Fluoro-Jade C Staining Kit with DAPI Counter Stain

PROTOCOL

Fluoro-Jade C stain is for neuronal degeneration with fluorescent Nissl counter stain. For the high contrast and resolution staining of degenerating neurons in formalin fixed brain tissue sections.

EQUIPMENT AND REAGENTS NEEDED

- Staining dishes or Copland jars
- Slide warmer
- Coverslips
- Convection oven or water bath
- Distilled water
- Ethanol
- Xylene
- DPX mounting media

Instructions

1. Mount brain tissue sections on gelatin coated slides and dry at 50-60 degrees C on a slide warmer for at least ½ hour. The slides are prepared by placing clean slides in a slide rack and placing in a solution of ethanol for 2 minutes, then placing in distilled water for 2 minutes. The slides are then transferred to a 1% pig-skin gelatin solution, (Sigma: 300 Bloom) which has been heated to 65 degrees C. Drain excess gelatin on paper towel and transfer to paraffin-free convection oven overnight at 60 degrees C.
2. Add 9 parts 70% ethanol to 1 part Solution A (sodium hydroxide), and incubate slides for 5 minutes.
3. Transfer the slides to 70% ethanol for 2 minutes.
4. Transfer slides to distilled water for 2 minutes.
5. Add 9 parts distilled water to 1 part Solution B (potassium permanganate) and incubate slides for 10 minutes.
6. Rinse slides for 2 minutes in distilled water.
7. Add 9 parts distilled water to 1 part Solution C (Fluoro-Jade C) and incubate slides for 10 minutes. OPTIONAL: add 1 part Solution D (DAPI) to staining solution above (Fluoro-Jade C) for blue fluorescent Nissl counter stain.
8. The slides are then rinsed for 1 minute in each of 3 distilled water rinses.
9. The slides are dried on a slide warmer at 50-60 degrees C for at least 5 minutes.
10. The dry slides are then cleared by brief (1-5 minutes) immersion in xylene.
11. The slides are then coverslipped with a non-aqueous, low fluorescence, styrene based mounting media, such as DPX. Mounting medias containing water or glycerol are not compatible.
12. Fluoro-Jade C labeled degenerating neurons are visualized with blue light excitation, while DAPI counter stained cell nuclei are visualized with ultra-violet illumination.

Comments on Additional and Alternative Procedural Variants

1. Concerning the basic ethanol pretreatment, this step can be omitted when using paraffin processed tissue.
2. Concerning the potassium permanganate pretreatment, it confirms a significant reduction in background staining. However, it can also denature some antigenic epitopes and therefore, the time in this solution may be reduced when combining with immunofluorescently labeled tissue.
3. Traditionally, the sections are air dried on a slide warmer, since ethanol dehydration can diffuse dye. However, it is possible to solvent dehydrate the sections using butanol as follows: transfer sections to distilled water for 1 minute, then into a mixture of equal parts ethanol and butanol for 1 minute and then through 2 five minute 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.

Troubleshooting

Q: The tissue wrinkles or falls off slides when processing.
A: Use proper slide gelling procedure (see

P.O. Box 183
Jefferson, Arkansas 72079 USA

histochem@centurytel.net
www.histo-chem.com

STAINING KITS

Black Gold II Staining Kit with Toluidine Blue O Counter Stain

Fluoro-Jade C Staining Kit with DAPI Counter Stain

SECURE ONLINE ORDERING

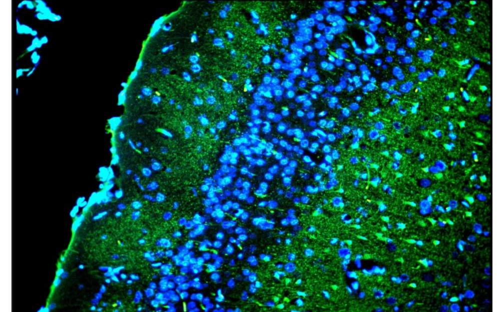
Histo-Chem, Inc. adheres to international PCI (payment card industry) compliance standards for data security. Visit us at www.histo-chem.com to view our products.

processing procedure described above).

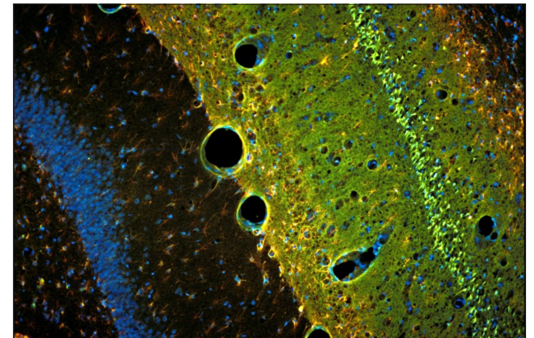
Q: The staining is present, but has low contrast (high background).
A: Reduce dye concentration or increase time in $KMnO_4$.

Q: The staining is present, but faint.
A: Increase the FJ-C concentration or reduce time in $KMnO_4$.
Q: The stain is present after final rinse, but lost following coverslipping.
A: Air dry slides rather than ethanol dehydration and avoid mounting media that contain polar solvents (eg. water, ethanol, glycerin).

Q: What if there is no staining.
A: May be due to absence of neurodegeneration – verify by running positive control (eg. kainic acid 10mg/kg i.p.) (Sigma). Fixative may consist of 10% formalin or 4% paraformaldhyde dissolved in either neutral phosphate buffer or physiological saline.



View of the superficial layers of the cingulated cortex of a rat exposed to kainic acid. Layer I contains conspicuous Fluoro-Jade C positive degenerating axon terminals. Layer II contains densely packed DAPI-positive viable granule cells. Layer III contains a mixture of Fluoro-Jade C positive degenerating pyramidal cells and DAPI positive viable pyramidal cells. Double exposure using combined blue and ultraviolet epi-fluorescent illumination. 20X mag.



Survey view of the hippocampus of a rat exposed to kainic acid. The section was triple labeled with Fluoro-Jade C and DAPI staining combined with GFAP immunohistochemistry. The section reveals extensive green Fluoro-Jade C positive neuronal degeneration throughout the entire CA-1 region of the hippocampus. The underlying blue viable positive granule cells of the dentate gyrus are only DAPI positive. Both regions exhibit red GFAP positive hypertrophied astrocytes. Triple exposure combining ultraviolet, blue and green light epi-fluorescent illumination. 10X mag.

Copyright © 2011
Histo-Chem, Inc.
All Rights Reserved

P.O. Box 183
Jefferson, Arkansas 72079 USA

histochem@centurytel.net
www.histo-chem.com

Histo-Chem Inc.