Protocol: Immunohistochemistry staining of frozen sections (IHC-Fr)

This protocol is a general guide for formaldehyde-based fixation, cryostat sectioning, and fluorescent staining of frozen tissue samples. Staining conditions for specific antibody must be optimized according to different antigens of interest.

Loading Control

For tissue preparation and cryosectioning

- PBS
- 4% paraformaldehyde (PFA)/PBS
- 30% sucrose/PBS
- OCT
- Mold
- Dry ice
- Coated slides (poly-L-Lysine)
- Frozen tissue sections
- Hydrophobic barrier pen for immunohistochemistry (GTX22601)
- Primary antibodies
- Secondary antibodies
- Blocking buffer: 5% normal animal serum + 0.5% Triton X-100 in PBS
- DAPI (GTX16206) or Fluoroshield™ with DAPI (GTX30920)

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PROTOCOL

I. Tissue preparation and fixation:

- Fix tissue by perfusing the animal with freshly prepared 4% PFA/PBS or by immersing the dissected tissue in 4% PFA/PBS for 4-24 hours at room temperature.

  Note: The temperature and time for fixation need to be optimized experimentally depending on the tissue type and size.

- Cryoprotect the tissue by incubating the tissue with 30% sucrose/PBS solution and allow it to sink to the bottom of the vial.

  Note: The larger the tissue, the longer time it takes to sink.

- Remove excess sucrose from tissue and place tissue in the center of mold filled with OCT.
- Orient the OCT-embedded tissue into the desired position in the mold.
- Freeze the tissue block on dry ice.
- Store the tissue block at -80° C until ready for sectioning.

  Note: Tissue blocks can be stored at -80° C for 6-12 months if necessary.

II. Tissue sectioning using cryostat

- Move the embedded tissue block from freezer to the cryostat machine and allow its temperature to equilibrate in the cryostat chamber for approximately 30 minutes.
- Adhere tissue block to specimen disc using OCT.
- Cut the tissue in 5-20 µm thick sections (usually 7µm, thickness should be determined experimentally).
- Mount tissue sections onto coated slides (usually poly-L-lysine coated) by placing the cold sections onto warm slides.
- Slides can be stored for 6-12 months at -80° C until ready for staining.

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III. Fluorescent staining of frozen sections

- Take slides with sections out from freezer and thaw at room temperature for 10-20 minutes.
- Wash slides with PBS for three times, each for 5 minutes.
- (Optional) Perform antigen retrieval if necessary.
  Be aware that many antigen retrieval techniques may be too harsh for frozen sections. The optimal method of antigen retrieval must be determined experimentally.
- Surround each tissue section with a hydrophobic barrier using a marking pen (GTX22601).
- Block the slides with blocking buffer (PBS with 5% serum and 0.5% Triton X-100) at room temperature for 1 hour.

  Note: We recommend using serum from the species the secondary antibody was raised in.
- Incubate the slides with primary antibody diluted in blocking buffer at 4° C overnight.

  Note: Keep the slides in a humidified sealed chamber during overnight incubation to prevent slides drying out.
- Wash slides with PBS three times, each for 5 minutes.
- Incubate the slides with secondary antibody diluted in blocking buffer at room temperature for 1 hour.

  Note: Keep slides in dark from this step if using fluorophore-conjugated secondary antibodies.
- Wash slides with PBS three times, each for 5 minutes.
- Mount the slides with Fluoroshield™ with DAPI anti-fade mounting medium (GTX30920) and cover with coverslips.

  Alternative method for nucleus staining and mounting:
  9-1. Incubate the slides in 0.1-1 µg/mL Hoechst or DAPI (GTX16206) for 5 minutes.
  9-2. Wash with PBS twice before mounting.
  9-3. Mount the slides with coverslips using anti-fade FluoroGel mounting medium (GTX28214).
- Seal the edges of the coverslip with nail polish and let it dry.

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