**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% H2O2 for 15 minutes (20 mL 30% H2O2 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 H2O** -------
   1. **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 H2O)

1. Wipe around edge and circle tissue with Immunedge pen.
2. Rinse 2 x 2 min with **AB**
3. Block with blocking diluent: **10% Normal Horse Serum (NHS)** in AB (Jackson Immunoresearch) for 30 min at room temp
   1. **Or block with 1% milk + 1% BSA +10% NHS in AB (SUPERBLOCK) all the way through primary antibody, biotinylated antibody, and EAP**
   2. **Recipe =** 10 mg milk + 10 mg BSA + 100 uL NHS + 1,000 uL AB
4. Dilute **Anti-Ki-67** **1:100 in 10% NHS (or in superblock)** in AB incubate at 1 h RT (4°C overnight)
   1. 1:100 **Mouse IgG** in Blocking diluent for Negative control
5. Rinse slides 3 x 5minutes with wash buffer.
6. Drain slides and apply **Biotinylated** **Horse Anti-Mouse IgG** (1:300 in **10% NHS in AB or in superblock**) 30 min at RT.
7. Rinse **3 x 5** min with **AB**
8. Apply **Vectastain R.T.U. Elite** (Vector Laboratories, PK7100) for 30 min at RT.
9. Rinse 3 x 5 min in wash buffer
10. Rinse 2x with dH2O - don’t forget to wash first!!
11. Mix DAB (1 drop/mL) and **apply to slides 5 min or until the sections turn brown then wash immediately**
12. Wash slides with water
13. Transfer slides to a dish and rinse in running water for 3 min.
14. Counterstain 30 seconds in hematoxylin; rinse in water 3 min.
15. Dunk in TBS (1 min) to turn the stain blue
16. Wash with water for 3 min.
17. 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