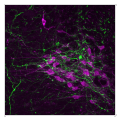

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Immunohistochemical labelling of spinal cord neurons involved in bladder activity

In 1 collection

Janet Keast[1], Peregrine Osborne[2], Nicole Wiedmann[1]

[1]University of Melbourne, [2]The University of Melbourne

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

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ABSTRACT

This protocol is used for immunohistochemical visualisation of immediate early gene expression (c-Fos or Egr-1) in cryosections of rat lumbosacral spinal cord. Free-floating sections are processed in a double labelling protocol to distinguish immediate early gene expression in different neurochemical classes of spinal cord neurons:

- ChAT [choline acetyltransferase]: preganglionic neurons
- TH [tyrosine hydroxyls]: dopaminergic neurons
- Pax2: inhibitory interneurons

MATERIALS

NAME	CATALOG #	VENDOR
Horse serum	12449C	Sigma Aldrich
OCT (Optimal Cutting Temperature compound)	4583	Sakura Finetek
Cy3 Donkey anti-goat IgG	705-165-147	Jackson Immunoresearch
AF488 Donkey anti-rabbit IgG	711-545-152	Jackson Immunoresearch
NeuroTrace™ 640/660 Deep-Red Fluorescent Nissl Stain - Solution in DMSO	N21483	Thermo Fisher
Mouse anti-cFos antibody	sc166940	Santa Cruz Biotechnology
Rabbit anti-Pax2 antibody	71-6000	Invitrogen - Thermo Fisher
Rabbit anti-Egr-1 (588) antibody	sc-110	Santa Cruz Biotechnology
Rabbit anti-TH antibody	AB152	Merck Millipore
Cy3 Donkey anti-mouse IgG	715-165-150	Jackson Immunoresearch
Goat anti-ChAT antibody	AB144P	Millipore

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 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:

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Abbreviation	Gene name	Synonym	RRID	Host Species	Dilution
cFos	Fos	cFos	AB_10609634	Mouse	1:100
ChAT	Chat	Choline acetyltransferase	AB_2079751	Goat	1:500
Egr-1/ Zif268	Egr1/ Zif268	Early growth receptor 1	AB_2097174	Rabbit	1:5000
Pax2	Pax2	Paired box gene 2	AB_2533990	Rabbit	1:1000
TH	Th	Tyrosine hydroxylase	AB_390204	Rabbit	1:2000

Secondary Antibodies:

Tag-antibody	Host species	Dilution
Cy3 anti-mouse	Donkey	1:2000
AF488 anti-rabbit	Donkey	1:1000
Cy3 anti-goat	Donkey	1:1000

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.

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8 Wash sections in PBS (3 x 10 min)

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

Microscope

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

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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