

Protocol for Fluoro-Jade B staining of frozen brain sections
MWS 1/25/06

Note: This staining works very poorly on paraffin sections... I saw only faint staining of reactive astrocytes, but the predominant (very strong!) staining was of vascular endothelial cells (visualized as thin rectangular chains of cells throughout the brain).

For this protocol, I am using 20 μm frozen sections cut w/ a cryostat and thaw-mounted onto Colorfrost Plus blue Fisher slides. I also cut 10 μm sections and stained them as well, but for the old PScDKO mutant brains there were many holes in the tissue. The 20 μm sections look better (fewer holes).

1. Air dry frozen sections on benchtop
2. Rinse slides in PBS 5 min on rotating shaker
3. Rinse slides quickly in dH_2O (2x)
4. Wick off excess dH_2O and dry slides on 50°C heat block 5 min
5. Incubate slides in 1% NaOH/ 80% EtOH 5 min on rotating shaker
6. Rinse slides in 70% EtOH 2 min on rotating shaker
7. Rinse slides in dH_2O 2 min on rotating shaker
8. Incubate slides in fresh 0.06% KMnO_4 20 min on rotating shaker
9. Rinse slides in dH_2O 2 min on rotating shaker
10. Incubate slides in 0.0004% Fluoro-Jade B [4 mL 0.01% FJB stock + 96 mL 0.1% acetic acid] for 20 min on rotating shaker. Cover with aluminum foil. [Note: You MUST prepare this solution less than 10 min before using!!]
11. Rinse slides in dH_2O 1 min on rotating shaker (3x)
12. Wick off excess dH_2O and dry slides on 50°C heat block 5 min
13. Immerse slides in HistoClear 3 min
14. Mount slides w/ Permount

Solutions

1. 0.01% Fluoro-Jade B stock solution: 0.01g per 100 mL dH_2O [Store at 4°C wrapped in foil]
2. 0.1% acetic acid (v/v): 500 μL per 500 mL dH_2O
3. 5% NaOH: 5g pellets per 100 mL dH_2O
4. 1% NaOH/ 80% EtOH: 100 mL 5% NaOH + 400 mL EtOH
5. 0.06% KMnO_4 : 0.12g per 200 mL dH_2O [Note: TOXIC!!! Store at 4°C, and use within 1 week]

Fluoro-Jade B is sold by Chemicon (Cat # AG310)

Note: If immunostaining is desired, KMnO_4 pretreatment time of 20 min will likely be too stringent; reduce time. Also, the 1% NaOH/ 80% EtOH step may have to be omitted.