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## IHC-Amplified Fluorescent Frozen Sections

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**Protocol status:** Working

**We use this protocol and it's working**

**Created:** October 25, 2019

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### Abstract

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

## Attachments



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<sup>®</sup>; 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

Biotin-SP-conjugated Affinipure Donkey Anti-Goat IgG from Jackson ImmunoResearch Catalog#705-065-147

Streptavidin from Thermo Scientific Catalog#21842

- 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 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 × 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