Biotinylation and Precipitation of Cell Surface Proteins

Reference

1. Paganoni, S., Anderson, K. L., & Ferreira, A. (2004). Differential subcellular localization of Ror tyrosine kinase receptors in cultured astrocytes. *Glia*, *46*(4), 456–466. https://doi.org/10.1002/glia.20023

Kits

1. Pierce EZ-Link-Sulfo-NHS-SS-Biotin, Cat# 21331
2. Pierce NeutrAvidin UltraLink Resin, Cat# 53150

Solutions

1. RIPA Homogenization Buffer: 50 mM Tris-HCl pH 7.4, 150 mM NaCl, 1 mM EDTA
   - Add to 2 ml aliquot before use: 12 mM NaF, 2.7 mM Na_3VO_4, 3 mM PMSF, 1% Protease Inhibitor Cocktail
2. RIPA Lysis Buffer: 50 mM Tris-HCl pH 8, 150 mM NaCl, 0.1% SDS, 1% NP-40
   - Add to 2 ml aliquot before use: 12 mM Sodium Deoxycholate ($C_{24}H_{39}NaO_4$), 119 mM NaF, 27 mM Na_3VO_4, 1% Protease Inhibitor Cocktail, 1% Triton X-100

Abbreviation

1. PBS: Phosphate Buffered Saline

Protocol

1. Remove medium and rinse with PBS (3 x 5 minutes).
2. Incubate with 1 mg/mL EZ-Link-Sulfo-NHS-SS-Biotin in PBS for 30 minutes in the cold room (with occasional stirring). One dish doesn't get biotin but only PBS (negative control).
3. Aspirate the saline solution and rinse cells 3 x 15 minutes in ice cold PBS buffer plus 100 mM glycine (glycine will quench any unbound biotin).
4. Incubate in 1 mL of RIPA buffer for 45 minutes in the cold room with agitation then scrape.
5. Spin for 20 minutes at 13,200 rpm to pellet the nuclei/debris.
6. Transfer the supernatant to a fresh tube (discard the pellet). Optional: save 50 µL of the supernatant, dilute it in 50 µL of 2X Laemmli buffer and boil for 10 minutes (this will be the "total cell lysate fraction").
7. Incubate the rest of the S/N with 300 µL of UltraLink Immobilized NeutrAvidin which had been pre-equilibrated with the lysing buffer. This incubation is performed in the cold room with shaking overnight.

8. Next day, spin at 14,000 x g for 15 minutes to pellet. Discard supernatant. Optional: save 50 µL of the supernatant, dilute it with 50 µL of 2X Laemmli buffer and boil it (this will be the "intracellular fraction").
9. Rinse the pellet at least 4 times with 500 µL of lysing buffer, discarding the supernatant after each rinse. After each rinse, centrifuge to collect pellet (as per step 9). The pellet contains the "biotinylated fraction".
10. After final rinse, aspirate and discard supernatant. Resuspend pellet in 100 µL of 2X Laemmli. Boil 5 minutes to dissociate complex.
11. Sample is now ready for SDS-PAGE.

NOTES

- Refer to the manufacturer's instructions for different applications
- Use PBS pH 8 (measured at 4°C).