



# EZLabel™ Protein Biotin Labeling Kit

6/15

(Store at 4°C)

Cat. No. K835-5, contains sufficient reagents to label and purify 5 x 1 mg of protein

## I. Introduction:

BioVision's EZLabel™ Protein Biotin Labeling Kit provides an easy way to label proteins with Biotin in a user-friendly spin column format. Due to its high affinity for tetrameric avidin proteins, biotin is ideal for labeling proteins. 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. Biotin-labeled protein can be directly used for multiple downstream applications including ELISA, western blot, Immunohistochemistry, Immunoassays, etc.

## II. Applications:

- Biotin labeled proteins can be used for ELISA, western blot, Immunohistochemistry, Immunoassays, etc.

## III. Kit Contents:

Components	K835-5	Cap Code	Part Number
EZLabel™ Biotin	5 vials	Red	K835-5-1
EZLabel™ Spin Column	10 columns	-	K835-5-2
EZLabel™ Elution Buffer	10 ml	NM	K835-5-3

## 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 Biotin 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 Biotin conjugation. Dialyze the protein using BioVision's DiaEasy™ Dialyzer tubes (Cat #s K1000 to K1022) against 0.1 M sodium bicarbonate buffer (pH 8.5-9.0) just before labeling experiment is performed to remove the primary amines.

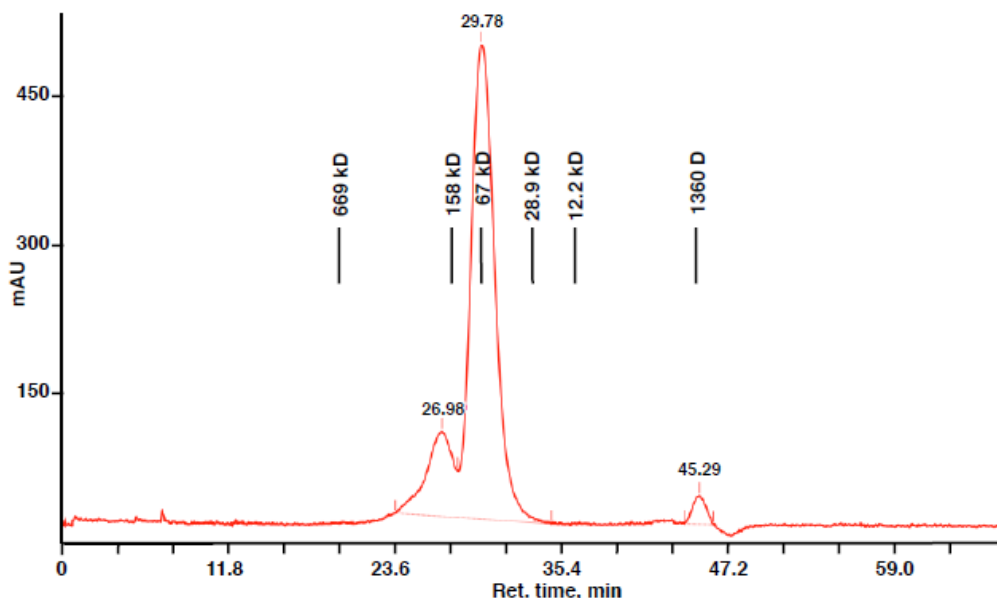
**B. Labeling Reaction:** Each vial of EZLabel™ Biotin is sufficient for labeling of 1 mg of protein. Reconstitute one vial of EZLabel™ Biotin with 5-10 µl of DMSO or DMF just before use. Dissolve completely by pipetting up and down. Transfer 100 µl of the prepared protein to a 1.5 ml microcentrifuge tube. 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.

**Note:** If the amount of protein is less than 1 mg, the amount of EZLabel™ Biotin 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 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.
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 Biotin per molecule of protein (the degree of labeling). For that, determine the concentration of the labeled protein by an appropriate method [ $A_{280}$ , BCA assay kit (K813), Bradford assay (K810) 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 BioVision's Biotin Quantitation Kit (Colorimetric) (BV# K811-100).



**Figure:** SEC chromatogram of a BSA labeled with Biotin 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. The spin column format ensured that the purification of protein was fast and there was no unreacted Biotin left after the protein was purified according to the kit protocol.

#### VII. RELATED PRODUCTS:

Biotin Quantitation Kit (Colorimetric) (K811)	Biotin-LC-NHS (2345)
Biotin-LC-LC-NHS (2346)	Biotin-NHS (2347)
Biotin-PEG <sub>4</sub> -amine (2791)	Biotinylated Bovine Serum Albumin (Biotin-LC-BSA) (7099)
Annexin V-FITC Apoptosis Kit (K101)	Hi-Bind™ Protein A-Agarose (6520)
Annexin V-FITC Reagent (1001)	Hi-Bind™ Albumin-IgG Depletion Beads (7933)
Human IgG (1296)	Mouse IgG (1265)
Rabbit IgG (1268)	Hi-Bind Ni QR Agarose Beads (6562)
Annexin V-Biotin Apoptosis Kit (K109)	Annexin V-Cy3 Apoptosis Kit (K102)
Annexin V- Cy5 Apoptosis Kit (K103)	Annexin V-EGFP Apoptosis Kit (K104)
Annexin V- PE Apoptosis Detection Kit (K128)	Annexin V-PE-Cy5 Apoptosis Detection Kit (K129)
Red Fluorescent Protein R-PE (R-Phycoerythrin) (6005)	Red Fluorescent Protein Monoclonal Antibody (3984)
EZlabel™ Antibody FITC Labeling Kit (K831)	EZlabel™ Protein FITC Labeling Kit (K832)
EZlabel™ Antibody Cy5 Labeling Kit (K838)	EZlabel™ Protein Cy5 Labeling Kit (K839)
EZlabel™ Antibody Cy3 Labeling Kit (K836)	EZlabel™ Protein Cy3 Labeling Kit (K837)
EZ-Desalt™ Spin Desalting Columns (6564)	EZlabel™ Protein Antibody Labeling Kit (K834)

**FOR RESEARCH USE ONLY! Not to be used on humans.**