

**Mouse anti-human Ki-67 (BD Pharmingen/BD Biosciences, San Jose, CA, Cat # 550609, Lot # 3165816) Paraffin/ formalin sections: Works on mouse tissue!**

**\*\*Block all the way through with 10% Normal Horse Serum if your tissue is extra juicy i.e. pregnant uteri, if not, just use 10% NHS or in some case use SUPERBLOCK through primary Ab, secondary AB, and EAP\*\***

1. Deparaffinize:
  - (1) Xylene 2 x 5 min
  - (2) 100% EtOH 2 x 4 min
  - (3) 95% EtOH 2 x 4 min
  - (4) 70% EtOH 4 min
  - (5) Rinse with water to remove EtOH 4 min
2. Place slides in plastic Coplin Jar with no lid. Fill with 1x citrate buffer (Biocare Medical). Place in decloaker with 500 ml of water, set for 3 min. Let slides cool for an extra 10 min when cycle is done. Rinse with water (add water, dump a little, repeat). For decloaker, turn the dial past 20 and then back to 3 min.
3. Block endogenous peroxidase with 3% H<sub>2</sub>O<sub>2</sub> for 15 minutes (20 mL 30% H<sub>2</sub>O<sub>2</sub> in 180 mL water). **Must not be more than a few months old.**
4. Rinse slides with 1x Wash Buffer (**AB**). ----- **Prepare fresh everytime with MQ H<sub>2</sub>O** -----
  - a. **50 mM Tris, 20 mM NaCl, 0.05% Tween-20**  
(50 mL 1 M Tris+4 mL 5M NaCl+500 µL Tween+ 945.5 mL MQ H<sub>2</sub>O)
5. Wipe around edge and circle tissue with Immunedge pen.
6. Rinse 2 x 2 min with **AB**
7. Block with blocking diluent: **10% Normal Horse Serum (NHS)** in AB (Jackson ImmunoResearch) for 30 min at room temp
  - a. **Or block with 1% milk + 1% BSA +10% NHS in AB (SUPERBLOCK) all the way through primary antibody, biotinylated antibody, and EAP**
  - b. **Recipe = 10 mg milk + 10 mg BSA + 100 uL NHS + 1,000 uL AB**
8. Dilute **Anti-Ki-67 1:100 in 10% NHS (or in superblock)** in AB incubate at 1 h RT (4°C overnight)
  - a. **1:100 Mouse IgG** in Blocking diluent for Negative control
9. Rinse slides 3 x 5 minutes with wash buffer.
10. Drain slides and apply **Biotinylated Horse Anti-Mouse IgG (1:300 in 10% NHS in AB or in superblock)** 30 min at RT.
11. Rinse **3 x 5 min** with **AB**
12. Apply **Vectastain R.T.U. Elite** (Vector Laboratories, PK7100) for 30 min at RT.
13. Rinse 3 x 5 min in wash buffer
14. **Rinse 2x with dH<sub>2</sub>O - don't forget to wash first!!**
15. Mix DAB (1 drop/mL) and **apply to slides 5 min or until the sections turn brown then wash immediately**
16. Wash slides with water
17. Transfer slides to a dish and rinse in running water for 3 min.
18. Counterstain 30 seconds in hematoxylin; rinse in water 3 min.
19. Dunk in TBS (1 min) to turn the stain blue
20. Wash with water for 3 min.
21. Dehydrate
  - (1) 70% EtOH 2 x 3 min
  - (2) 95% EtOH 2 x 3 min
  - (3) 100% EtOH 2 x 3 min
  - (4) Xylene 2 x 3 min; 1 x 5 min
  - (5) Coverslip with permount
  - (6) Let the permount dry under the hood