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IHC Frozen Tissue—Indirect Method

Staining using a purified antibody

Research Use Only

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Materials

- Tris buffered saline (TBS) (50 mM Tris, 150 mM NaCl, pH 7.4) or phosphate buffered saline (PBS) (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.76 mM KH_2PO_4, pH 7.4)
- Acetone, reagent grade
- Blocking reagent: IHC/ICC blocking buffer-low protein (cat. no. 00-4952) or high protein (cat. no. 00-4953) or 10% Normal Serum (using the species from which the secondary antibody was made) in PBS or TBS
- Primary antibody: purified format
- Secondary antibody: fluorophore-conjugated
- Nuclear counterstain: DAPI or DRAQ5 (cat. no. 65-0880)
- Mounting medium: Fluoromount-G (cat. no. 00-4958) or Fluoromount-G with DAPI (cat. no. 00-4959)

Accessories

- Humidified container in which to place the samples during incubations
- Coplin jars
- Parafilm
- Glass coverslips (size appropriate to tissue section size)
- Clear nail polish

Experimental Procedure

1. Air dry cut sections for 20 min.
2. Fix the sections by immersing in acetone for 10 min using a coplin jar.
3. Rehydrate the tissue in a coplin jar in PBS or TBS for 10 min at room temperature.
   NOTE: It is critical from this point on that the tissue does not dry out as this will result in high levels of background staining and difficulty interpreting staining results.
4. Cover tissue with blocking reagent for 1 hour at room temperature (100 µL/ tissue section). To limit evaporation of blocking reagent and help evenly spread the blocking solution over the tissue, use forceps to gently overlay the tissue section with a piece of parafilm cut to the dimension of the tissue. It is not necessary to stretch the parafilm or cover edges of the slide.
   Note: PAP Pen ink can interfere with eFluor® Nanocrystal signal and is not recommended.
5. Using forceps, gently lift and remove the parafilm without disturbing the tissue section. Immerse the slide with the tissue into a coplin jar containing PBS or TBS. Using an

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orbital shaker set to low speed, gently agitate, changing the PBS or TBS wash solution 2 more times for a total of 3 washes (5 min/wash).

6. Dilute the purified antibody, at manufacturer's recommended dilution, in blocking reagent. Overlay the primary antibody solution on the tissue and cover with parafilm as described in step 4. Incubate in a humidified chamber overnight at 4°C.
7. Gently wash the tissue 3 times in PBS or TBS (5 min/wash) as described in step 5.
8. Dilute the fluorophore-conjugated secondary antibody, at manufacturer's recommended dilution, in blocking reagent, protecting from light. Overlay the secondary antibody solution on the tissue and cover with parafilm as described in step 4. Incubate in a humidified chamber for 1 hour at room temperature.
9. Gently wash the tissue 3 times in PBS or TBS (5 min/wash) as described in step 5.
10. Optional: Nuclei can be counterstained using DAPI (see step 15) or DRAQ5. It is necessary to select a counterstaining agent with a fluorescent emission spectra that does not overlap with the other fluorophores used in the experiment.
11. Mount and coverslip using Fluoromount-G or Fluoromount-G with DAPI. Seal the edge of the coverglass with clear nail polish.
12. Allow slides to dry for 1-2 hours before visualizing.
13. Slides can be stored at 4°C protected from light if needed.

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