

## FLUORO-JADE® C

Ordering Information	Storage Conditions
Product Number: 23062 (5 mg)	Store desiccated at -20 °C. Expiration date is 12 months from the date of receipt.

### General Properties

Molecular Weight: 823  
Appearance: Coffee brown to brick red powder  
Maximum excitation: 485 nm  
Maximum Emission: 525 nm  
Solvents: H<sub>2</sub>O

### Biological Applications

Fluoro-Jade® C, like its predecessors, Fluoro-Jade and Fluoro-Jade B, stains all degenerating neurons, regardless of specific insult or mechanism of cell death. Therefore, the patterns of neuronal degeneration seen following exposure to either the glutamate agonist, kainic acid, or the inhibitor of mitochondrial respiration, 3-NPA, were the same for all of the Fluoro-Jade dyes. However, there was a qualitative difference in the staining characteristics of the three fluorochromes. Specifically, Fluoro-Jade C exhibited the greatest signal to background ratio, as well as the highest resolution. This translates to a stain of maximal contrast and affinity for degenerating neurons. This makes it ideal for localizing not only degenerating nerve cell bodies, but also distal dendrites, axons and terminals. The dye is highly resistant to fading and is compatible with virtually all histological processing and staining protocols. Triple labeling can be accomplished by staining degenerating neurons with Fluoro-Jade C, cell nuclei with DAPI and activated astrocytes with GFAP immunofluorescence.

### Storage Conditions

Store the powder at -20 °C, avoid light and moisture. Desiccator is recommended when possible. The liquid stock solution (0.01%) in distilled water can be stored at 2-8 °C for up to 3 months. The .0002-.0001% working solution in 0.1% acetic acid should be used within 4 hrs of preparation.

### Sample Protocol

1. Tissue sections are mounted onto pre-treated slides and allowed to dry at room temperature overnight, for example, mount brain tissue sections on gelatin coated slides and dry at 50-60 °C on slide warmer for 30 minutes.
2. Immerse slides in basic alcohol solution (20 mL of 1% NaOH to 80 mL of 100% ethanol) for 5 min.
3. Transfer the slides into 70% ethanol for 2 min, and then into distilled water for 2 min.
4. Incubate in 0.06% potassium permanganate solution for 10 min.
5. Rinse slides in distilled water for 2 min.
6. Incubate the slides in 0.0001% solution of Fluoro-Jade® C dissolved in 0.1% acetic acid for 10 minutes.  
*Note 1: To make 0.0001% solution of Fluoro-Jade® C, first making a 0.01% stock solution of the dye in distilled water and then adding 1 mL of the stock solution to 99 mL of 0.1% acetic acid vehicle.*  
*Note 2: The working solution was used within 2 h of preparation. The stock solution, when refrigerated, can be kept for long periods but should be discarded if the solution becomes cloudy.*
7. Rinse the slides with distilled water for 1min per change for 3 times.
8. Drain excess water onto a paper towel, and air-dry the slides on a slide warmer at 50 °C for at least 5 min.
9. Clean the air dried slides in xylene for at least 1 min (1-5 min).
10. Coverslip with DPX (Fluka or Sigma) nonfluorescent mounting media. Do not use polar cover slipping media, such as those that contain water, alcohol or glycerol.  
*Note 1: For best preservation of sections, apply a clear coat of nail polish around the edges of the coverslip, store in the cold.*

*Note2: To protect Fluor-jade from photo-bleaching, mix the solutions in dim light. When the slides are staining, wrap the staining trays in aluminum foil.*

### Comments on additional and alternative procedural variants

- 1 When working with paraffin processed tissue, the sections are first deparaffinized through two 10 min changes of xylene and then the sections are rehydrated through a graduated alcohol series, omitting the basic alcohol solution. Once in distilled water, the sections are transferred to the potassium permanganate solution (30 ml of 0.1-0.2M tripotassium phosphate solution to 70 mL 100% ethanol) at which point the staining procedure is identical to that described for frozen sections.
- 2 The potassium permanganate pretreatment confirms a significant reduction in background staining. However, it can also denature some antigenic epitopes and therefore reduced the time in this solution when combining with immunofluorescently labeled tissue.
- 3 The working solution should be made up fresh daily, and used within 2 h of preparation. The stock solution, when refrigerated, can be kept for long periods but should be discarded if the solution becomes cloudy. The dye working concentration typically ranges from 0.0001% to 0.0002% depending on properties of the tissue and microscope.
- 4 A DNA counterstain can be achieved by the addition of 1 ml of 0.01% DAPI stock solution to 99 ml of *Fluoro-Jade® C* solution.
- 5 Traditionally the sections are air dried on a slide warmer, since the tracer is not compatible with ethanol dehydration. However, it is possible to solvent dehydrate the sections using butanol as follows: transfer sections to distilled H<sub>2</sub>O for 1 minute, then into a mixture of equal parts ethanol and butanol for 1 min, and then through 2 five minutes changes of butanol. The slides are then transferred to xylene and coverslipped as described above. Solvent dehydration allows for simultaneous processing of larger volumes of slides.

### References:

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

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