Immunoprecipitation Protocol for Tissue Samples

Reference

1. Paganoni, S., Bernstein, J., & Ferreira, A. (2010). Ror1-Ror2 complexes modulate synapse formation in hippocampal neurons. Neuroscience, 165(4), 1261–1274. https://doi.org/10.1016/j.neuroscience.2009.11.056

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. BSA: Bovine Serum Albumin

Protocol (All steps should be done at 4°C or on ice to minimize protease activity)

1. Homogenize tissue sample in 500 µL of RIPA buffer in 1.5 mL Eppendorf tube.
2. Centrifuge @15,000 x g, 4°C for 30 min to pellet nuclei. The supernatant is the total cell lysate.
3. Transfer supernatant to fresh Eppendorf tube, add 15 µL of a 1 mg/ml BSA stock solution (made in RIPA buffer) and rock for 1h at 4°C.
4. In fresh Eppendorf tube, combine supernatant with 10 µL of primary antibody and incubate for 1-2 hours at 4°C rocking.
5. Add 25 µL of pre-equilibrated Protein resin and incubate while rocking for 1 hour at 4°C.
   a. Neutravidin comes in 50% v/v slurry, to pre-equilibrate in RIPA:
      i. Aliquot ~26 µL slurry into Eppendorf tube
      ii. Centrifuge at medium speed ~4,000 x g for 1 minute
      iii. Decant supernatant
      iv. Resuspend pellet in 13 µL RIPA buffer and repeat washes ~3 times
      v. After final spin, decant supernatant and resuspend pellet in 13 µL RIPA
6. Spin at 14,000 x g for 1 minute to pellet, discard supernatant.
7. Wash at least 4 times with the lysing buffer (RIPA), centrifuge to collect pellet, discard the supernatant after each wash.
8. After final wash, discard the supernatant and resuspend pellet in 30-40 µL of 2X Laemmli buffer.
9. Boil samples for 5-10 minutes to dissociate the complex.