Immunofluorescence on FFPE sections
Janes Lab Protocols


1. Formalin-fixed paraffin-embedded human tumor tissue blocks are received by the Biorepository and Tissue Research Facility (BTRF)
   - The BTRF removes any patient identifiers from blocks and performs 5 μm tissue sectioning
   - The BTRF deparaffinizes the sections and performs High pH TR Dako antigen retrieval for 20 mins at 97 °C with 0 psi. The antigen retrieval time may be cut in half if the formalin fixation was weak for a particular specimen.
   - It is recommended to validate antibodies by immunohistochemistry before performing immunofluorescence.
   - It is recommended to have an adjacent section stained by hematoxylin and eosin for comparison.

2. Deparaffinized and antigen-retrieved slides are received from the BTRF in PBS.
   - Once slides are received, proceed with the protocol immediately. Do not allow slides to sit in PBS for an extended period of time.
   - Take note of the tissue fragility throughout staining procedure

3. Wash slides 2 x 5 min in PBS.

4. Block slides in a humid chamber for 1 hr at room temperature in blocking solution: 1x Western Blocking Reagent (Roche #11921673001) diluted in PBS + 0.1% Tween-20.
   - Humid chambers can be constructed with an inverted pipette-tip box containing a paper town saturated with water
   - 100–200 μl of blocking solution is sufficient for each slide
   - A piece of parafilm should be placed on each slide to ensure that the solution is evenly dispersed

5. Add primary antibody at the appropriate dilution in blocking solution overnight in a humid chamber at room temperature.
   - 100–200 μl of blocking solution is sufficient for each slide
   - A piece of parafilm should be placed on each slide to ensure that the solution is evenly dispersed

6. Wash slides 3 x 5 min in PBS.

7. Add secondary antibody at the appropriate dilution in blocking solution for 1 hr in a humid chamber at room temperature.
   - 100–200 μl of secondary-antibody solution is sufficient for each slide
   - 1:200 dilution of species-specific, highly-crosslinked Alexa-labeled antibodies from Molecular Probes; these fluorochromes do not need to be protected from ambient light during the staining process
   - A piece of parafilm should be placed on each slide to ensure that the solution is evenly dispersed

8. Wash slides 3 x 5 min in PBS.

9. Counterstain cells with 0.5 μg/ml DAPI and DyLight750-labeled wheat-germ agglutinin (1:350 dilution) in PBS for 5 min.
   - 100–200 μl of counterstaining solution is sufficient for each slide
   - A piece of parafilm should be placed on each slide to ensure that the solution is evenly dispersed
   - Slides may rest on the bench top at this time

10. Wash slides 2 x 5 min in PBS.

11. Incubate slides with 10 mM CuSO4 in 50 nM NH4Ac (pH 5.0; acidify with acetic acid) for 10 min.
    - CuSO4 treatment dramatically reduces autofluorescence of sections, while only slightly diminishing the fluorescence of Cy- and Alexa-family dyes (Schnell et al. J. Histochem. Cytochem. 47:719 (1999))
    - Higher concentrations of CuSO4 reduced signal, whereas lower concentrations are less effective at quenching autofluorescence
    - Apply 100–500 μl of CuSO4 treatment per slide and ensure tissue is completely immersed in solution.
• **Slides may rest on the bench top at this time**

12. Wash slides 5 min in PBS.

13. Carefully remove residual liquid on slide and mount with an antifade mounting medium.
   - *We use 0.5% N-propyl gallate in 90% glycerol + 1x phosphate buffer (pH 8.0)*
   - *To reduce spherical aberration, use a minimal amount of cells suspended in mounting medium on each coverslip. We use 10–20 μl per 24 x 50 mm coverslip depending on tissue section size (allow to spread slowly across the entire surface of the coverslip once coverslip is mounted on slide).*
   - *Remove any drops of PBS on slide with a Kimwipe before mounting. These will dilute out the mounting medium and lower its refractive index. Careful not to disturb the tissue.*

14. Seal the edges of the coverslip in clear nail polish and allow to air dry.

15. Remove residual salts from coverslip with a wet Kimwipe and air dry.

16. Store slides in 4°C until imaging.
   - *Autofluorescence will be suppressed in mounted sections for a few days, but it will increase thereafter as Cu²⁺ ions diffuse out of the tissue*
   - *Slides may be imaged for up to one week*