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IHC Fluorescent Frozen Sections

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Protocol status: Working
We use this protocol and it's working

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Abstract

Immunohistochemistry protocol used for staining with fluorescent secondary antibodies to highlight specific tissue structures.

Attachments

DOCX

Fluor Immun Protocol...

20KB

Guidelines

Frozen tissues cut in 30 micron intervals were used in this protocol. Do not touch the tissue on the slide or it will come off.

Materials

MATERIALS

- Normal Donkey Serum **Catalog #**017-000-121
- Citifluor AF-1 anti-fading solution **Electron Microscopy Sciences**
- KimWipes **Fischer Scientific**
- Triton X-100 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**93426
- PBS
- ImmEdge hydrophobic barrier pap pen **Vector Laboratories Catalog #**H-4000
- Superfrost™ Disposable Microscope Slides, White; 3 × 1 in. x 1mm **Thermo Fisher Catalog #**12550123
- Bovine Serum Albumin **Thermo Fisher Catalog #**15561020
- Coplin Staining Jar **Thermo Fisher Catalog #**194
- Scientific Device Humidity/Slide Moisture Chamber **Thermo Fisher Catalog #**23769522

Slides boxes wrapped in tinfoil to store slides in -20 degrees Celsius prior to cutting.
Various primaries and secondaries dependent upon structure of interest.
Glass coverslips in various sizes depending on tissue size.
Primaries:
Neuropeptide Y (NPY) Antibody from Immunostar Catalog#22940
Anti-Tyrosine Hydroxylase (TH) from Millipore Catalog#AB1542
VAChT from Synaptic Systems Catalog#139 103
Anti-PGP9.5 antibody from abcam Catalog#ab108986
Secondaries:
Alexa Fluor 488 conjugated AffiniPure Donkey Anti-Rabbit IgG from Jackson ImmunoResearch Catalog#711-545-152
Alexa Fluor 555 conjugated Donkey Anti-Goat IgG from ThermoFisher Catalog#A-21432
Alexa Fluor 555 conjugated Donkey Anti-Rabbit IgG from ThermoFisher Catalog#A-31572
Alexa Fluor 594 conjugated Donkey Anti-Rabbit IgG from Jackson ImmunoResearch Catalog#711-585-152
Alexa Fluor 594 conjugated Donkey Anti-Goat IgG from ThermoFisher Catalog#A-11058

1 Day 1: Using the PAP Pen, carefully draw a water barrier circle around the tissue sections on the slide – allow this circle to dry for several seconds or up to approx. one minute

2 Rinse slides with PBS (pH 7.3-7.4): 4 × 5 min each

3 Rinse slides with 0.5% BSA + 0.4% Triton X-100 in PBS): 1 × 10 min

4 Remove slides one at a time and using a clean Kimwipe, carefully wipe around the tissue sections to dry the slide

5 Place the slides into a black, covered slide incubation box/humidity box

6 Cover the tissue sections with blocking buffer (10% normal donkey serum in 1.0% BSA + 0.4% Triton X-100 + PBS)

7 Allow the sections to remain in blocking buffer for 1.5-2 hrs. at RT

8 Pour off the blocking buffer

9 Replace with primary antibody solution (antibody of choice diluted in 1.0% BSA + 0.4% Triton X-100 + PBS)

10 Incubate tissue with primary antibody overnight in incubation box

11 Day 2: Rinse slides with PBS: 4 × 5 min

12 Rinse slides with 0.5% BSA + 0.4% Triton X-100 + PBS: 1 × 10 min

13 Place the slides into a black, covered slide incubation box/humidity box

14 Cover the tissue sections with blocking buffer (10% normal donkey serum in 1.0% BSA + 0.4% Triton X-100 + PBS)

15 Allow the sections to remain in blocking buffer for 1.5-2 hrs. at RT

16 Prepare fluorescent secondary antibody (secondary antibody should be diluted in 1.0% BSA + 0.4% Triton X-100 + PBS)

17 Cover the tissue with the secondary antibody solution and incubate for 2 hrs. at RT in the incubation box. *From this point on, use low light and/or cover tissues.*

18 Rinse slides with PBS: 4 × 5 min

19 Remove excess PBS with a Kimwipe

20 Carefully add a drop of mounting medium to the center of the tissue and apply cover glass

21 Seal cover glass with clear nail polish. For thicker tissue, add a weight before sealing