

## Fluoro Jade B Protocol

From Justin Botterill (May, 2018)

### Procedure:

- |  |        |
|--|--------|
| 1) Mount tissue onto gelatin-treated microscope slides and heat slides at 50°C using a slide warmer (or incubator).  | 30 min |
| 2) Immerse slides into a solution of 100% EtOH for 5 minutes.<br><i>*some labs use 1% sodium hydroxide in 80% EtOH instead for this step. However, this can occasionally cause tissue to fold/wrinkle.</i> | 5 min  |
| 3) Immerse in 70% EtOH   | 2 min  |
| 4) Rinse slides with double-distilled (dd)H <sub>2</sub> O   | 2 min  |
| 5) 0.06% Potassium permanganate (Shaking gently)   | 15 min |
| <u>250mL working solution:</u>   |        |
| KMnO <sub>4</sub> (150mg)  |        |
| ddH <sub>2</sub> O (250mL)   |        |
| 6) Rinse slides with ddH <sub>2</sub> O  | 2 min  |

**All subsequent steps must be performed with as little light as possible. Always protect against photobleaching. Cover all staining dishes with aluminum foil.**

- |   |                |
|---|----------------|
| 7) Stain slides in 0.001% Fluoro-Jade working solution (gently shaking)   | 30 min         |
| 8) Rinse slides with ddH <sub>2</sub> O   | 3 x 1 min each |
| 9) Remove slides from rack. Lay flat and dry at room temp.<br><i>**Do not begin next step until slides are completely dry**</i> |                |
| 10) Rinse slides in xylene  | 3 x 2 min each |
| 11) Coverslip with mounting medium (i.e., Permount).<br>Keep slides in the dark and store in the cold.                          |                |

### FJB Stock and Working Solutions

#### **0.01% FJ Stock solution (expires in 2 months; store at 4°C)**

Fluoro-Jade	50mg	10mg	7.5mg	5mg
ddH <sub>2</sub> O	500 mL	100 mL	75 mL	50 mL

#### **0.001% FJ Working Solution (Make up 10 minutes before use)**

Fluoro-Jade (stock)	25 mL	20 mL	10 mL
0.1% acetic acid	225 mL	180 mL	90 mL

#### **0.1% Acetic Acid Solution**

Glacial Acetic Acid	250 µl	225 µl	180 µl	100 µl
ddH <sub>2</sub> O	250 mL	225 mL	180 mL	100 mL

## Fluoro-Jade C Staining Protocol

*From Swati Jain (May, 2018)*

To coat glass slides, the slides should be immersed for 2 minutes in a solution of 1% gelatin (porcine skin gelatin from Sigma) heated to 60°C. Drain off the excess gelatin from the slide rack and dry the slides in an oven at 60°C overnight.

### Procedure to mount the sections:

1. Mount the sections on 1% gelatin coated slides with distilled water. Let the slides dry on a hot plate at 50-60°C for an hour. Make sure the temperature does not exceed 60°C.
2. Allow the slides to cool to room temperature for ~20 minutes.

### Solutions for staining:

1. Basic absolute alcohol (1% NaOH in 80% ethanol)
  - 20 ml of 1% NaOH\*
  - 80 ml absolute alcohol (100% ethanol)
  - \*To make 1% NaOH:
    - 3g of NaOH pellets (J.T.Baker Cat. # 3722)
    - 60 ml of distilled water (dH<sub>2</sub>O)
2. 0.06% Potassium Permanganate (KMnO<sub>4</sub>; Fisher Cat. # P-279)
  - KMnO<sub>4</sub> 180 mg
  - Distilled Water 300 ml

### 3. Fluoro-Jade C solution

First make a stock solution (0.01%, Histo-Chem). Note that this solution should be made every 3 months and stored at 4°C. It is photosensitive and hygroscopic so cover it in aluminum foil in an airtight container.

Fluoro-Jade C 5 mg  
Distilled Water 50 ml.

Second make the staining solution (0.0002%) Make it fresh and use within 10 minutes of preparation.

Fluoro-Jade C Stock Solution 6 ml  
0.1% acetic acid 294 ml

\*To make 0.1% acetic acid:

acetic acid 294 µl  
dH<sub>2</sub>O 294 ml

### Procedure for staining:

Place slides in a staining rack for even staining. All steps are at room temperature.

1. Immerse slides in basic absolute alcohol for 5 min.

Note: This is different from the standard procedure because there is no pre-treatment with basic alcohol.

2. Immerse slides in 70% alcohol for 2 min.

3. Rinse slides in distilled water once.

4. Let the sections dry (place slides on a hot plate at ~40°C for 10 minutes) and then let them cool to room temperature.

5. Immerse slides in a solution of 0.06% KMnO<sub>4</sub> for 10 min, preferably on a shaker to ensure equal exposure of slides to the solution.

Note:

Gentle shaking is preferable (e.g., a "belly dancer" (Stovall) set on ½ setting).

KMnO<sub>4</sub> pretreatment minimizes background staining and confers considerable resistance to fading.

6. Rinse slides in distilled water (2 washes, 1 min each).

7. Immerse slides in Fluoro-Jade C staining solution for 20 min in the dark.

**Protect slides from light from this step onwards.**

8. Rinse in distilled water (3 washes, 1 min each).

9. Dry in the dark (e.g., a drawer) overnight at room temperature on a paper towel.

10. The next day, slides are cleared by immersion in xylene 3 times for at least 1 min each time and coverslipped with DPX mounting medium. DPX is a non-aqueous, non-fluorescent plastic mounting media (Fluka Cat. # 44581).

**References:**

Schmued LC, Hopkins KJ (2000) Fluoro-Jade B: a high affinity fluorescent marker for the localization of neuronal degeneration. *Brain Research* 874: 123–130.

Schmued LC, Stowers CC, Scallet AC, Xu L (2005) Fluoro-Jade C results in ultra high resolution and contrast labeling of degenerating neurons. *Brain Res* 1035:24–31.