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Tissue preparation and immunohistochemistry-Alcacer et al 2024

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We use this protocol and it's working

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Abstract

This protocol describes the tissue preparation and immunohistochemistry protocol to verify the extent of dopamine denervation from 6-OHDA injections and check the transfection efficiency of GCamp6f-GFP vial construct that was injected into mouse brains.

Materials

-Perfusion pump
-4% (w/v) paraformaldehyde in phosphate buffered saline (PBS), pH 7.4
-Storage solution: 30% (w/v) ethylene glycol, 30% (w/v) glycerol, and 0.1 M PBS
-Tris-buffered saline (TBS; 0.10 M Tris and 0.14 M NaCl, pH 7.4)
-GFP Polyclonal Antibody, Alexa Fluor 488, Invitrogen (Molecular Probes) Cat#A-21311)
-Rabbit polyclonal α TH antibody Rabbit anti-TH: Peel Freez Biological, Product code: P40101-150
-Alexa Fluor 594 goat anti-rabbit, Jackson ImmunoResearch Labs Cat#111-585-045
-Polyvinyl alcohol mounting medium (PVA-DABCO, Sigma Aldrich)
-Zeiss Axio Imager M2 fluorescence microscope (for imaging final slices)

Before start

Twenty minutes prior the mouse is given an intraperitoneal injection of vehicle (physiological saline) or L-DOPA (6 mg/kg). The mouse is then rapidly anesthetized with pentobarbital (500 mg/kg intraperitoneal injection).

Perfusion & Dissection

1 Stabilize mouse and open the abdomenal cavity of the anesthetized mouse and expose the heart.

2 Using forceps, stabilize the heart and insert a blunted needle into the left ventricle. The needle is connected to tubing for the perfusion pump that contains 4% (w/v) paraformaldehyde in saline phosphate buffer (PBS), pH 7.4.

3 Make a small incision on the right atrium using fine scissors.

4 Start the perfusion pump with PFA and perfuse until liquid is clear and not bloody (for a total of 5 min infusing 100mL per minute).

5 Once perfusion is complete, dissect out the brain.

Brain Slice preparation

6 Post-fix the brain overnight in 4% (w/v) paraformaldehyde in saline phosphate buffer (PBS), pH 7.4 and store at 4 °C

7 Cut brains at 30um sections with a vibratome and store at -20 °C in 30% (v/v) ethylene glycol, 30% (v/v) glycerol, and 0.1 M sodium phosphate buffer until sections are processed.

Immunohistochemistry (IHC)

8 Rinse free floating sections in Tris-buffered saline (TBS; 0.10 M Tris and 0.14 M NaCl, pH 7.4).

9 Incubate for 5 minutes in TBS containing 3% H2O2 and 10% methanol.

10 Rinse in TBS (0.10 M Tris and 0.14 M NaCl, pH 7.4).

11 Incubate for 15 minutes in TBS containing 0.2% Triton X-100.

12 Block sections in bovine serum solution (BSA).

13 Incubate overnight at 4 °C with 1:1000, GFP Polyclonal Antibody, Alexa Fluor 488 and 1:1000 rabbit polyclonal α TH antibody.

14 Rinse in TBS (0.10 M Tris and 0.14 M NaCl, pH 7.4).

15 Incubate for 45 minutes with 1:400 secondary antibody goat-anti rabbit Alexa Fluor 594 at room temperature.

16 Rinse **twice** in TBS (0.10 M Tris and 0.14 M NaCl, pH 7.4).

17 Rinse **twice** in TB (0.10 M Tris, pH 7.4).

18 Mount sections in a polyvinyl alcohol mounting medium.

Imaging confirmation

19 Use Zeiss Axio Imager M2 fluorescence microscope to confirm lens placement, viral transduction and dopaminergic denervation.