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FLUORO-JADE® B

CATALOG NUMBER: AG310

LOT NUMBER:

QUANTITY: 50 mg

DESCRIPTION: Fluoro-Jade® B

BACKGROUND: Fluoro-Jade® B is a polyanionic fluorescein derivitave which sensitively and specifically binds to degenerating neurons. It is a dark red power that has a green iridescence with excitation peak at 480 nm and emission peak at 525 nm. The filter used for visualizing Fluoro-Jade® B is a fluorescein/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 µm. 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.

APPEARANCE: Dark red powder with green iridescence.

PURITY: This layer chromatography using cellulose plates and a solvent system of n-propinol, water, and ammonium hydroxide (6:5:2) revealed the presence of two fluorescent isomers and two trace non-fluorescent bands. No amount of fluorescein or Fluoro-Jade® was present.

MOLECULAR WEIGHT: 681

EXCITATION PEAK: 480 nm

EMISSION PEAK: 525 nm

FILTER SYSTEM: Fluorescein / FITC

SOLUBILITY: Very soluble in water and bases, moderately soluble in alcohol and weak acids.

STORAGE/HANDLING: The powder should be stored well sealed at room temperature, preferable in a desiccator, due to its hygroscopic nature. The liquid stock solution (0.01%) in distilled water can be stored at 2-8°C for up to 3 months. The working solution (0.0004%) in 0.1% acetic acid should be prepared fresh and not be stored or reused.

TOXICITY: Although the compound appears to be of low toxicity, it has not been extensively evaluated and therefore routine laboratory caution should be exercised. Not intended for human consumption.

METHODS REFERENCES:

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

28820 Single Oak Drive • Temecula, CA 92590
Technical Support: T: 1-800-MILLIPORE (1-800-645-5476) • F: 1-800-437-7502
www.millipore.com

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SUGGESTED PROTOCOL FOR USING FLUORO-JADE® B

Processing:

Following appropriate survival interval, animals were perfused with 300 mL of 0.1 M neutral phosphate buffered 10% formalin (4% formaldehyde) via the ascending aorta, while clamping off the descending aorta. The brains were postfixed at least overnight in the same fixative solution plus 20% sucrose. Tissue was cut on a freezing sliding microtome at a thickness of 25 um. The sections were collected in 0.1 M neutral phosphate buffer. The sections were typically mounted on 2% gelatin coated slides and then air dried on a slide warmer at 50 degrees C for at least half an hour. The slides were first immersed in a solution containing 1% sodium hydroxide in 80% alcohol (20 mL of 5% NaOH added to 80 mL absolute alcohol) for 5 minutes. This was followed by 2 minutes in 70% alcohol and 2 minutes in distilled water. The slides were then transferred to a solution of 0.06% potassium permanganate for 10 minutes, preferably on a shaker table to insure consistent background suppression between sections. The slides were then rinsed in distilled water for 2 minutes. The staining solution was prepared from a 0.01% stock solution for Fluoro-Jade® B that was made by adding 10 mg of the dye powder to 100 mL of distilled water. To make up 100 mL of staining solution, 4 mL of the stock solution was added to
96 mL of 0.1% acetic acid vehicle. This results in a final dye concentration of 0.0004%. The stock solution, when stored in the refrigerator was stable for months, whereas the staining solution was typically prepared within 10 minutes of use and was not reused. After 20 minutes in the staining solution, the slides were rinsed for one minute in each of three distilled water washes. Excess water was removed by briefly (about 15 s) draining the slides vertically on a paper towel. The slides were then placed on a slide warmer, set at approximately 50 degrees C, until they were fully dry, (eg. 5-10 min). The dry slides were cleared by immersion in xylene for at least a minute before coverslipping with DPX (Fluka, Milwaukee WI, or Sigma Chem. Co., St. Louis, MO), a non-aqueous non-fluorescent plastic mounting media.

Analysis:

The tissue was then examined using an epifluorescent microscope with blue (450-490 nm) excitation light. A barrier filter that allows passage of all wavelengths longer than 515 nm will result in a yellow-green emission color, where as a notch filter, (eg. 515-565 nm) will result in a green emission color. Most filters designed for visualizing fluorescein or FITC (eg. the Nikon B-2A or the B-3A filter cubes) will be suitable for visualizing Fluoro-Jade® B.

Frequently Asked Questions:

1) What can be done if the background level is too high relative to specific staining?

Answer: Leave in fresh potassium permanganate longer, about twenty minutes, or dilute the Fluoro-Jade® concentration by half, Fluoro-Jade® B (.0002%)

2) Can Fluoro-Jade® be used with paraffin processed tissue?

Answer: Yes. Use xylene to remove paraffin, then rinse twice with alcohol. Also works with cryostat cut unfixed tissue.

3) Can it be combined with immunofluorescence?

Answer: Yes. 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.

Fluoro-Jade® is a registered trademark of Histo-Chem, Inc.

Important Note: *During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. For products with volumes of 200 µL or less, we recommend gently tapping the vial on a hard surface or briefly centrifuging the vial in a tabletop centrifuge to dislodge any liquid in the container's cap.*

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28820 Single Oak Drive • Temecula, CA 92590
Technical Support: T: 1-800-MILLIPORE (1-800-645-5476) • F: 1-800-437-7502
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