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Immunohistochemical staining of wholemount major pelvic ganglia (MPG) for analysis of myelinated bladder afferents

Forked from a private protocol

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We use this protocol and it's working

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Abstract

This protocol describes immunohistochemical procedures applied to wholemount major pelvic ganglia (MPG) for the visualization of myelinated axons filled with cholera toxin subunit B (CTB) and/or neurons transfected with adeno-associated virus (AAV) expressing TdTomato. In this protocol, samples were obtained from rats in which CTB was microinjected into the bladder, and AAV-PHP.S was intravenously administered to preferentially and sparsely label peripheral neurons. In this context, neurofascin (paranode marker) identifies myelinated axons.

Materials

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

Triton X-100 **Sigma Aldrich Catalog #**T8787-50ML

Anti-RFP antibody (guinea pig) **Synaptic Systems Catalog #**390004

Anti-cholera toxin subunit B antibody (goat) **List Labs Catalog #**703

Anti-neurofascin antibody (rabbit) **Alomone Labs Catalog #**AIP-025

Cy3 Donkey anti-guinea pig IgG **Jackson ImmunoResearch Laboratories, Inc. Catalog #**706-165-148

AF488 Donkey anti-goat IgG **Jackson ImmunoResearch Laboratories, Inc. Catalog #**705-545-147

AF647 Donkey anti-rabbit antibody **Invitrogen Catalog #**A32795

Solutions:

- PBS: phosphate-buffered saline, 0.1 M, pH 7.2
- PBS containing 0.1% sodium azide
- PB: phosphate-buffer, 0.1M, pH7.2
- 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
	RFP	Red fluorescent protein	AB_2737052	Guinea pig	1:500
	CTB	Cholera toxin subunit B	AB_10013220	Goat	1:10,000
	Neurofascin	NF155	AB_2756657	Rabbit	1:1000

Secondary Antibodies:

	A	B	C	D
	Tag-antibody	Host Species	RRID	Dilution
	Cy3 anti-guinea pig	Donkey	AB_2340460	1:2000
	AF488 anti-goat	Donkey	AB_2336933	1:1000
	AF647 anti-rabbit	Donkey	AB_2762835	1:1000

Immunohistochemistry

1 Wash whole MPGs in phosphate buffer (PB; 0.1M; pH 7.2) (3 x 30 min)

2 Incubate sections in blocking solution (PB; 10% horse serum; and 0.5% Triton-X) at room temperature for 2 h

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

4 Wash tissue in PBS (3 x 30 min)

5 Incubate sections in appropriate dilutions of secondary antibodies (or combinations of secondary antibodies) 24 h in the dark. Antibodies are diluted in PBS containing 2% horse serum, and 0.5% triton-X.

6 Wash tissue in PBS (3 x 30 min)

7 Mount tissue onto glass slides and coverslip in preferred anti-fade mountant.