TUNEL Assay Staining for Fixed Tissue Sections

Reference

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

Kit

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

Abbreviations

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

Protocol

1. Block sections in 5% normal goat serum + 1% BSA in PBS-T for 1 hour at RT.
2. Add primary antibody in PBS-T (0.2% Triton-X) containing 1% normal goat serum and 1% BSA, incubate at 4°C overnight.
3. Wash 4 x 20 mins in PBS at RT.
4. Prepare TUNEL/2°antibody solution (1 ml per well): WEAR GLOVES
    a. Add 450 µl of Label Solution to new 1.6 ml Eppi tube (from kit)
    b. Add 50 µl of Enzyme Solution (from kit)
    c. Add 5 µl of Secondary Antibody
    d. Mix by vortexing (light sensitive, minimize exposure)
5. Cover 12 well plate in aluminum foil and incubate at 37°C for 1 hour.
6. Wash 3 x 15 mins in PBS at RT.
7. Mount on gelatin coated slides.
8. Examine cells by fluorescence at an excitation wavelength in the range of 520-560 nm or confocal microscopy.