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Immunohistochemistry for astrocytes, microglia, and c-Fos

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We use this protocol and it's working

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Abstract

Performing immunohistochemistry for astrocytes and microglia allows us to study changes in their morphology or cell density, which reflects glial response to damage, inflammation, or neuronal plasticity. Immunohistochemistry for c-Fos is used as a marker of neuronal activity induced by specific stimuli, behaviors, or experiences. Below we describe a protocol for performing immunohistochemistry for astrocytes [glial fibrillary acidic protein (GFAP)], microglia [ionized calcium-binding adaptor molecule 1 (Iba1)], and c-Fos.

Guidelines

It is important that the sections are stirred continuously during incubation.

Materials

Vibratome, 1x phosphate buffer solution, hydrogen peroxide (H_2O_2), normal rabbit serum, normal goat serum, primary antibodies (goat anti-GFAP ab53554, rabbit anti-Iba-1 cell signaling), secondary antibodies (Goat IgG VECTASTAIN, Rabbit IgG VECTASTAIN), avidin-biotin complex, 3-3'-diaminobenzidine, peroxidase substrate kit DAB (SK-4100), Permount mounting medium, coverglass, slide, pasteur pipette, micropipette, oscillator.

Before start

Check the availability of equipment and necessary supplies. Thaw the antibody aliquots if necessary.

Brain preparation, tissue collection, and storage

1 Anesthetize the rats by intraperitoneal injection of a ketamine/xylazine cocktail (0.75 ml ketamine + 0.25 ml xylazine + 5 ml sterile saline), administering a dose of 0.125 ml per 20 g of body weight.

2 Perform transcardial perfusion with 300 ml of fresh cold phosphate buffered saline (1x PBS, pH 7.4), followed by 300 ml of 4% paraformaldehyde.

3 Remove brains and postfix 4% paraformaldehyde for 48h in a Falcon tube.

4 Perform coronal brain slices of the region of interest at 40 µm using a vibratome (Leica, VT1000S microsystem, USA).

5 Collect slices sequentially in four-row wells coated with 1x PBS.

6 After sectioning all brains, remove 1x PBS and place sections in cryoprotectant solution [glycerol: ethylene glycol: 1x PBS, (3:3:4)] and store at -20 °C until further use.

Immunohistochemistry (GFAP or Iba1)

1d 5h 37m 30s

7 Wash slices in phosphate buffered saline (1x PBS, pH 7.4), 4 times for 5 minutes. 20m

8 Incubate slices in 1.5% hydrogen peroxide dissolved in 1x PBS for 5 minutes. 5m

9 Rinse slices in 1x PBS, 2 times for 5 minutes. 10m

10 Incubate slices in blocking solution [1x PBS + 0.3 % Triton X-100 + 3% normal rabbit serum (for GFAP) or 3% normal goat serum (for Iba1)] for 2 hours. 2h

11 Add primary antibody [goat anti-GFAP ab53554, abcam [1:500] (for astrocytes) or rabbit anti-Iba-1 cell signaling [1:1000] (for microglia)] in diluent antiserum [1x PBS + 0.3 Triton X-100 + 1% normal rabbit serum (for GFAP) or 1% normal goat serum (for Iba1)] and incubate for 24 hours at room temperature. 1d

12 Wash slices in 1x PBS, 3 times for 5 minutes. 15m

13 Incubate slices in secondary antibody: anti-goat [1:250] using a Goat IgG VECTASTAIN, Elite, ABC-HRP Kit, Peroxidase, PK-6105, CA, USA (for GFAP) or anti-rabbit [1:500] using a Rabbit IgG VECTASTAIN, Elite, ABC-HRP Kit, Peroxidase, PK-6101, CA, USA (for Iba1) dissolved in diluent antiserum for GFAP or Iba1, for 1 hour at room temperature. 1h

14 Wash slices in 1x PBS 3 times for 5 minutes. 15m

15 Incubate slices in avidin-biotin complex for 1 hour at room temperature. 1h

16 Wash slices in 1x PBS, 3 times for 5 minutes. 15m

17 Incubate slices with 3-3'-diaminobenzidine for 2:30 minutes (Peroxidase substrate kit DAB, SK-4100, Vector Laboratories Inc., CA, USA). 2m 30s

18 Wash slices in 1x PBS twice for 5 minutes and once in distilled water for 5 minutes. 15m

19 Mount slices on gelatinized slides and cover with Permount mounting medium and coverglass.

Immunohistochemistry (c-Fos) 1d 5h 37m 30s

20 Wash slices in phosphate buffered saline (1x PBS, pH 7.4), 4 times for 5 minutes. 20m

21 Incubate slices in 1.5% hydrogen peroxide dissolved in 1x PBS for 5 minutes. 5m

22 Rinse slices in 1x PBS 2 times for 5 minutes. 10m

23 Incubate slices in blocking solution (1x PBS + 3% normal rabbit serum + 0.3% Triton X-100) for 120 min. 2h

24 Add primary antibody (Abcam goat anti c-Fos polyclonal ab156802 at [1:500]) in diluent antiserum (1x PBS + 1% normal rabbit serum + 0.3% Triton X-100) and incubate for 24 hours at room temperature. 1d

25 Wash slices in 1x PBS, 3 times for 5 minutes. 15m

26 Incubate slices in secondary antibody (anti goat [1:250]) using a Goat IgG, VECTASTAIN, Elite, ABC-HRP Kit, Peroxidase, PK-6105, CA, USA for 1 hour at room temperature. 1h

27 Wash slices in 1x PBS, 3 times for 5 minutes. 15m

28 Incubate slices in avidin-biotin complex for 1 hour at room temperature. 1h

29 Wash slices in 1x PBS, 3 times for 5 minutes. 15m

30 Incubate slices with 3-3'-diaminobenzidine for 2.5 minutes (Peroxidase substrate kit DAB, SK-4100, Vector Laboratories Inc., Burlingame, CA, USA). 2m 30s

31 Wash slices in 1x PBS twice for 5 minutes and once in distilled water for 5 minutes. 15m

32 Mount slices on gelatinized slides and covered with Permount mounting medium and coverglass.

Protocol references

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