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IHC FFPE Tissue Trypsin Digestion—Direct Method

Staining using a fluorophore-conjugated antibody

Research Use Only

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Materials

- 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)
- Clearing and rehydration/dehydration reagents: Histoclear II or Xylene, 100% Ethanol (Histology grade: 100%, 90%, and 70%)
- Antigen Retrieval reagent: 2.5% Trypsin (Fisher Scientific)
- Blocking reagent: IHC/ICC Blocking Buffer-Low Protein (cat. no. 00-4953) or High Protein (cat. no. 00-4952)
- Primary antibody: Fluorophore-conjugated format
- 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

Methods

1. Paraffin-embedded sections are cut and mounted on Superfrost plus slides. Slides are placed in plastic vertical slide holders.
2. Slides are heated for 20 min for 50-60°C in a dry oven to facilitate attachment of tissue and soften the paraffin.
   NOTE: To prevent damage to target antigens temperature should not exceed 60°C.
3. Remove paraffin and rehydrate tissue using the following slide wash/incubation sequence:
   Histoclear II (3 x 5 min each)
   100% Ethanol (2 x 5 min each)
   90% Ethanol (5 min)
   70% Ethanol (5 min)
   ddH2O (5 min)
   When moving the slides through the solutions, be sure to adequately mix the reagents and remove bubbles collecting on the slides by dipping the slide holder, with slides, up and down in the solution several times. From this point on, it is critical that the tissue does not dry out as this will result in difficulty interpreting staining results.
4. Submerge slides in 0.1% Trypsin diluted in 1X PBS for 10-20 min at 37°C.
   Incubation time must be optimized and is antigen and tissue dependent.

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5. Wash the slides in a coplin jar with 1X PBS for 5 min using gentle agitation in an orbital shaker set to low speed.
6. Cover the 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 the edges of the slide.
   NOTE: PAP Pen ink can interfere with eFluor® Nanocrystal signal and is not recommended.
7. 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. Using an orbital shaker set to low speed, gently agitate, changing the PBS wash solution 2 more times for a total of 3 washes (5 min/wash).
8. Dilute the fluorophore-conjugated antibody or combination of multiple antibodies, at manufacturer's recommended dilution, in blocking reagent, protecting from light. Overlay the primary antibody solution on the tissue and cover with parafilm as described in step 6. Incubate in a humidified chamber overnight at 4°C.
9. Gently wash the tissue 3 times in PBS (5 min/wash) as described in step 7.
10. Optional: Nuclei can be counterstained using DAPI (see step 11) 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 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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