# Immunohistochemistry for Carbon Fiber Thread Electrodes

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ABSTRACT

Methods for immunofluorescent staining of brain tissue with indwelling electrodes are described.

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**Protocol status:** Working
We use this protocol and it's working

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1 Free floating 25-µm sections from rat A were rinsed three times for 5 min each with 0.01 M phosphate buffered saline with 0.2% Triton X-100 (PBS-Tx), and then blocked for 60 min in TSA Blocking reagent (Akoya science FP1012) diluted in PBS-Tx (TSA block) on a shaker.

2 Sections were left shaking for one night at 4°C in primary antibodies diluted in TSA block.

3 Primary antibody and concentration used was: rabbit anti-MOR1(Abcam Ab134054, 1:500).

4 Sections were rinsed three times for 5 min each with PBS-Tx and then were incubated in secondary antibodies diluted in TSA block for 2 hours on the shaker at room temperature.

5 Secondary antibodies and concentrations used were: goat anti-rat conjugated with AlexaFluor 546 (Invitrogen A-11081, 1:300), and goat anti-rabbit conjugated with AlexaFluor 647 (Invitrogen A-21245, 1:300).

6 Sections were rinsed three times for 5 min in 0.1 M PB, then incubated for 2 min in DAPI (Invitrogen 62248, 1:1000) diluted in PBS.

7 After rinsing three times for 5 min in 0.1 M PB, all sections were mounted onto glass slides and cover slipped with ProLong Gold antifade reagent (Invitrogen, P36930).

8 Slides were covered with aluminum foil and stored in 4°C until imaging.

9 100-µm sections on the glass slides from rat A were cover slipped without any staining with ProLong Gold antifade reagent.

10 The 100-µm sections on the glass slides from rat B were rinsed three times for 5 min each with 0.01 M PBS-Tx, and blocked endogenous peroxidase activity with 3% H2O2 in PBS-Tx.

11 After three times washing with PBS-Tx, slides were blocked in TSA block in the humidity-controlled chamber.

12 After removing the excess liquid from slides, rabbit anti-MOR1 (Abcam Ab134054, 1:500) antibody diluted in TSA block was applied on the slides and slides were left for one night at 4°C in a humidity-controlled chamber.

13 Slides were then rinsed three times for 5 min each with PBS-Tx and were incubated with goat anti-rabbit antibody conjugated with polymer HRP (Thermofisher B40962) for 45 minutes at room temperature.

14 After rinsing three times for 5 min each with PBS-Tx, slides were incubated with TSA-Plus Fluorescein (PerkinElemer NEL745001KT) diluted at 1:100 in 1X Plus Amplification Diluent for 15 minutes at room temperature.

15 Slides were rinsed three times for 5 min in 0.1 M PB, then incubated for 2 min in DAPI (1:1000, Invitrogen 62248) diluted in PBS.

16 After rinsing three times for 5 min in 0.1 M PB, slides were air dried, and then were cover slipped with ProLong Gold antifade reagent.

17 Slides were covered with aluminum foil and stored at 4°C until imaging was done.

18 The TissueFAXS Whole Slide Scanning System (TissueGnostics) with x20 objective lens was used to obtain tiled-images from rat A.

19 Cameras equipped with this system are Baumer HXG40c (HX series) CMOS camera 16 bit (2048 × 2048) for brightfield imaging and Hamamatsu Orca Flash 4.0 V2 cooled digital CMOS camera C11440-22CU for fluorescence imaging.

20 Brightfield images were obtained from 100-µm sections to see carbon fibers left in the brains and fluorescence images were obtained from 25-µm sections to visualize MOR1 enriched striosome and endogenous rat IgG.