TUNEL Assay Staining for Cultured Cells on Coverslips

References

1. Lang, A. E., Riherd Methner, D. N., & Ferreira, A. (2014). Neuronal degeneration, synaptic defects, and behavioral abnormalities in tau_{45-230} transgenic mice. *Neuroscience*, *275*, 322–339. https://doi.org/10.1016/j.neuroscience.2014.06.017
2. Park, SY., & Ferreira, A. (2005). The Generation of a 17 kDa Neurotoxic Fragment: An Alternative Mechanism by which Tau Mediates β-Amyloid-Induced Neurodegeneration. Neuroscience, 22, 5365-5375. https://doi.org/10.1523/JNEUROSCI.1125-05.2005

Kit

1. Roche, In Situ Cell Death Detection Kit, TMR red, Cat# 12156792910

Abbreviations

1. PBS: Phosphate Buffered Saline
2. BSA: Bovine Serum Albumin
3. RT: Room Temperature
4. PBS-T: Phosphate Buffered Saline w/ 0.1% Tween 20

Protocol

1. Fix coverslips containing hippocampal neurons by covering with 4% paraformaldehyde (4% Sucrose) for 20 min at room temperature.
2. Rinse slides with PBS. Coverslips may be stored in PBS at 4°C.
3. Permeabilize cells with 0.3% Triton X-100 made in PBS for 4 min at RT.
4. Rinse coverslips with 3 x 5 mins with PBS.
5. Block non-specific binding with 100 µl/coverslip of 10% BSA made in PBS for 1 hr at RT.
6. Add primary antibody in PBS-T containing 1% BSA, incubate at 4°C overnight.
7. Wash coverslips 3 x 5 mins in PBS at RT.
8. Prepare TUNEL Reaction Mixture/2°antibody solution (55 µl/coverslip): WEAR GLOVES
    a. Add 45 µL of Label Solution (from kit)
    b. Add 5 µL of Enzyme Solution (from kit)
    c. Add 5 µL of Secondary Antibody
    d. Mix by vortexing (light sensitive, minimize exposure)
9. Incubate coverslips in a humidified chamber at 37°C for 1 hr in the dark.
10. Rinse coverslips 3 x 5 min with PBS, air dry, and mount coverslips using antifade mounting medium.
11. Examine cells by fluorescence at an excitation wavelength in the range of 520-560 nm or confocal microscopy.