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Protocol for Investigating Periglomerular Afferent Innervation in Mouse Renal Cortex

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We use this protocol and it's working

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Abstract

This protocol aims to investigate the anatomical relationship between renal glomeruli and afferent axons in the mouse kidney, focusing on those identified by TRPV1 lineage and CGRP immunolabeling. The study seeks to elucidate the potential mechanosensory role of periglomerular afferent axons in monitoring glomerular pressure.

Materials

Materials -
C57BL/6J Mice
Phosphate Buffered Saline (PBS)
Paraformaldehyde (PFA)
Triton-X100
Bovine Serum Albumin (BSA)
Normal Donkey Serum
CUBIC-L and CUBIC-R+
Gelatin-coated slides
Xylenes
DPX Mountant

Reagents -
Paraformaldehyde
PBS with Triton-X100, BSA, and Donkey Serum

Primary Antibodies -

	A	B	C	D	E	F	G
	Name	**Target**	**Host**	**Vendor**	**Catalog number**	**RRID**	**Dilution**
	Living Colors DsRed Polyclonal Antibody	DsRed	rabbit	Takara Bio	632496	RRID:AB_10013483	1:500
	Mouse Nephrin Antibody	Nephrin - mouse	goat	R and D Systems	AF3159	RRID:AB_2155023	1:500
	Sheep Anti-Tyrosine Hydroxylase (TH, Tyrosine Monooxygenase) Polyclonal antibody, Unconjugated	Tyrosine Hydroxylase	sheep	Millipore	AB1542	RRID:AB_90755	1:500
	Anti-Tyrosine	Tyrosine Hydroxyl	rabbit	Millipore	AB152	RRID:AB_390204	1:500

	A	B	C	D	E	F	G
	Hydroxylase Antibody	ase					
	CGRP (Calcitonin Gene Related Peptide) Antibody	rat alpha-CGRP	rabbit	Immuno Star	24112	RRID:AB_572217	1:500

Primary antibodies

rabbit anti-DsRed, Takara Bio, San Jose, CA, USA, cat# 632496
goat anti-nephrin, R&D Systems, Minneapolis, MN, USA, cat# AF3159
sheep anti-TH, Millipore, Burlington, MA, USA, cat# AB1542
rabbit anti-TH, Millipore, Burlington, MA, USA, cat# AB152
rabbit anti-CGRP, Immunostar, Hudson, WI, USA, cat#24112

Secondary Antibodies -

	A	B	C	D	E	F	H
	Name	**Target**	**Host**	**Vendor**	**Catalog number**	**RRID**	**Dilution**
	Cy3-AffiniPure Donkey Anti-Rabbit IgG (H+L)	rabbit IgG (H+L)	donkey	Jackson ImmunoResearch Labs	711-165-152	RRID:AB_2307443	1:300
	Cy3-AffiniPure Donkey Anti-Sheep IgG (H+L)	sheep IgG (H+L)	donkey	Jackson ImmunoResearch Labs	713-165-147	RRID:AB_2315778	1:300
	Alexa Fluor 488-AffiniPure Donkey Anti-Goat IgG (H+L)	goat IgG	donkey	Jackson ImmunoResearch Labs	705-545-147	RRID:AB_2336933	1:300
	Cy5-AffiniPure Donkey Anti-Rabbit IgG (H+L)	rabbit IgG (H+L)	donkey	Jackson ImmunoResearch Labs	711-175-152	RRID:AB_2340607	1:300

	A	B	C	D	E	F	H
	Cy5-AffiniPure Donkey Anti-Sheep IgG (H+L)	sheep IgG (H+L)	donkey	Jackson ImmunoResearch Labs	713-175-147	RRID:AB_2340730	1:300

Cy3-conjugated donkey anti-rabbit, Jackson ImmunoResearch, West Grove, PA, cat# 711-165-152
Cy3-conjugated donkey anti-sheep, Jackson ImmunoResearch, West Grove, PA, cat# 713-165-147;
Alexa 488-conjugated donkey anti-goat, Jackson ImmunoResearch, West Grove, PA, cat# 705-545-147
Cy5-conjugated donkey anti-rabbit, Jackson ImmunoResearch, West Grove, PA, cat# 711-175-152
Cy5-conjugated donkey anti-sheep, Jackson ImmunoResearch, West Grove, PA, cat# 713-175-147

Troubleshooting

Safety warnings

Handle all reagents and biological materials according to institutional biosafety guidelines. Use personal protective equipment (PPE) including gloves, lab coats, and safety goggles.

Ethics statement

The animal study was reviewed and approved by University of Minnesota Institutional Animal Care and Use Committee.

1 Anesthetize mice using isoflurane.

STEP CASE

Preparation for glomerular scoring analyses 15 steps

If renal slices are intended to be used to analyze fiber prevalence near glomeruli *en masse*, follow this step-case.

2 Perform cardiac perfusion with ice-cold calcium-free Tyrode's solution (in mM: NaCl 116, KCl 5.4, MgCl2 1.6, MgSO4 0.4, NaH2PO4 1.4, glucose 5.6, NaHCO3 26) followed by Lana's fixative (4% paraformaldehyde and 0.2% picric acid in 0.1 M phosphate buffer pH 6.9).

Tissue Sectioning

3 Remove kidneys and store in PBS until sectioned.

4 Decapsulate kidneys and section coronally into 150 µm slices using a Vibratome. Store serial sections in PBS at 4 °C until staining.

Immunostaining and Xylenes Clearing 4d 0h 30m

5 Incubate sections in blocking buffer (PBS with 0.3% Triton-X100; 1% BSA, 1% normal donkey serum) at. 4 °C Overnight . 1d

6 Incubate sections in primary antibodies diluted in blocking buffer for 48:00:00 at 4 °C . 2d

7 Wash sections 3 times for 00:30:00 each in PBS at Room temperature . 30m

8 Incubate in secondary antibodies for 24:00:00 at Room temperature . 1d

9 Wash sections again in PBS and mount on gelatin-coated slides.

10 Dehydrate slide-mounted sections with increasing concentrations of ethanol (50, 75, 100, and 100% in diH2O, 30 minutes each).

11 Clear slide-mounted sections in Xylenes until tissue appears transparent.

12 Coverslip using DPX Mountant.

Imaging

13 Use a Nikon A1R FLIM Confocal Microscope equipped with the A1R GaAsP Confocal system to capture images of immunolabeled sections.

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> Note
>
> Most slices will be imaged as 5-15 optical sections with a z-step between 5 and 15 µm.

Analysis of whole-slice, low-resolution images ("Glomerular Scoring")

15 Process composite images into multiple maximum intensity projections of 2-6 optical sections, taking care to ensure that individual glomeruli are not duplicated between projections.

16 Load each composite image into the custom MATLAB scripts that localizes immunofluorescently labeled glomeruli. Individual glomeruli will be presented for the user to score whether a fine fiber-like structure is closely apposed to the nephrin+ labeling. Scripts used: Glomerular_Depth_Characterization.m; SmartBorder.m; simpleGlomFinder.m; play_script.m

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