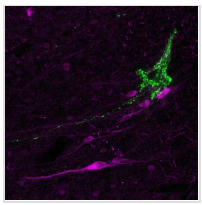

Dec 15, 2021

Immunohistochemical labelling of lower urinary tract afferents in spinal cord

Immunohistochemical labelling of spinal cord neurons involved in bladder activity

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This protocol is used for immunohistochemical visualisation of cholera toxin subunit B within afferents innervating the lower urinary tract in cryosections of rat lumbosacral spinal cord. Free-floating sections are processed in a double labelling protocol to distinguish regions of innervation by these afferents.

- Cholera toxin B antibody [lower urinary tract afferents]
- Choline acetyltransferase antibody [preganglionic autonomic neurons and motoneurons]

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Visualizing lower urinary tract afferent projections in the lumbosacral spinal cord in rats

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Part of collection

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MATERIALS

Horse serum **Sigma Aldrich Catalog #12449C**

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

Goat anti-ChAT antibody **Millipore Catalog #AB144P**

Rabbit anti-cholera toxin antibody **Sigma Aldrich Catalog #C3062**

AF647 Donkey anti-sheep IgG **Thermo Fisher Scientific Catalog #A21448**

Cy3 Donkey anti-rabbit IgG **Jackson Immunoresearch Catalog #711-165-152**

NeuroTrace™ 435/455 Blue Fluorescent Nissl Stain **Thermo Fisher Catalog #N21479**

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 10% normal horse serum and 0.5% triton X-100
- PBS containing 0.1% sodium azide, 2% normal horse serum and 0.5% triton X-100

Primary Antibodies:

A	B	C	D	E
Abbreviation	**Synonym**	**RRID**	**Host Species**	**Dilution**
CTB	Cholera toxin subunit B	AB_10609634	Rabbit	1:30,000
ChAT	Choline acetyltransferase	AB_2079751	Goat	1:500

Secondary Antibodies:

A	B	C
Tag-antibody	Host species	Dilution
Cy3 anti-rabbit	Donkey	1:2000
AF647 anti-sheep	Donkey	1:1000

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Preparation of cryosections

1 Cryoprotect fixed tissue (L5-S2 spinal cord) 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 (40 µm), collecting sections progressively across sets of 4 wells to collect 160 µm spaced series.

Immunostaining

3 Wash sections in PBS (3 x 10 min)

4 Incubate sections in blocking solution at room temperature for 2 h

5 Incubate sections in appropriate dilutions of primary antibodies (or combinations of primary antibodies) for 48-72h. Antibodies are diluted in PBS containing 0.1% sodium azide, 2% horse serum, and 0.5% triton-X.

6 Wash sections in PBS (3 x 10 min)

7 Incubate sections in appropriate dilutions of secondary antibodies (or combinations of secondary antibodies) 4 h in the dark. Antibodies are diluted in PBS containing 2% horse serum, and 0.5% triton-X.

A useful counterstain to visualise spinal regions can be included here, by adding NeuroTrace (fluorescent Nissl stain; 1:100) to the secondary antibody incubation.

8 Wash sections in PBS (3 x 10 min)

9 Mount sections onto glass slides and coverslip in preferred anti-fade mountant.

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Microscope

10 Labeled lower urinary tract afferents with cholera toxin subunit B should be most visible along the boundaries of each dorsal horn. Look for the lateral projections, which are the most prominent, as well as the medial projections, which are comparatively fainter.

Urethra injections of cholera toxin subunit B will occasionally result in the uptake of tracer by preganglionic neurons if the tracer injection site is near intramural or serosal pelvic ganglion neurons. These neurons will be visible within the sacral preganglionic nucleus in the intermediolateral column. If the urethral rhabdosphincter is exposed to tracer, this will be evident by labelling of motor neurons in the ventral horn.

For digital analysis, tile-scanning of complete spinal cord sections is recommended, ensuring that the order of sections (rostral to caudal) is noted.

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