Immunocytochemistry - Cultured Cells

References

1. Afreen, S., & Ferreira, A. (2022). The formation of small aggregates contributes to the neurotoxic effects of tau_{45-230}. *Neurochemistry international*, *152*, 105252. https://doi.org/10.1016/j.neuint.2021.105252
2. Afreen, S., & Ferreira, A. (2019). Altered Cytoskeletal Composition and Delayed Neurite Elongation in tau_{45-230}-Expressing Hippocampal Neurons. *Neuroscience*, *412*, 1–15. https://doi.org/10.1016/j.neuroscience.2019.05.046
3. 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

Solutions

1. Fixative: 4% Paraformaldehyde prepared in PBS w/ 4% Sucrose, pH 7.4 w/ NaOH
2. Blocking Buffer: 10% Bovine Serum Albumin prepared in PBS

Abbreviations

1. NaOH: Sodium Hydroxide
2. PBS: Phosphate Buffered Saline
3. PFA: Paraformaldehyde
4. BSA: Bovine Serum Albumin
5. RT: Room Temperature

Protocol

1. Rinse cells grown on coverslips once in PBS then fix cells for 15 min in 4% PFA w/ 4% sucrose (pre-warmed at 37°C).
2. Remove the PFA and rinse 3 x 15 minutes with PBS. These can be stored at 4°C in PBS.
3. Day 1 - In a 30 or 60 mm dish, permeabilize the cells with 0.3% Triton X-100 prepared in PBS for 4 min at RT.
4. Rinse 3 x 5 min with PBS.
    a. Skip steps 1 and 2 if using antibodies with extracellular epitopes to visualize protein membrane. Just rinse once in PBS.
5. With the coverslips placed separately on wax sheet, cells-up, block non-specific binding with 100 µl of 10% BSA prepared in PBS per coverslip for 1 h at RT. Cover the wax sheet to minimize evaporation.
6. Prepare the primary antibody solution (100 µl per coverslip) at the correct concentration in 1% BSA (dilute the 10% BSA with PBS). NOTE: If using more than one primary antibody, make sure they were prepared from different species.

7. Remove the coverslips from the blocking solution and place cells-up on a wax sheet in a humid container (can be made out of a Petri dish with wet filter paper under the wax sheet).
8. Cover each coverslip with 100 µl primary antibody solution.
9. Store overnight at 4°C.
10. Day 2 - Rinse 3 x 5 min with PBS.
11. Prepare secondary antibody solution (100 µl per coverslip) at the appropriate concentration in 1% BSA (in PBS). Always make 50 µl more than you need per secondary antibody (or avidin) and spin in benchtop centrifuge for 5 min and pipet from the top.
    a. NOTE: If using more than one secondary, make sure they're from different species and can be seen under different filters, ex: fluorescein vs rhodamine.
    b. If secondary antibody is not biotinylated, proceed to step 14.
12. Place coverslips cells-up in a humid box as described above.
13. Cover each coverslip with 100 µl secondary antibody solution.
14. Incubate at 37°C for 1 h.
15. If secondary is biotinylated, rinse after incubation 3 x 5 min and then add the avidin for 1 h at 37°C.
16. Rinse 2 x 5 min with PBS.
17. Carefully remove paraplast dots with forceps and rinse 1 x 5 min with PBS.
18. Mount coverslips on clean labeled microscope slides using ~5-10 µl slow-fade mounting media.
19. Allow coverslips to dry in the dark overnight at RT.
20. Next day, rinse coverslips with MilliQ water to dissolve dried salts.
21. Store the coverslips at 4°C.