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Immunohistochemical labelling of spinal cord sections for chemoarchitectural analysis of segments

 Forked from [Immunohistochemical labelling of spinal cord neurons involved in bladder activity](#)

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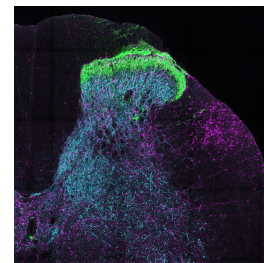
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We use this protocol and it's working

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Disclaimer

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The [protocols.io](https://dx.doi.org/10.17504/protocols.io.14egn6376l5d/v1) team notes that research involving animals and humans must be conducted according to internationally-accepted standards and should always have prior approval from an Institutional Ethics Committee or Board.

Abstract

This protocol is used for immunohistochemical visualisation of the chemoarchitecture of the adult rat lumbosacral spinal cord (and other segments for comparison). Spinal cords were sub-dissected into segments, and transverse sections were obtained from across the rostrocaudal axis of each segment. Two combinations of antibodies were used:

- Combination 1: neuronal nitric oxide synthase (nNOS), choline acetyltransferase (ChAT) and NeuN
- Combination 2: calcitonin gene-related peptide (CGRP), vesicular glutamate transporter 1 (VGLut1) and tyrosine hydroxylase (TH)

Materials

MATERIALS

- ⊗ Horse serum **Merck MilliporeSigma (Sigma-Aldrich) Catalog #12449C**
- ⊗ OCT (Optimal Cutting Temperature compound) **Sakura Finetek Catalog #4583**
- ⊗ Goat anti-ChAT antibody **Merck Millipore (EMD Millipore) Catalog #AB144P**
- ⊗ Anti-NeuN Antibody, clone A60 **Merck Millipore (EMD Millipore) Catalog #MAB377**
- ⊗ Anti-neuronal nitric oxide synthase antibody (rabbit) **Invitrogen Catalog #61-7000**
- ⊗ Goat anti-CGRP antibody; AB_2290729 **Bio-Rad Laboratories Catalog #1720-9007**
- ⊗ Anti-VGlut1 antibody (guinea pig) **Merck Millipore (EMD Millipore) Catalog #AB5905**
- ⊗ Mouse anti-TH (tyrosine hydroxylase) antibody **Catalog #22941**
- ⊗ AF488 Donkey anti-goat IgG **Jackson ImmunoResearch Laboratories, Inc. Catalog #705-545-147**
- ⊗ AF488 Donkey anti-mouse IgG **Jackson ImmunoResearch Laboratories, Inc. Catalog #715-545-150**
- ⊗ Cy3 Donkey anti-rabbit IgG **Jackson ImmunoResearch Laboratories, Inc. Catalog #711-165-152**
- ⊗ Cy3 Donkey anti-guinea pig IgG **Jackson ImmunoResearch Laboratories, Inc. Catalog #706-165-148**
- ⊗ AF647 Donkey anti-sheep IgG **Molecular Probes Catalog #A21448**
- ⊗ AF647 Donkey anti-mouse IgG **Invitrogen Catalog #A31571**

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	F
	Abbreviation	Gene name	Synonym	RRID	Host Species	Dilution
	NeuN	FOX3	NeuN	AB_2298772	Mouse	1:2000



	A	B	C	D	E	F
	ChAT	chat	Choline acetyltransferase	AB_2079751	Goat	1:500
	nNOS	nnos	Neuronal nitric oxide synthase	AB_2313734	Rabbit	1:2000
	CGRP	calca	Calcitonin gene-related peptide	AB_2290729	Goat	1:2000
	TH	th	Tyrosine hydroxylase	AB_572268	Mouse	1:2000
	VGluT1	slc17a7	vesicular glutamate transporter 1	AB_2301751	Guinea pig	1:5000

Secondary Antibodies:

	A	B	C	D
	Tag-antibody	Host species	RRID	Dilution
	Anti-mouse AF488	Donkey	AB_2340846	1:2000
	Anti-goat	Donkey	AB_233	1:1000



	A	B	C	D
	AF488		6933	
	Anti-rabbit Cy3	Donkey	AB_2307443	1:3000
	Anti-guinea pig Cy3	Donkey	AB_2340460	1:2000
	Anti-sheep AF647	Donkey	AB_2535865	1:500
	Anti-mouse AF647	Donkey	AB_162542	1:1000

Sub-dissection of spinal cord into segments

- 1 In a silicone gel-lined petri dish, immerse the fixed spinal cord in phosphate-buffered saline (PBS; 0.1 M, pH7.2).
- 2 If still present on the spinal cord, carefully remove the dura mater from the outside of the spinal cord using fine forceps and iris scissors. Take care not to damage the spinal cord or remove the spinal roots as these will be needed as landmarks.
- 3 Pin the spinal cord flat by laying the spinal cord dorsal surface facing the gel, and individually pinning all of the ventral spinal roots out perpendicularly.
- 4 Identify each of the spinal cord segments using the following landmarks:
 - Each segment is defined by a ventral root, with the boundaries between segments where one set of rootlets ends, and another begins.
 - In the lumbar spinal cord, the lumbar enlargement is the widest portion, containing segments L3-L5.
 - The ventral roots of the sacral segments are much thinner than those of the lumbar segments.
- 5 Starting with the most caudal segments, use a scalpel blade to sub-dissect each segment, cutting at the exact point between two sets of rootlets. Store the segments in separate tubes of PBS containing 0.1% sodium azide, labelled appropriately until further use.

Preparation of cryosections

- 6 Cryoprotect fixed spinal cord segments in PBS containing 30% sucrose. This should be performed at 4 °C, 24-72h prior to cutting.
- 7 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

- 8 Wash sections in PBS (3 × 10 min)
- 9 Incubate sections in blocking solution at room temperature for 2 h



- 10 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.
- 11 Wash sections in PBS (3 × 10 min)
- 12 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.
- 13 Wash sections in PBS (3 × 10 min)
- 14 Mount sections onto glass slides and coverslip in preferred anti-fade mountant.

Microscope

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

Note

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