DISCOVERIES THAT MAKE A DIFFERENCE

Core Imaging Facility

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Brief Immunofluorescence Protocol

(Non-conjugated primary and conjugated secondary)

1. Remove embedding matrix
   a) paraffin - run through deparaffinization process
   b) cryo - rinse in water

2. Dry slightly (don't let tissue dry out; can be done with a KimWipe) and circle tissue with PapPen. Do this for one slide at a time.

3. Add PBS to tissue and hydrate until ready for next step

4. Block with 2% BSA (or blocking solution of choice) for 45 minutes

5. 1 Ab (or PBS for control) for 45 minutes

6. Rinse 4X, 5 min. each with PBS

7. 2 Ab** (or PBS for control) for 1 hour

8. Rinse 2X, 5 min. each with PBS

9. DAPI counterstain if applicable; 1 minute

10. Rinse 2X, 5 min. each with PBS

11. Mount coverslip; seal if necessary

12. Store covered (protected from light) at 4C. Temperature of storage may be dependent on fluorophore.

* Each set of experiments should contain a negative control (the slide is subjected to the same procedure, but instead of adding antibodies all steps receive buffer) and a 2 Ab control (no primary is added, but 2 Ab is added.) If desired, a non-specific primary antibody control may also be performed.

* All steps are performed at room temperature.

** Samples should be protected from light as much as possible after addition of 2 Ab. The easiest way to do this is to cover them with a box or other non-transparent object.