

PGR- progesterone receptor (Santa Cruz) Paraffin/ formalin sections

1. Deparaffinize:
 - a. Xylene 2 x 5 min
 - b. 100% EtOH 2 x 3 min
 - c. 95% EtOH 2 x 3 min
 - d. 70% EtOH 3 min
 - e. Automation buffer 2 x 5 min
2. Heat-Induced Epitope Retrieval Using the Decloaker.
 - a. Fill canister with **1x citrate buffer** (10mM Sodium Citrate, 0.05% Tween 20, pH 6.0)
 - i. Tri-sodium citrate (dehydrate) 2.94 g + MQ water 1000 ml, Mix to dissolve. Adjust pH to 6.0 with 1N HCl and then add 0.5 ml of Tween 20 and mix well. Store this solution at room temperature for 3 months or at 4°C for longer storage.
 - b. Place in decloaker with 500 mL of water. Fill the other 2 canisters with 250 mL water
 - c. Use **program 5**: 110°C 15 min cycle – wait until the cycle is done
 - d. Let slides cool for an extra **10 min** in the decloaker ☐ and outside the decloaker for another **10 min**
 - e. Rinse slide in running **dH₂O** for **3 min**
3. Block endogenous peroxidase with 3% H₂O₂ for 15 minutes.
 - a. Rinse **2 x 5 min** in 1x automation buffer (**AB**) –Make fresh--- 50 mM Tris, 20 mM NaCl, 0.05% Tween-20 (50 mL 1 M Tris + 4 mL 5M NaCl + 0.5 mL Tween+ 945.5 mL MQ H₂O)
 - b. Draw a circle around the tissue with Pap pen ~ 5 mm away from the tissue
4. Block with **10% Normal Horse Serum in AB (10% NHS)** for 20 min RT (50-100 µL/section)
5. Block with **Avidin D/Biotin** solutions (Avidin-Biotin blocking Kit, Vector Laboratories, SP2001)
 - a. Avidin D solution for **15 min RT, quick rinse with AB**
 - b. Biotin solution for **15 min RT, DO NOT RINSE** slides, only wipe excess block
6. **anti-PR (1:50, SC-398898 mouse monoclonal PR)** in **10% NHS** at **1h 40 min** RT or 4°C O/N
 - a. Rinse **2 x 5 min** in **AB**
7. Drain slides and apply **Biotinylated horse anti-mouse (1:1000)** in **10% NHS**, incubate 35 min at RT.
 - a. Rinse **2 x 5 min** in **AB**
8. Apply **Vectastain R.T.U. Elite** (Vector Laboratories, PK7100) 30 min RT.
 - a. Rinse **2 x 5 min** in **AB** – Then quick rinse with **MQ H₂O**
9. Apply the **DAB** Chromogen (Dako). Incubate RT 5 min
 - a. Wash with dH₂O & Rinse in **running dH₂O** for **3 min**.
10. Counterstain **30 seconds** in **hematoxylin**; rinse slides in tap water until water is clear
 - a. Gently agitate slides in **AB** until the tissues turn blue (I did **30 sec**).
11. Dehydrate
 - a. 95% EtOH 1 x 3 min
 - b. 100% EtOH 3 x 3 min
 - c. Xylene 2 x 5 min & Coverslip with Permount®