FLUORO-JADE® B

Ordering Information	*Storage Conditions*
Product Number: 23061 (5 mg)	Store desiccated at -20 °C

General Properties

Molecular Weight: 681
Appearance: Dark red powder with green iridescence
Maximum excitation: 480 nm
Maximum Emission: 525 nm
Solvent: H_2O

Biological Applications

Fluoro-Jade® B is a polyanionic fluorescein derivative which sensitively and specifically binds to degenerating neurons. It has a green fluorescence with excitation peak at 480 nm and emission peak at 525 nm. The filter used for visualizing Fluoro-Jade B is the FITC filter. Fluoro-Jade B can be used on most tissue section types and thicknesses including frozen, vibratomed cryostat or paraffin-embedded sections from 3-50 um. Fluoro-Jade B is faster and more reliable than older methods (e.g. suppressed silver) for the unequivocal qualitative detection and quantitative measurement of both gross and fine scale neuronal degeneration. Fluoro-Jade B will not distinguish between apoptotic and necrotic neurons. Astrocytic labeling is occasionally observed with Fluoro-Jade B, especially in chronic studies. Fluoro-Jade® is a registered trademark of Histo-Chem, Inc.

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 0.0004% working solution in 0.1% acetic acid should be used within 10 minutes of preparation.

Sample Protocol

1. Treat animals with appropriate procedures as recommended by your animal vendors.
2. Perfuse the animal with 300 mL of 0.1 M neutral phosphate buffered 10% formalin (4% formaldehyde) via the ascending aorta, while clamping off the descending aorta.
3. Postfix the brains at least overnight in the same fixative solution plus 20% sucrose.
4. Cut the tissue on a freezing sliding microtome at a thickness of 25 um.
5. Collected the sections in 0.1 M phosphate buffer, pH 7.4. Mounted on 2% gelatin coated slides and then air dried on a slide warmer at 50 degrees C for at least half an hour.
6. Immerse the slides in 1% NaOH with 80% alcohol (20 mL of 5% NaOH to 80 mL absolute alcohol) for 5 minutes, and then in 70% alcohol for 2 minutes followed by 2 minutes in ddH_2O.
7. Transfer the slides into 0.06% potassium permanganate solution for 10 minutes, shake it gently.
8. Rinse the slides in ddH_2O for 2 minutes.
9. Prepare a 0.01% Fluoro-Jade® B stock solution by adding 10 mg of the dye powder to 100 mL of ddH_2O, mix it well.
10. Prepare 100 mL of 0.0004% Fluoro-Jade® B staining solution by adding 4 mL of the stock solution to 96 mL of 0.1% acetic acid .
11. Stain the slides in the staining solution for 20 minutes, rinse with ddH_2O for one minute X 3 times.
12. Drain the slides vertically on a paper towel. Dry the slides on a slide warmer (~50 degrees C) for 5-10 min until they were fully dry.
13. Clear the dry slides by immersion in xylene for at least 1 minute before cover slip with a non-aqueous non-fluorescent plastic mounting media such as DPX (Sigma Chemicals Co., St. Louis, MO).

Data Analysis

Examine the tissue using an epifluorescent microscope with a filter set at Ex/Em = 490/525 nm.

Note 1: If the background level is high, one can leave in fresh potassium permanganate longer (~20 minutes), or dilute the Fluoro-Jade® B concentration by half (0.0002%).
Note 2: For paraffin processed tissues, use xylene to remove paraffin, and then rinse twice with alcohol.
Note 3: It can be combined with immunofluorescence, although sometimes pretreatment procedures can attenuate immunofluorescent labeling. If so, the time in potassium permanganate solution should be reduced as necessary. Dye concentration may also need to be reduced.

References:

1. L. Schmued, W. Slikker, G. Wang, Soc. Neuroscience Ab. 24 (1998) 1064.
2. L. Schmued and K. Hopkins, *Brain Res*. (2000) **874**:123-130.