CHRISTIE LAB
UNIVERSITY OF VICTORIA

Ki-67 Immunohistochemistry
(Free-Floating Brain Sections)

1 DAY 1

1. Rinse 3 x 10 min in 0.1 M TBS. (5 mL/well)
2. Antigen Unmasking Technique - Incubation with Citric Acid.
   Citric Acid Solution: 10 mM (100 mL: 210.1 mg/100 mL TBS), pH=6.0
   Incubate at 95°C 2 x 5 min (change citric acid solution in between and allow the vials to cool for approx. 20 min) (5 mL/well)
3. Rinse 3 x 10 min in 0.1 M TBS. (5 mL/well)
4. Quench in 3% H_2O_2 and 10% MetOH in 0.1 M TBS for 10 min. (5 mL/well)
   (1 mL solution: 100 µL 30% H_2O_2, 100 µL MetOH and 800 µL 0.1 M TBS)
5. Rinse 3 x 10 min in 0.1 M TBS. (5 mL/well)
6. Preincubate in 5% normal goat serum (NGS) and 0.25% Triton X-100 in 0.1 M TBS (Blocking Solution) for 1 h at RT. (5 mL/well)
   (1 mL solution: 50 µL NGS, 25 µL 10% Triton X-100, 925 µL 0.1 M TBS)
7. Incubate with primary antibody (rabbit polyclonal anti-Ki-67) (1:500; Vector) in 5% Blocking Solution at +4°C for 48 hours.
   (1 mL solution: 2 µL anti-Ki-67, 998 µL 5% Blocking Solution)
   (Transfer brain sections from nets to 12 or 24-well plates. 500 or 1000 µL/well)

2 DAY 2

8. Rinse 2 x 10 min in 0.1 M TBS with 0.25% Triton X-100. (5 mL/well)
   (1 mL solution: 25 µL 10% Triton X-100, 975 µL 0.1 M TBS)
9. Rinse 1 x 10 min in 2% NGS Blocking Solution. (5 mL/well)
   (1 mL solution: 20 µL NGS, 25 µL 10% Triton X-100, 955 µL 0.1 M TBS)
10. Incubate with secondary antibody (biotin-conjugated goat anti-rabbit IgG) (1:200; Vector) in 5% Blocking Solution at RT for 2 hours.
    (1 mL solution: 5 µL secondary antibody, 50 µL NGS, 25 µL 10% Triton X-100, 920 µL 0.1 M TBS)
    (Transfer brain sections from nets to 12 or 24-well plates. 500 or 1000µL/well)
11. Rinse 3 x 10 min in 0.1 M TBS. (5 mL/well)
12. Incubate in Vectastain Elite ABC solution for 1 h at RT.
    (5 mL solution: 1drp A + 1 drp B in 5 ml 0.1 M TBS; vortex and let stand for 30 min before using)
    (Transfer brain sections from nets to 12 or 24-well plates. 500 or 1000 µL/well)

13. Rinse 3 x 10 min in 0.1 M TBS. (5 mL/well)

14. Develop in DAB for about 2-3 min.
    (5 mL solution: 2 drops of buffer, 4 drops of DAB, 2 drops of H_2O_2, 2 drops of niquel sol. in 5ml of dH_2O; mix well in between) (4 mL/well)

15. Rinse 3 x 10 min in 0.1 M TBS. (5 mL/well)

16. Mount brain sections (in 0.1 M TBS) onto 2% gelatin-coated microscope slides. Let them dry on a rack over-night (covered with foil).

17. Dehydrate the slides on:
    - 50% EtOH (5 min)
    - 70% EtOH (5 min)
    - 100% EtOH (5 min)
    - CitriSolv (5 min)

18. Coverslip with Paramount. Let slides dry overnight (covered with foil).