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Immunohistochemical analysis of ganglion neurons innervating the lower urinary tract [keast-001-stage03]

In 1 collection

Janet R Keast¹, Peregrine B Osborne¹

¹University of Melbourne

SPARC

Tech. support email: info@neuinfo.org

Janet R Keast
University of Melbourne

ABSTRACT

This protocol is used for studies of neurons containing retrograde tracer dye in adult rat dorsal root ganglia (DRG) or pelvic ganglia (PG; synonym, major pelvic ganglia). Cryosections of ganglia are mounted directly on slides for immunohistochemical staining. Antibodies have been selected to distinguish different neurochemical classes of PG neurons or to specifically identify subclasses of sensory neurons that are myelinated (immunoreactive for NF200 [neurofilament, 200 kD]) or nociceptive (immunoreactive for TRPV1).

MATERIALS

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- ⊗ Horse serum **Sigma Aldrich Catalog #12449C**
- ⊗ OCT (Optimal Cutting Temperature compound) **Sakura Finetek Catalog #458**
- ⊗ Chicken anti-neurofilament-200 antibody; AB_304560 **Abcam Catalog #4680**
- ⊗ Rabbit anti-NECAB2 antibody; AB_1234217 **Atlas Antibodies Catalog #HPA013998**
- ⊗ Goat anti-CALB1 antibody; AB_2254256 **R&D Systems Catalog #AF3320**
- ⊗ Goat anti-CGRP antibody; AB_2290729 **Bio-rad Laboratories Catalog #1720-9007**
- ⊗ Guinea pig anti-Nav1.8 antibody; AB_2340965 **Alomone Labs Catalog #AGP-029**
- ⊗ Goat anti-TRPV1 antibody; AB_1624144 **Neuromics Catalog #GT15129**
- ⊗ Rabbit anti-substance P antibody; AB_572266 **Immunostar Catalog #20064**
- ⊗ Rabbit anti-neurofilament-200 antibody; AB_477272 **Sigma Aldrich Catalog #N4142**

Rabbit anti-tyrosine hydroxylase antibody; AB_390204 Merck Millipore Catalog #AB152
Sheep anti-neuronal nitric oxide synthase antibody; AB_90743 Merck Millipore Catalog #AB1529
Rabbit anti-CART antibody; AB_11211921 Phoenix Pharmaceuticals Catalog #H-003-62
Goat anti-calretinin antibody; AB_2231996 Swant Catalog #CG1
Cy3 Donkey anti-rabbit IgG Jackson ImmunoResearch Catalog #711-165-152
AF647 Donkey anti-chicken IgY (IgG) Jackson ImmunoResearch Catalog #703-605-155
Cy3 Donkey anti-goat IgG Jackson ImmunoResearch Catalog #705-165-147
Cy3 Donkey anti-guinea pig IgG Jackson ImmunoResearch Catalog #706-165-148
AF647 Donkey anti-sheep IgG Thermo Fisher Scientific Catalog #A21448
AF488 Donkey anti-goat IgG Jackson ImmunoResearch Catalog #705-545-147
AF594 Donkey anti-rabbit IgG Jackson ImmunoResearch Catalog #711-585-152
AF488 Donkey anti-rabbit IgG Jackson ImmunoResearch Catalog #711-545-152
AF594 Donkey anti-goat IgG Jackson ImmunoResearch Catalog #705-585-003
Rabbit anti-somatostatin antibody; AB_2687415 Phoenix Pharmaceuticals Catalog #H-060-03

Solutions:

- PBS: phosphate-buffered saline, 0.1 M, pH 7,2
- PBS containing 0.1% sodium azide
- PBS containing 30% sucrose (w/v)
- Blocking solution: PBS containing normal horse serum and 0.1% triton X-100
- Hypertonic PBS: PBS containing 17g NaCl per liter

Primary antibodies:

	Abbreviation	Gene name	Synonym	RRID	Host species	Dilution
	CALB1	Calb1	calbindin 1	AB_2254256	goat	1:500

	calretinin	Calb2	calbindin 2	AB_22319 96	goat	1:500
	CART	Cartpt	Cocaine- and amphetamine regulated transcript	AB_11211 921	rabbit	1:3000
	CGRP	Calca	Calcitonin gene-related peptide	AB_22907 29	goat	1:2000
	Nav1.8	Scn10a	Voltage-gated sodium channel 1.8	AB_23409 65	guinea pig	1:1000
	NECAB2	Necab2	N-terminal EF-hand calcium binding protein 2	AB_12342 17	rabbit	1:2000
	NF200	Nefh	Neurofilament protein, 200 kD	AB_30456 0	chicken	1:2000
	NF200	Nefh	Neurofilament protein, 200 kD	AB_47727 2	rabbit	1:2000
	nNOS	Nos1	Neuronal nitric oxide synthase	AB_90743	sheep	1:2000
	SOM	Sst	Somatostatin	AB_26874 15	rabbit	1:2000
	SP	Tac1	Substance P	AB_57226 6	rabbit	1:5000
	TH	TH	Tyrosine hydroxylase	AB_39020 4	rabbit	1:2000
	TRPV1	Trpv1	Transient receptor potential cation channel subfamily V member 1	AB_16241 44	goat	1:500

Primary antibodies used for dorsal root ganglia (DRG):

NF200 with CALB1, NECAB2 or CGRP

TRPV1 with Nav1.8, SP or SOM

Primary antibodies used for pelvic ganglia (PG):

CALB1, calretinin, CART, NF200, nNOS, TH

Secondary antibodies:

Tag-antibody	Host species	Dilution
AF647 anti-chicken	Donkey	1:1000

AF488 anti-goat	Donkey	1:2000
AF594 anti-goat	Donkey	1:1000
Cy3 anti-goat	Donkey	1:1000
Cy3 anti-guinea pig	Donkey	1:2000
AF488 anti-rabbit	Donkey	1:1000
AF594 anti-rabbit	Donkey	1:1000
Cy3 anti-rabbit	Donkey	1:1500
AF647 anti-sheep	Donkey	1:1000

Preparation of cryosections

- 1 Cryoprotect tissues 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 tissues in cryomold using OCT, freeze in cryostat and cut sections (14 μ m), distributing sections progressively across sets of 3 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 Wash sections in PBS (10 min).

- 5 Incubate sections in blocking solution at room temperature in a humidified dark chamber for 1-2 h.
- 6 Incubate sections in appropriate dilutions of primary antibodies (or combinations of primary antibodies) for 18-24h in a humidified dark chamber. Antibodies are diluted in hypertonic PBS.
- 7 Wash sections in PBS (15 min)
- 8 Incubate sections in appropriate dilutions of secondary antibodies (or combinations of secondary antibodies) for 2-3h in a humidified dark chamber. Antibodies are diluted in hypertonic PBS.
- 9 Wash sections in PBS (15 min)
- 10 Coverslip in preferred anti-fade mountant.

Microscopy

- 11 Retrogradely labeled neurons are counted and classified according to their immunoreactivity, including only nucleated neuronal profiles in the analysis.