Biotinylation of Cell Surface Proteins

Guo N. Huang*

Program in Biochemistry, Cellular and Molecular Biology, Johns Hopkins University School of Medicine, Baltimore, USA

*For correspondence: guo.n.huang@gmail.com

[Abstract] Membrane proteins are major sensors of extracellular stimuli and initiators of intracellular signal transduction, and their abundance on the cell surface in particular is often dynamically regulated even when there are no significant changes of their total abundance in a cell. This protocol is designed to biochemically label and separate membrane proteins on the plasma membrane from those in the intracellular compartments. In conjunction with co-immunoprecipitation and western blot analysis, functional analysis of dynamic interaction of membrane proteins with other membrane proteins or intracellular adaptor and effector proteins can be achieved.

Materials and Reagents

1. HEK293 cells
2. Poly-dL-Ornithine (PLO) (Sigma-Aldrich, catalog number: P8638)
3. Boric Acid (Sigma-Aldrich, catalog number: B0394)
4. Sulfo-NHS-SS-biotin solution (Thermo Fisher Scientific, Pierce Antibodies, catalog number: 21331)
5. Neuroavidin beads (Thermo Fisher Scientific, Pierce Antibodies, catalog number: 29200)
6. $EDTA{\cdot}2H_2O$ (MW 372.2) (Sigma-Aldrich, catalog number: E1644)
7. $EGTA{\cdot}2H_2O$ (MW 380.4) (Sigma-Aldrich, catalog number: E4378)
8. NaPyrophophate (Sigma-Aldrich, catalog number: S9515)
9. NaF (Sigma-Aldrich, catalog number: S7920)
10. $Na_3VO_4{\cdot}10H_2O$ (MW 446.1) (Sigma-Aldrich, catalog number: 5-9515)
11. Protease inhibitor (F. Hoffmann-La Roche, catalog number: 1873580)
12. $NaVO_3$
13. NaOH
14. PBS
15. $CaCl_2$
16. $MgCl_2$
17. Glycine

18. Triton
19. HCl
20. 4x loading buffer
21. Biotin quenching solution (see Recipes)
22. PBS/$CaCl_2$/$MgCl_2$ (see Recipes)
23. IP buffer (see Recipes)
24. Stock (see Recipes)

Equipment

1. 0.22 μm filters
2. 12-well plates
3. Western blot equipment

Procedure

1. Prepare PLO solution: Add 90 mg PLO into 180 ml borate buffer (dissolve 1.668 g borate acid in 180 ml H_2O and adjust pH to 8.3 using NaOH), and sterilize by filtering through 0.22 μm filters.
2. Coat 12-well plates with PLO solution at room temperature overnight in the culture hood (1 ml per well). Wash three times with sterile water on the second day before cell plating.
3. Plate HEK293 cells on poly-ornithine coated 12-well plates and transfect as needed.
4. On the day of cell harvest, dissolve Sulfo-NHS-SS-Biotin in PBS/$CaCl_2$/$MgCl_2$ at 0.5 mg ml^{-1} and store on ice.
5. Wash cells with 1 ml ice-cold PBS/$CaCl_2$/$MgCl_2$ twice.
6. Biotinylation
   a. Add 0.3 ml of 0.5 mg/ml Sulfo-NHS-SS-biotin solution.
   b. Incubate on ice for 30 min, with occasional shaking.
7. Wash three times with ice-cold quenching solution and incubate 5 min before each solution removal to allow neutralization of uncrosslinked reagents.
8. Lyse cells with 120 μl IP buffer and incubate 10 min on ice.
9. Transfer all contents of each well into a 1.5-ml tube and spin at Vmax (about 14,000 rpm) on a 4 °C desktop centrifuge for 10 min.
10. Save 10 μl supernatant (added 10 μl 4x loading buffer) as offers.
    Mix 100 μl supernatants with 50 μl 50% slurry of immobilized Neutravidin and rotate for 2-3 h at 4 °C.

11. Spin 1 min at 5,000 rpm in a 4 °C centrifuge. Remove the supernatant and wash the beads with 1 ml IP buffer by rotating the mixture for 5 min in the 4 °C room. Wash three times in total.
12. After the final wash, remove the supernatant and add 25 µl 4x loading buffer to the beads. Mix well and put the tubes in a 65 °C water bath for 10 min to denature proteins (65 °C is chosen because we find that in boiling water, many membrane proteins form aggregates, which results in failure of proteins to migrate into acrylamide gels). Load 10 µl for western blot analysis.

Recipes

1. Biotin quenching solution (pH 7.4)
   50 mM glycine (0.1877 g) in 50 ml PBS/$CaCl_2$/$MgCl_2$
2. PBS/$CaCl_2$/$MgCl_2$
   PBS (+2.5 mM $CaCl_2$, 1 mM $MgCl_2$, pH 7.4)
3. IP buffer (pH 7.4)

PBS		50 ml
EDTA	5 mM	0.5 ml x 0.5 M (pH 7.4)
EGTA	5 mM	0.5 ml x 0.5 M (pH 7.4)
NaPyrophophate	10 mM	0.223 g
NaF	50 mM	0.1 g
$NaVO_3$	1 mM	0.5 ml x 100 mM

   1% Triton
   1 tablet Protease inhibitor
4. Stock
   a. 0.5 M EDTA (pH 7.4)
      93.05 g EDTA·$2H_2O$
      500 ml ddH_2O
      Add NaOH until EDTA dissolves and add HCl to adjust pH back to 7.4.
   b. 0.5 M EGTA (pH 7.4)
      95.1 g EGTA·$2H_2O$
      500 ml ddH_2O
      Add NaOH until EDTA dissolves and add HCl to adjust pH back to 7.4.
   c. 100 mM Na_3VO_4
      1.84 g Na_3VO_4·10 H_2O
      Add HCl to pH less than 10 (yellow solution). Boil the solution until clear and colorless.

d. 10 ml aliquots and freeze at -20 °C.

Acknowledgments

This work was supported by National Institute on Drug Abuse (NIDA; DA00266, DA10309) and the National Institute of Mental Health (NIMH; MH068830). This protocol was previously used in and adapted from Huang *et al.* (2006).

References

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