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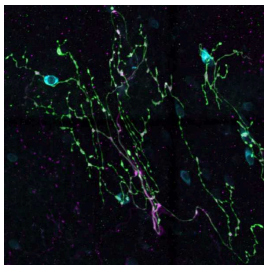
Immunohistochemical staining of wholemount female rat urethra preparations



Forked from a private protocol

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Abstract

This protocol describes the tissue preparation and immunohistochemical procedures applied to wholemount urethra from the female rat. Wholemount immunohistochemistry keeps structures within the tissue intact, but keeps the tissue flat enough that high resolution confocal microscopy can be employed. This is only suitable for the female rat, as the male rat urethra is too thick and muscular. The antibodies recommended in this protocol are to visualize AAV-PHP.S transfected neuron axons, as well as myelin basic protein and S100.

Materials

- Anti-CGRP antibody **Abcam** **Catalog #**AB81887
- Horse serum **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**12449C
- Triton X-100 **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**T8787-50ML
- Anti-S100 antibody (rabbit) **Dako** **Catalog #**Z0311
- Anti-CGRP antibody (mouse) **Abcam** **Catalog #**AB81887
- Anti-MBP antibody (human) conjugated to FITC **Miltenyi Biotec** **Catalog #**130-120-341
- Anti-RFP antibody (guinea pig) **Synaptic Systems** **Catalog #**390004
- Anti-TH antibody (rabbit) **Merck Millipore (EMD Millipore)** **Catalog #**AB152
- Anti-VAChT antibody (rabbit) **Synaptic Systems** **Catalog #**139103
- AF647 Donkey anti-rabbit antibody **Invitrogen** **Catalog #**A32795
- Cy3 Donkey anti-guinea pig IgG **Jackson ImmunoResearch Laboratories, Inc.** **Catalog #**706-165-148
- AF488 Donkey anti-mouse IgG **Jackson ImmunoResearch Laboratories, Inc.** **Catalog #**715-545-150

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
- 4% paraformaldehyde in 0.1M phosphate buffer, pH 7.4
- 0.9% sodium chloride, 1% sodium nitrite (w/v) and 0.11 ml heparin (5000 IU/ml)

Primary Antibodies:

	A	B	C	D	E
	Abbreviation	**Synonym**	**RRID**	**Host Species**	**Dilution**
	S100	S100	AB_10013383	Rabbit	1:1000
	MBP-FITC	Myelin basic protein conjugated to Fluorescein	AB_2857520	Human	1:2000

	A	B	C	D	E
		isothiocyanate			
	RFP	Red fluorescent protein	AB_2737052	Guinea pig	1:500
	TH	Tyrosine hydroxylase	AB_390204	Rabbit	1:2000
	CGRP	Calcitonin gene-related peptide	AB_1658411	Mouse	1:1000
	VAChT	Vesicular acetylcholine transporter	AB2247684	Rabbit	1:4000

Secondary Antibodies:

	Tag-antibody	Host species	RRID	Dilution
	Cy3 anti-guinea-pig	donkey	AB_2340460	1:2000
	AF647 anti-rabbit	donkey	AB_2762835	1:1000
	AF488 anti-mouse	donkey	AB_2340846	1:2000

Preparation of sample

1 Following 'Intracardiac perfusion with buffer for anatomical studies'' dissect urethra from the female rat and place it in a petri dish containing prewash solution.

2 Bisect the urethra longitudinally on the dorsal midline.

3 Pin the urethra out flat on a Sylgard-lined Petri dish with the urothelial surface facing upwards and gently stretch equally on both edges of the tissue by moving the pinned tissue out until the tissue is completely flat. Do this under stereomicroscope to ensure no damage is done to tissue during this procedure.

4 Replace the prewash solution with freshly made 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer (PB) and post-fix overnight at 4 °C

5 Remove the pins and wash the urethra in PB (3 × 30 min)

6 Store in PBS containing 0.1% sodium azide at 4 °C until used for immunohistochemistry

Immunohistochemistry

7 Wash urethra in phosphate buffer (PB; 0.1M; pH 7.2) (3 × 10 min)

8 Wash urethra in 50% ethanol/ H_2O (2 × 30 min)

9 Incubate sections in blocking solution (PB; 10% normal horse serum) at room temperature for 2 h

10 Incubate sections in appropriate dilutions of primary antibodies (or combinations of primary antibodies) for 5 days. Antibodies are diluted in PBS containing 0.1% sodium azide, 10% normal horse serum, and 0.5% Triton-X100.

11 Wash tissue in PBS (3 × 30 min)

12 Incubate sections in appropriate dilutions of secondary antibodies (or combinations of secondary antibodies) 5 days in protected from light. Antibodies are diluted in PBS-azide containing 10% horse serum, and 0.5% triton-X.

13 Wash tissue in PBS (3 × 30 min), protected from light

14 1 × 1 hour wash in buffered glycerol

15 Mount the flat urethra on a glass slide using buffered glycerol and coverslip, seal the slide with clear nail polish. For optimal imaging ensure the tissue layer of interest is facing up towards the coverslip. For example, if the lamina propria and urothelium are to be imaged, place the sample with urothelium up.