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Dissection and immunohistochemistry of mouse brainstem

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We use this protocol and it's working

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Abstract

Mice are euthanized, perfused with fixative for brainstem tissue collection. Mouse brainstem is cryosectioned and slices are stained for protein expression using immunohistochemistry. Expression of specific proteins and reporter proteins are visualized using microscopy.

Troubleshooting

Tissue collection and cryosection

1 Mice are euthanized by CO_2inhalation and transcardially perfused with ice-cold PBS followed by perfusion fixation with ice-cold 3.7% formaldehyde (FA).

2 Brainstem is dissected out and post-fixed for 4 h in 3.7% FA at 4°C. The brainstem is washed in PBS to remove residual FA and transferred to 30% sucrose solution for cryoprotection.

3 The brainstem is mounted in OCT (optimal cutting temperature) compound and snap frozen in dry ice. The brainstem is sectioned in 40 µm slices.

4 All slices are collected onto SuperFrost Plus slides. Slides are then air dried at room temperature in the dark overnight.

Immunohistochemistry

5 Tissue are permeabilized with 0.3% Triton X-100 in PBS (PBSTx) for 15 min.

6 Tissue are blocked with 1% bovine serum albumin (BSA)/10% donkey serum (DS)/0.3% PBSTx. for 1 h at room temperature.

7 Tissue are incubated with primary antibodies diluted in blocking buffer overnight at 4°C.

8 Tissue are washed with 0.2% Tween 20 in PBS (PBST) three times for 10 min.

9 Tissue are incubated with secondary antibodies in 1% BSA/5% DS in 0.2% PBST for 1 h at room temperature.

10 Tissue are washed with 0.2% PBST three times for 10 min and rinsed briefly with H_2O.

11 Slides are air-dried and mounted with DPX Mounting Medium (Sigma-Aldrich) for imaging.