

Immunofluorescence

Paraffin Sections

(w/o Trilogy)

Note: This IF method is for use on tissues prone to falling off slides (e.g. adult skulls), which cannot withstand the high-pressure, 15-minute antigen retrieval step. See IF for Paraffin w/ Trilogy for samples with better slide-adhesion. Trilogy shortens the dehydration and antigen retrieval steps, so it is faster than this classical method, and also is reportedly a more effective antigen retrieval (e.g. more positive cells based on cleaved Caspase staining)

Tissue Processing Procedure

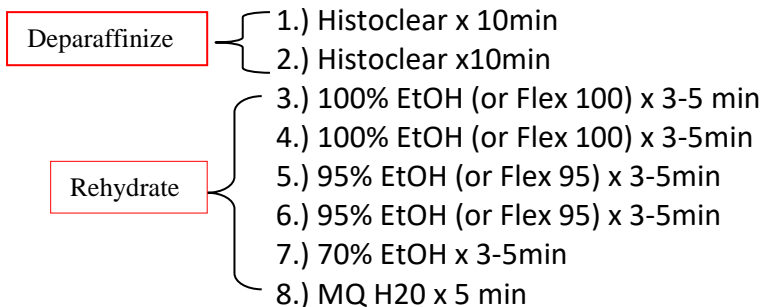
1. Fix tissues in formalin (or 4%PFA) immediately after collection 1hr/mm of tissue (perfused tissue is best)
2. Wash in 1X PBS for 3x10 min
3. Put in 70% EtOH, process through EtOH steps, HistoClear & Wax (see Manual Processing Protocol)
4. Section embedded tissues to make 6um thick sections

Day 1 (~2.5 hours)

5. Deparaffinize slides **60min**:

Done using 250 ml of solution in staining buckets with slide rack

Note – never let your slides dry out!!!!!! Throughout this whole procedure



Note all of the above steps do not have to be exact... you can leave for longer

6. Antigen retrieval (Heat Induced Epitope Retrieval) **30min**:
 - a. In **Plastic** Coplin jar(s), place slides in **Dako Antigen Retrieval Buffer** (To prep: in fridge, make excess of 1:10 dilution with distilled water, pH to 6.0-6.2, optimal is pH 6.1, using HCl if necessary.) (Alternatively, may use 10 mM **sodium citrate buffer**); antigen retrieve for 10 min at low pressure in the pressure cooker (filled between the 6 and 8 mark with MQ water).
 - i. When pressure cooker is done (**takes roughly 20 minutes**) – quick release the steam, allowing pressure to normalize

- ii. Take off lid, remove Coplin jar(s) – careful! They'll be hot, use oven mitt and set them on bench, remove lids and allow them to cool to ~tepid RT. Wait for water to cool before discarding from the cooker.
- iii. Pull Coplin jar out and let cool to warmish RT – 20min maybe?

7. Wash in PBS 1x **5 min** in Coplin jar, on bench, RT
8. Block for 1-2 hours at room temperature with fresh (no longer than 4 weeks old) 5% BSA/0.3% Triton X-100 (**OR** 10% heat-inactivated Goat Serum with 0.3% TritonX)
9. Incubate samples with primary antibodies overnight (>16 hrs) at 4°C in 1% BSA/0.3% Triton X-100 **OR** 5% HIGS/0.1% Triton X-100. Use 50-200 ul of primary antibody solution per slide (sample size dependent). Best if in a level, moist container (blue slide containers, holds 20 slides each, or use tip boxes with 1 inch MQ water inside)

Primary Antibody	Concentration
Pax 5	1:200
F4/80	1:400
E-cadherin	1:500

****Do NOT add Ab to your Secondary alone control here!**

Instead, incubate in 1% BSA/0.3% Triton X-100 **or** HIGS/Triton o/n

Day 2 (~3.5 hrs-4 hours)

10. Save primary Abs label with concentration and date – use for 3-4wks, keep at 4C
11. PBS wash 5 x 5 min, RT
12. Secondary antibodies for 2 hours at room temperature in 1% BSA/0.3% Triton X-100 **OR** 5% HIGS/0.3% Triton X-100. [50-200 ul per slide] Use moist container and **KEEP DARK with foil. (Most secondary antibodies are diluted 1:500)**
13. PBS wash 5 x 5 min with agitation, RT
14. DAPI stain (from 5000x stock → dilute to 1x in PBS) for 5 min (still protect from light)
15. Coverslip with Prolong Gold Antifade or Fluoromount G (Aqueous, anti-fade mounting medium); Wipe off excess media, Seal with nail polish; Let dry at least 1 hour before imaging; store/ dry away from light