Dissection and immunohistochemistry of mouse vagal ganglia

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dx.doi.org/10.17504/protocols.io.w6cfhaw

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ABSTRACT

Mice are euthanized, perfused with fixative and the vagal ganglia extracted. Vagal ganglia are then cyosectioned. Slices are stained for protein expression using immunohistochemistry. Expression of specific proteins and reporter proteins isarevisualized using microscopy.

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

MATERIALS

NAME	CATALOG #	VENDOR
Triton X-100	1610407	Bio-rad Laboratories
Bovine Serum Albumin (BSA)	A7906	Sigma Aldrich
DPX	1.00579.0500	Merck Millipore
Sucrose		
Tween 20	P1379	Sigma
Paraformaldehyde	P6148	Sigma Aldrich
Phosphate buffered saline powder, pH 7.4, for preparing 1 L solutions	P3813	Millipore Sigma
alexa fluor 647 chicken anti-goat	A21469	Invitrogen - Thermo Fisher

1 6 to 8 weeks old mice (male) were euthanized by CO_2 inhalation

2 Mice were transcardially perfused with phosphate buffered saline (PBS) to remove blood followed by 3.7% formaldehyde (PFA) in PBS

3 Vagal ganglia (including both jugular and nodose ganglia) were collected and fixed with 3.7% PFA for 2 hours on ice

4 Vagal ganglia were transferred to 18% sucrose solution for overnight at 4 °C

5 Tissue were mounted in optimal cutting temperature compound and frozen

6 Tissue were cut at 20 µm thickness and collected onto superfrost plus microscopy slide glass

7 Tissue were washed with PBS three times for 10 min

8 Tissue were blocked with blocking buffer (1% bovine serum albumin/10% normal donkey serum/0.3% triton x-100 in PBS) for 45 min at room temperature

9 Tissue were incubated with primary antibody (1:150, goat anti-VR1, SC-12498, Santa Cruz) diluted in blocking buffer overnight at 4 °C

10 Tissue were washed with 0.2% tween20 in PBS (PBST) for 10 min for three times at room temperature

11 Secondary antibody (1:300, Alexa flour 647 Chicken anti-goat, A21469, Invitrogen) was diluted in the 1% bovine serum albumin/5% normal donkey serum/0.2% PBST and tissue were incubated with it for 1 hour at room temperature

12 Tissue were washed with 0.2% tween20 in PBS (PBST) for 10 min for three times at room temperature

13 Tissue were cover-slipped with DPX for imaging and left to dry in the dark overnight.