FT-CD0860

FLUORO-JADE C

Product Description

.

Name :	**FLUORO-JADE C**
Catalog Number :	FP-CD0860, 30 mg
Molecular Weight :	MW= 823
Solubility:	Highly soluble in water and bases; moderately soluble in alcohol and weak acids.
Absorption / Emission :	$\lambda_{exc}\backslash\lambda_{em}$ = 485/525 nm

Storage: The powder should be stored well sealed at room temperature, preferably in a desiccator, due to its hygroscopic nature.
The 0.01% stock solution in distilled water should be stored at +4°C.
The 0.001% working solution in 0.1% acetic acid should be prepared fresh and not be stored or reused.

Introduction

The causes and effects of neuronal degeneration are of major interest to a wide variety of neuroscientists. Paralleling this growing interest is an increasing number of methods applicable to the detection of neuronal degeneration. The earliest methods employing aniline dyes were methodologically simple, but difficult to interpret due to a lack of staining specificity. In an attempt to circumvent this problem, numerous suppressed silver methods have been introduced. However, these methods are labor intensive, incompatible with most other histochemical procedures and notoriously capricious. In an attempt to develop a tracer with the methodological simplicity and reliability of conventional stains but with the specificity of an ideal suppressed silver preparation, the Fluoro-Jade dyes were developed. Fluoro-Jade C, like its predecessors, Fluoro-Jade and Fluoro-Jade B, were found to stain 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 actrocytes with GFAP immunofluorescence.

Directions for use

Histological staining

Half of each group of brains were paraffin embedded and cut on a rotary microtome while the remainder were cut on a freezing sliding microtome. Paraffin sections were 10um in thickness while frozen sections were cut at a thickness of 25um. Prior to staining, sections were mounted from distilled water onto gelled slides. Gelatin

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coated slides were prepared by immersion in a 60 degree C solution of 1% pig skin gelatin (type A, 300 Bloom, # 24539S) and then oven dried overnight at the same temperature. The sections were mounted onto the slides from distilled water and then air dried for at least 30 min on a slide warmer at 50 degrees C. Slides bearing frozen cut tissue sections were first immersed in a basic alcohol solution consisting of 1% sodium hydroxide in 80% ethanol for 5 min. They were then rinsed for 2 min in 70% ethanol, for 2 min in distilled water, and then incubated in 0.06% potassium permanganate solution for 10 min. Following a 1-2 min water rinse, the slides were then transferred for 10 min to a 0.0001% solution of Fluoro-Jade C dissolved in 0.1% acetic acid vehicle. The proper dilution was accomplished by first making a 0.01% stock solution of the dye in distilled water and then adding 1ml of the stock solution to 99ml of 0.1% acetic acid vehicle. 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. The slides were then rinsed through three changes of distilled water for 1 min per change. Excess water was drained onto a paper towel, and the slides were then air dried on a slide warmer at 50 degrees C for at least 5 min. The air dried slides were then cleared in xylene for at least 1 min and then coverslipped with DPX (# 263630) nonfluorescent mounting media. Polar coverslipping media, such as those that contain water, alcohol or glycerol were never used.

For comparative purposes, some slides were stained with Fluoro-Jade B according to the previously described procedure. 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 at which point the staining procedure is identical to that described for frozen sections.

Multilabeling

Fluoro-Jade C can readily be combined with other fluorescent markers. Multiple labeling was achieved using anti-glial fibrillary acidic protein (GFAP; DiaSorin,Stillwater MN) immunocytochemistry to label activated astrocytes while using DAPI to label nuclear DNA. Incorporating 4',6-diamidino-2-phenylindole (DAPI; # FP-371862) as a fluorescent nuclear stain is accomplished by simply incorporating 0.0001% into the Fluoro-Jade C staining solution. This is accomplished by the addition of 1 ml of 0.01% DAPI stock solution to 99 ml of 0.1% acetic acid. Fluoro-Jade C was also combined with immunofluorescent labeling of GFAP according to the following procedure. Loose frozen tissue sections were incubated in a prediluted solution of anti-GFAP at about 5 degrees C in the refrigerator for 1-3 days. It should be mentioned that although in this study all immunocytochemistry was performed on frozen sections, the methods are fully compatible with paraffin processed tissue as well. Sections were rinsed in two changes of buffered saline for 10 min each and then transferred to a tetramethylrhodamine isothiocynate (TRITC) or FluoProbes 547H labeled secondary antibody (FluoProbes), diluted 1:100 in buffered saline, for 1 h at room temperature. Sections were rinsed in two changes of buffered saline for 10 min each and then the sections were mounted onto gelled slides from distilled water and air dried on a slide warmer at 50 degrees C for 30 min. To combine with Fluoro-Jade C, the slide mounted sections were rehydrated for 2 min in distilled water and then transferred to the 0.06% potassium permanganate solution for 10 min. It is worth mentioning that the incubation time in potassium permanganate may need to be reduced when co-localizing those antigenic epitopes susceptible to chemical oxidation. The slides were then rinsed for 2 min in distilled water, transferred to the Fluoro-Jade C working solution for 10 min and then rinsed, air dehydrated, xylene cleared and coverslipped with DPX, as previously described. The blue nuclear label conferred by DAPI is visualized via ultraviolet light excitation, while the red TRITC or FP547H labeled antibody is visualized by green light excitation.

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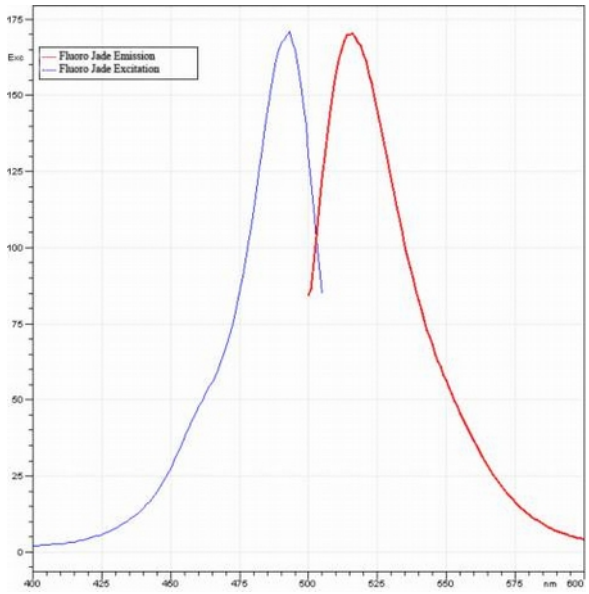
EXCITATION AND EMISSION PROFILE FOR FLUORO-JADE



References

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

Ordering information

Catalog size quantities and prices may be found at www.interchim.com/
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