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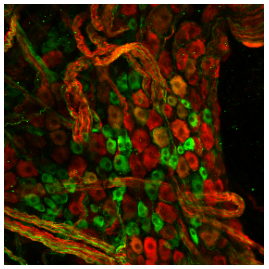
Immunohistochemical labeling of thick cryosections from pelvic ganglia



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We use this protocol and it's working

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Abstract

This protocol describes immunohistochemical procedures applied to thick (50 µm) cryosections mounted directly on slides. It is used when the structures to be analysed are too large to remain intact within thin (10-20 µm) cryosections. Antibodies have been selected to distinguish different neurochemical classes of autonomic ganglion neurons and synaptic boutons associated with these neurons. The protocol can also be used to characterize neurons containing retrograde tracer.

Materials

MATERIALS

Horse serum **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**12449C

OCT (Optimal Cutting Temperature compound) **Sakura Finetek** **Catalog #**4583

Sheep anti-neuronal nitric oxide synthase antibody; AB_90743 **Merck Millipore (EMD Millipore)** **Catalog #**AB1529

Cy3 Donkey anti-goat IgG **Jackson ImmunoResearch Laboratories, Inc.** **Catalog #**705-165-147

AF488 Donkey anti-rabbit IgG **Jackson ImmunoResearch Laboratories, Inc.** **Catalog #**711-545-152

PAP hydrophobic barrier pen **Enzo Life Sciences** **Catalog #**ADI-950-233-0001

Triton X-100 **Merck Millipore (EMD Millipore)** **Catalog #**X100

Vectorshield anti-fade mounting medium **Vector Laboratories** **Catalog #**H-1000

Mouse anti-synaptophysin antibody **Agilent Technologies** **Catalog #**M0776

Rabbit anti-protein gene product 9.5 antibody **Merck Millipore (EMD Millipore)** **Catalog #**B5925

Sheep anti-tyrosine hydroxylase antibody **Merck Millipore (EMD Millipore)** **Catalog #**AB1542

AF647 Donkey anti-mouse IgG **Thermofisher** **Catalog #**A-31571

Solutions:

- PBS: phosphate-buffered saline, 0.1 M, pH 7,2
- PBS containing 30% sucrose (w/v)
- PBS containing 0.1% sodium azide
- Blocking solution: PBS containing 10% non-immune horse serum and 0.5% triton X-100

Primary antibodies:

	Abbreviation	Gene name	Synonym	RRID	Host species	Dilution
	nNOS	Nos1	Neuronal nitric oxide synthase	AB_90743	sheep	1:2000
	PGP	Uchl1	Protein gene product 9.5; ubiquitin C-terminal hydrolase 1	AB_11214054	rabbit	1:2000
	SYP	Syp	Synaptophysin	AB_2199013	mouse	1:300
	TH	Th	Tyrosine hydroxylase	AB_90755	sheep	1:1000

Neuronal cell bodies are labelled with PGP, nNOS or TH antibodies. Synaptic boutons associated with these cells are identified with synaptophysin-immunoreactivity.

Secondary antibodies:

	Tag-antibody	Host species	Dilution
	AF488 anti-rabbit	Donkey	1:1000
	AF647 anti-mouse	Donkey	1:500
	Cy3 anti-goat	Donkey	1:1000

Troubleshooting

Preparation of cryosections

1 Cryoprotect fixed tissue in phosphate-buffered saline (PBS; 0.1 M, pH7.2) containing 30% sucrose. This should be performed at 4C, 24-72h prior to cutting.

2 Embed tissue in cryomold using OCT, freeze in cryostat and cut sections (50 µm), distributing sections progressively across sets of 3-4 slides so that each slide has non-consecutive sections.

Note

Chrome-alum treated slides are pre-coated (subbed) with 1% gelatin, dried and stored at room temperature. This slide treatment enhances section adhesion while retaining good surface tension of the antibody droplets.

Immunostaining

3 Air-dry slides at room temperature for at least 10 minutes.

4 Draw around sections with hydrophobic barrier pen (PAP pen); wait for 10 min to dry.

5 Wash sections in PBS (10 min).

6 Incubate sections in blocking solution (PBS containing 10% non-immune horse serum and 0.5% triton X-100) at room temperature in a humidified dark chamber for 2 h.

7 Incubate sections in appropriate dilutions of primary antibodies (or combinations of primary antibodies) for 48h in a humidified dark chamber. Antibodies are diluted in PBS containing 2% horse serum, 0.5% Triton and 0.1% sodium azide.

8 Wash sections in PBS (30 min)

9 Incubate sections in appropriate dilutions of secondary antibodies (or combinations of secondary antibodies) for 4h in a humidified dark chamber. Antibodies are diluted in PBS containing 2% horse serum, 0.5% Triton and 0.1% sodium azide.

10 Wash sections in PBS (30 min)

11 Coverslip in Vectorshield or preferred anti-fade mountant.