IHC Fluorescent Frozen Sections

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Nov 15, 2019

1 Works for me

dx.doi.org/10.17504/protocols.io.7ayhifw

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ABSTRACT

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

Fluor Immun Protocol-Slides.docx

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

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

MATERIALS TEXT

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

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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 x 5 min each

3 Rinse slides with 0.5% BSA + 0.4% Triton X-100 in PBS): 1 x 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 x 5 min

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

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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 x 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

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