



Nov 08,
2019

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

In 1 collection

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1 Works for me dx.doi.org/10.17504/protocols.io.w3efgje

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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

NAME	CATALOG #	VENDOR
Horse serum	12449C	Sigma Aldrich
OCT (Optimal Cutting Temperature compound)	4583	Sakura Finetek
Chicken anti-neurofilament-200 antibody; AB_304560	4680	Abcam
Rabbit anti-NECAB2 antibody; AB_1234217	HPA013998	Atlas Antibodies
Goat anti-CALB1 antibody; AB_2254256	AF3320	R&D Systems
Goat anti-CGRP antibody; AB_2290729	1720-9007	Bio-rad Laboratories
Guinea pig anti-Nav1.8 antibody; AB_2340965	AGP-029	Alomone Labs
Goat anti-TRPV1 antibody; AB_1624144	GT15129	Neuromics
Rabbit anti-substance P antibody; AB_572266	20064	Immunostar
Rabbit anti-neurofilament-200 antibody; AB_477272	N4142	Sigma Aldrich
Rabbit anti-tyrosine hydroxylase antibody; AB_390204	AB152	Merck Millipore
Sheep anti-neuronal nitric oxide synthase antibody; AB_90743	AB1529	Merck Millipore
Rabbit anti-CART antibody; AB_11211921	H-003-62	Phoenix Pharmaceuticals
Goat anti-calretinin antibody; AB_2231996	CG1	Swant
Cy3 Donkey anti-rabbit IgG	711-165-152	Jackson ImmunoResearch
AF647 Donkey anti-chicken IgY (IgG)	703-605-155	Jackson ImmunoResearch
Cy3 Donkey anti-goat IgG	705-165-147	Jackson ImmunoResearch
Cy3 Donkey anti-guinea pig IgG	706-165-148	Jackson ImmunoResearch
AF647 Donkey anti-sheep IgG	A21448	Thermo Fisher Scientific
AF488 Donkey anti-goat IgG	705-545-147	Jackson ImmunoResearch
AF594 Donkey anti-rabbit IgG	711-585-152	Jackson ImmunoResearch

NAME ▾	CATALOG # ▾	VENDOR ▾
AF488 Donkey anti-rabbit IgG	711-545-152	Jackson ImmunoResearch
AF594 Donkey anti-goat IgG	705-585-003	Jackson ImmunoResearch
Rabbit anti-somatostatin antibody; AB_2687415	H-060-03	Phoenix Pharmaceuticals

MATERIALS TEXT

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

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.



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.

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



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