Surface protein biotinylation

Authors: Daehun Park[1,2], Pietro De Camilli[1,2]

[1]Departments of Neuroscience and of Cell Biology, Howard Hughes Medical Institute, Program in Cellular Neuroscience, Neurodegeneration and Repair, Yale University School of Medicine, New Haven, Connecticut 06510, USA;

[2]Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD, 20815

Abstract

This protocol describes surface protein labeling with biotin using EZ-Link Sulfo-NHS-LC-Biotin. This chemical reacts with primary amines such as lysine but does not permeate cell membranes because of the charge. Thus, it only biotinylates surface proteins.

Solutions to prepare

1. EZ-Link Sulfo-NHS-LC-Biotin

    0.25 mg/ml EZ-Link Sulfo-NHS-LC-Biotin (Thermo) in ice-cold PBS. This solution should be made fresh just before use.

2. Quenching solution

    50 mM glycine in ice-cold PBS (keep at 4 °C).

3. 1% triton X-100 lysis buffer

    20 mM Tris-HCl, pH 8, 1% triton X-100, 10% glycerol, 137 mM NaCl, 2 mM EDTA (keep at 4 °C and add protease inhibitor cocktails just before use)

4. 2x sample buffer

    100 mM Tris, 4% SDS, 0.2% bromophenol blue, 20% glycerol in DW.

Protocol

1. Wash cells three times with ice-cold PBS and incubate 30 min at 4 °C to label surface proteins. A rocking platform is recommended.

Note1. There are many washing steps. Thus, cells may detach from the dishes. If this occurs, coat dishes with poly-D-lysine.

2. Discard biotin containing medium. Quench and remove unbound biotin using 50 mM glycine in ice-cold PBS for 10 min at 4 °C.

3. Wash 2-3 times and lysis cells with 1% triton X-100 lysis buffer and centrifuge the samples at 14,000 g for 20 min at 4°C.

4. Collect supernatants and discard the pellets.

5. Measure the protein concentrations using BCA Protein Assay Kit.

6. Incubate the same amount of lysates (500-1000μg) with streptavidin or NeutraAvidin beads for 2 hours to overnight at 4 °C to pull-down the biotinylated proteins.

7. Wash the beads three times with lysis buffer by cycles of suspension and centrifugation and elute proteins from the packed beads by adding an equal volume of 2x sample buffer and boiling for 5 min 95 °C.

8. Run eluate samples on a SDS polyacrylamide gel and perform western blotting to visualize labeling (and thus evidence of surface expression) of the protein of interest. Total cell lysates can be used to determine the expression level.