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

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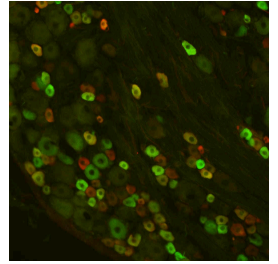
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Keywords: immunohistochemistry; immunofluorescence, immunohistochemical analysis of ganglion neuron, adult rat dorsal root ganglia, pelvic ganglia, major pelvic ganglia, ganglion neuron, cryosections of ganglia, different neurochemical classes of pg neuron, ganglia, lower urinary tract, studies of neuron, pg neuron, subclasses of sensory neuron, immunohistochemical staining, sensory neuron, neurofilament, slides for immunohistochemical staining, immunoreactive for trpv1, neuron, different neurochemical class, immunohistochemical analysis, containing retrograde tracer dye

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

MATERIALS

- ☒ Horse serum **Merck MilliporeSigma (Sigma-Aldrich) Catalog #12449C**
- ☒ OCT (Optimal Cutting Temperature compound) **Sakura Finetek Catalog #4583**
- ☒ 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 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #N4142**
- ☒ Rabbit anti-tyrosine hydroxylase antibody; AB_390204 **Merck Millipore (EMD Millipore) Catalog #AB152**
- ☒ Sheep anti-neuronal nitric oxide synthase antibody; AB_90743 **Merck Millipore (EMD 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 Laboratories, Inc. Catalog #711-165-152**
- ☒ AF647 Donkey anti-chicken IgY (IgG) **Jackson ImmunoResearch Laboratories, Inc. Catalog #703-605-155**
- ☒ Cy3 Donkey anti-goat IgG **Jackson ImmunoResearch Laboratories, Inc. Catalog #705-165-147**
- ☒ Cy3 Donkey anti-guinea pig IgG **Jackson ImmunoResearch Laboratories, Inc. Catalog #706-165-148**
- ☒ AF647 Donkey anti-sheep IgG **Thermo Fisher Scientific Catalog #A21448**
- ☒ AF488 Donkey anti-goat IgG **Jackson ImmunoResearch Laboratories, Inc. Catalog #705-545-147**
- ☒ AF594 Donkey anti-rabbit IgG **Jackson ImmunoResearch Laboratories, Inc. Catalog #711-585-152**
- ☒ AF488 Donkey anti-rabbit IgG **Jackson ImmunoResearch Laboratories, Inc. Catalog #711-545-152**
- ☒ AF594 Donkey anti-goat IgG **Jackson ImmunoResearch Laboratories, Inc. 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_2231996	goat	1:500
	CART	Cartpt	Cocaine- and amphetamine regulated transcript	AB_11211921	rabbit	1:3000
	CGRP	Calca	Calcitonin gene-related peptide	AB_2290729	goat	1:2000
	Nav1.8	Scn10a	Voltage-gated sodium channel 1.8	AB_2340965	guinea pig	1:1000
	NECAB2	Necab2	N-terminal EF-hand calcium binding protein 2	AB_1234217	rabbit	1:2000
	NF200	Nefh	Neurofilament protein, 200 kD	AB_304560	chicken	1:2000
	NF200	Nefh	Neurofilament protein, 200 kD	AB_477272	rabbit	1:2000
	nNOS	Nos1	Neuronal nitric oxide synthase	AB_90743	sheep	1:2000
	SOM	Sst	Somatostatin	AB_2687415	rabbit	1:2000
	SP	Tac1	Substance P	AB_572266	rabbit	1:5000
	TH	TH	Tyrosine hydroxylase	AB_390204	rabbit	1:2000
	TRPV1	Trpv1	Transient receptor potential cation channel subfamily V member 1	AB_1624144	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

Troubleshooting

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.