

ER(Thermo #MA5-13191) 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)
Recipe = Tri-sodium citrate (dehydrate) 2.94 g + MQ H₂O 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 RT for 3 months. Place in decloaker with 500 mL of water. Fill the other 2 canisters with 250 mL water
 - b. Use **program 5**: 110°C 15 min cycle – wait until the cycle is done
 - c. Let slides cool for an extra **10 min** in the decloaker and outside the decloaker for another **10 min**
 - d. 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 -
 - c. Dap or wipe off excess AB with Kimwipe
4. Block with **10% Normal Horse Serum in AB (10% NHS)** for 20 min RT (50 µL/section)
5. **Apply 1° Ab: Anti-ERα (1:200, Thermo #MA5-13191) in AB** at 1h RT or 4°C O/N
 - a. Rinse **3 x 5 min** in **AB** (⇒ Wipe off excess AB)
6. Drain slides and apply **2° Ab: Biotinylated horse anti-mouse (1:400) in AB**, incubate 30 min at RT.
 - a. Rinse **3 x 5 min** in **AB** (⇒ Wipe off excess AB)
7. Apply **Vectastain R.T.U. Elite** (Vector Laboratories, PK7100) 30 min RT.
 - a. Rinse **3 x 5 min** in **AB** – Then quick rinse with **MQ H₂O twice** (⇒ Wipe off excess MQ H₂O)
8. Apply the **ImmPACT** (Vector Lab, ImmPACT DAB Peroxidase (HRP) Substrate SK-4105), black box @ 4°C.
 - a. By adding 30 µL of DAB Chromogen concentrate/ 1,000 µL of DAB Diluent
 - b. Incubate DAB mixture = 2 min ⇒ Then, Wash with dH₂O & Rinse in **running dH₂O for 3 min**.
9. Counterstain **1 min** in **hematoxylin**; rinse slides in **running dH₂O** until water is clear
 - a. Gently agitate slides in **AB** until the tissues turn blue (~**1 min**).
10. Dehydrate
 - a. 70% EtOH 1 x 3 min ⇒ 95% EtOH 2 x 3 min ⇒ 100% EtOH 2 x 3 min ⇒ Xylene 2 x 5 min
 - b. Coverslip with Permount®