Staining for Ki67 in paraffin-embedded tissue sections

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Purpose: This protocol describes how to stain sections of formalin-fixed paraffin-embedded tissue for Ki67, a protein marker of proliferation expressed during S, G2, and M phase of the cell cycle. A primary antibody is used to identify and bind to Ki67, and a secondary antibody conjugated to a fluorescent probe identifies and binds the primary antibody for imaging by fluorescence.

Materials

- Slide racks
- A microwaveable box with a lid to hold slide rack (e.g., large tip box with rack removed)
- Humidified chamber(s) with lids (pipette tip boxes with water in the bottom work well)
- Xylenes
- 100% Ethanol (EtOH)
- 90% EtOH
- 70% EtOH
- 50% EtOH
- Deionized water
- 1x Phosphate buffered saline (PBS)
- Dako Target Retrieval Solution (Agilent, S1700)
- Hydrophobic PAP pen
- Blocking buffer: 5% Bovine serum albumin (BSA) + 0.3% Triton X-100 in PBS
- Staining buffer: 1% BSA + 0.3% Triton X-100 in PBS
- DAPI with ProLong Gold Antifade (ThermoFisher, P36941)
- Ki67 primary antibody: Abcam ab15580 (rabbit host)
- Ki67 secondary antibody: select a secondary antibody that will detect rabbit antibodies and fluoresces at a wavelength your microscope can detect.

Note: Do not let tissue sections dry out!

Note: Every time you stain for immunofluorescence, four controls are required: 1 slide to receive primary antibody (Ab) only, 1 slide to receive secondary Ab only, 1 negative control (untreated) sample, and 1 sample treated with a positive control that is certain to induce proliferation.

Slides used

- Samples:
- Primary Ab-only control:

- Secondary Ab-only control:

Procedure

1. Deparaffinize slides in chemical fume hood
    a. 3x xylene, 5min each, swirling occasionally
    b. 2x 100% EtOH, 5 min each
    c. 10 min each 90% EtOH, then 70% EtOH, then 50% EtOH
    d. 2x in diH2O, 2 min each
2. Antigen retrieval – boil for ~30 minutes in Dako citrate buffer
    a. Place slide rack containing buffer into a large tip box. Fill tip box up to buffer level with diH2O.
    b. Start buffer for 2 min at full power in microwave while slides are in H2O
    c. Add slides to hot buffer and boil at power 2 for 30 minutes
        i. Check buffer every 5-10 minutes to make sure it doesn't boil down
    d. Let cool ~20 mins
3. Wash 3x with diH2O for 5 min on shaker
4. Draw hydrophobic barrier around tissue section with PAP pen and lay slides flat, face up, in the humidified chamber
5. Add blocking buffer to tissue section (5% BSA + 0.3% Triton in PBS) for 1 hr @ room temp
6. Drain blocking buffer, redraw hydrophobic barrier if needed, and return to humidified chamber. Add primary antibody
    a. Dilute antibody 1:200 in tube of staining buffer (1% BSA + 0.3% Triton in PBS)
        i. Make just slightly more Ab solution than you will need to cover tissue sections to conserve Abs
    b. Add diluted Ab to tissue section
    c. Incubate @ 4C overnight
7. Wash 3x with PBS + .05% Tween for 5 mins on shaker
8. Redraw hydrophobic barrier if needed, return slides to humidified chamber, and add secondary Ab
    a. Dilute antibody 1:400 in tube of staining buffer (1% BSA + 0.3% Triton in PBS)
    b. Add diluted Ab to tissue section
    c. Incubate @ 1 hr @ room temp
9. Wash 5x in PBS + .05% Tween for 5 mins on shaker
10. Dry off slide around tissue, add a drop of DAPI + Prolong Gold Antifade, and gently lay a coverslip on top, avoiding bubbles
11. Store slides in refrigerator until imaging.
12. Image samples on a fluorescence microscope with fixed exposure settings for both DAPI and secondary fluorophore filters. Identify different regions of the tissue on the DAPI channel to avoid biased selection of tissue regions, then capture images with both filters.

Sample Results

Blue = DAPI, nuclei

Green = Ki67, proliferating cells