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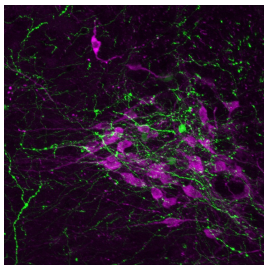
Immunohistochemical labelling of spinal cord neurons involved in bladder activity



In 1 collection

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Protocol status: Working
We use this protocol and it's working

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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

MATERIALS

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

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:

	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_253390	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 × 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 × 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.

Note

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 × 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.

Note

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