Immunofluorescence on FFPE sections Entered by Leen Jamal
Janes Lab Protocols 05/30/12

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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 × 5 min in PBS.
4. Block slides in a humid chamber for 1 hr at room temperature in blocking solution: 1× 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 × 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 × 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 × 5 min in PBS.
11. Incubate slides with 10 mM $CuSO_4$ in 50 nM NH_4Ac (pH 5.0; acidify with acetic acid) for 10 min.
    - *$CuSO_4$ 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 $CuSO_4$ 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 4ºC until imaging.
    - *Autofluorescence will be suppressed in mounted sections for a few days, but it will increase thereafter as Cu^{2+} ions diffuse out of the tissue*
    - *Slides may be imaged for up to one week*