

Ki-67 Staining

Reagents Needed:

VectaStain Elite Kit (Vector Laboratories, Item: PK-6100)
DAB Peroxidase Substrate Kit (Vector Laboratories: SK-4100)
Antibodies
PBS
Normal Goat Serum
Methanol
Hydrogen Peroxide
Hematoxylin QS (Vector Laboratories, H3404)
Ethanol
Xylene or HistoClear
Permount

Use frozen sections (cut with Cryostat on Superfrost Plus slides)

1. Thaw sections briefly
2. Rinse in PBS
3. Quench endogenous peroxidase activity: 0.3% H₂O₂ in MeOH x 30 min
4. Rinse in PBS
5. Block in PBS + 2% Normal Goat Serum (PBS+NGS)
6. Dilute 1° antibody 1:100 in PBS+NGS. Leave O/N @ 4°C (cover slides with cover slips to keep antibody solution from evaporating)
7. Wash 3X in PBS + NGS (10 min each wash)
8. Add Biotin α-Mouse 2° diluted 1:200 in PBS+NGS. Incubate for 1 hr at RT
9. Wash 3X 10 min each in PBS
10. Make ABC solution 30 min before use! (from VectaStain kit)
 - Incubate slides in ABC solution for 1 hr at RT
 - ABC solution:
 - per 5 ml PBS add:
 - 2 drops reagent A
 - 2 drops reagent B
11. Wash 3X 10 min each in PBS
12. DAB staining (from DAB substrate kit)
 - Incubate slides in DAB solution for 10 min at RT
 - DAB solution:
 - per 5 ml ddH₂O:
 - 2 drops buffer
 - 2 drops DAB
 - 2 drops H₂O₂
13. Rinse 3x in ddH₂O
14. Counterstain and dehydrate
 - Dilute Hematoxylin QS (20 drops in 2 ml ddH₂O).

- Apply to sections. Incubate ~ 1 min.
 - Rinse in ddH₂O
 - 70% EtOH 1 min
 - 85% EtOH 1 min
 - 95% EtOH 1 min
 - Xylenes or HistoClear 1 min
15. Use Permount and cover with coverslip