ST. MICHAEL'S
UNITY HEALTH TORONTO

RCF
HISTOLOGY

Immunofluorescence Protocol

1. Preparation of Slides

A. Cell Lines

- Grow cultured cells on sterile glass cover slips or slides overnight at 37 ° C
- Wash briefly with PBS
- Fix as desired. Possible procedures include:

  10 minutes with 10% formalin in PBS (keep wet)
  5 minutes with ice cold methanol, allow to air dry
  5 minutes with ice cold acetone, allow to air dry

- Wash in PBS

B. Frozen Sections

- Snap frozen fresh tissues in liquid nitrogen or isopentane pre-cooled in liquid nitrogen, embedded in OCT compound in cryomolds. Store frozen blocks at - 80 °C.
- Cut 4-8 um thick cryostat sections and mount on superfrost plus slides or gelatin coated slides. Store slides at - 80 °C until needed.
- Before staining, warm slides at room temperature for 30 minutes and fix in ice cold acetone for 5 minutes. Air dry for 30 minutes.
- Wash in PBS

C. Paraffin Sections

- Deparaffinize sections in xylene, 2x5min.
- Hydrate with 100% ethanol, 2x3min.
- Hydrate with 95% ethanol, 1min.
- Rinse in distilled water.
- Follow procedure for pretreatment as required.

(Last reviewed May 23rd, 2025)

2. Pretreatments of Tissue Sections

Antigenic determinants masked by formalin-fixation and paraffin-embedding often may be exposed by epitope umasking, enzymatic digestion or saponin, etc. Do not use this pretreatment with frozen sections or cultured cells that are not paraffin-embedded.

3. Procedure

1. Rinse sections in PBS-Tween 20 for 2x2min
2. **Serum Blocking**: incubate sections with normal serum block – species same as secondary antibody, for 30 minutes to block non-specific binding of immunoglobulin. Note: since this protocol uses avidin-biotin detection system, avidin/biotin block may be needed based on tissue type. If you do, the avidin/biotin block should be done after normal serum block.
3. **Primary Antibody**: incubate sections with primary antibody at appropriate dilution in primary antibody dilution buffer for 1 hour at room temperature or overnight at 4 C.
4. Rinse in PBS-Tween 20.
5. Secondary Antibody: incubate sections with biotinylated secondary antibody at appropriate dilution in PBS for 30 minutes at room temperature.
6. Rinse in PBS-Tween 20 for 3x2min.
7. **Detection:** incubate sections in FITC-Avidin D in PBS for 30 minutes at room temperature. Protecting slides from light starting from this step to the end by covering slides with aluminum foil or black box.
8. Rinse in PBS-Tween 20 for 3x2min.
9. **Counterstain** with PI or DAPI if desire.
10. Rinse in PBS-Tween 20.
11. Dehydrate through 95% ethanol for 2 min, 100% ethanol for 2x3min.
12. Coverslip with anti-fade mounting medium.