg of Protein with Biotin for multiple applications such as ELISA, western blot, Immunohistochemistry, Immunofluorescence and FACS analysis.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
https://www.abcam.com/ab288093

Storage and Stability

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

Materials Supplied

Item	Quantity	Storage Condition
EZLabeL Biotin	5 vials	+4°C
EZLabel Spin Column	10 columns	+4°C
EZLabel Elution Buffer	10 ml	+4°C

Materials Required, Not Supplied

These materials are not included in the kit but will be required to successfully utilize this assay:

- Microcentrifuge, DMSO/DMF, and 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.

Sample Preparation

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.

Δ Note: *Buffers that contain primary amines (e.g. Tris or glycine) interfere with the intended Biotin conjugation. It is recommended to Dialyze the protein against 0.1 M sodium bicarbonate buffer (pH 8.5-9.0) just before labeling experiment is performed to remove the primary amines.*

Assay Protocol

- Each vial of EZLabel Biotin is sufficient for labeling of 1 mg of protein.

Labeling Reaction:

1. Reconstitute one vial of EZLabel Biotin 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.
2. Add reconstituted EZLabel Biotin 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.

   Δ Note: *If the amount of protein is less than 1 mg, the amount of EZLabel Biotin also needs to be lowered accordingly to avoid overlabeling of protein.*

Purification of Labeled Protein:

1. During the labeling reaction, snap off the bottom closure of an EZLabel Spin Column and place in a fresh microcentrifuge tube.
2. 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.
3. 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.
4. 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.

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).

Calculation

In some cases, it is advantageous to determine the number of molecules of Biotin per molecule of protein (the degree of labeling). For that, determine the concentration of the labeled protein by an appropriate method (ab102535 - Bradford assay etc.) It may be necessary to dilute the labeled protein in EZLabel Elution Buffer for protein measurement. Calculate the number of Biotin(s) per molecule of protein using a Biotin Assay Kit (Colorimetric) (ab185441).

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)