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IHC-Amplified Fluorescent Frozen Sections

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1 Works for me [dx.doi.org/10.17504/protocols.io.8rmhv46](https://doi.org/10.17504/protocols.io.8rmhv46)

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ABSTRACT

Immunohistochemistry protocol used for staining with fluorescent secondary antibodies to highlight specific tissue structures and amplify specific antibody signals.

Fluor Immun Protocol-
Biotin_Streptavidin-Slides
(amplification).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 1 mm	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

- 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
- 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 Prepare fluorescent Streptavidin secondary antibody (should be diluted in 1.0% BSA + 0.4% Triton X-100 + PBS)
- 21 Cover the tissue with the fluorescent Streptavidin antibody solution and incubate for 2 hrs. at RT in the incubation box.
- 22 Rinse slides with PBS: 4 x 5 min
- 23 Remove excess PBS with a Kimwipe
- 24 Carefully add a drop of mounting medium to the center of the tissue and apply cover glass
- 25 Seal cover glass with clear nail polish. For thicker tissue, add a weight before sealing



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