Chromate/Fluoro-Jade fluorescent myelin staining kit for paraffin embedded tissue sections, with Ethidium Bromide counterstain & Ruthenium Red contrast enhancer.

What is the Chromate/Fluoro-Jade staining kit?

The present Chromate/Fluoro-Jade (Cr/F-J) stain was developed to enable the stable, high contrast and resolution fluorescent labeling of myelin in paraffin processed tissue sections. This stain exhibits the high contrast fluorescent labeling of both large myelinated tracts as well as fine individual myelinated axons. Omission of the chromium pretreatment abolishes all myelin staining. The Cr/F-J stain can also be used to demonstrate myelinopathies and can be combined with a number of other histological methodologies including fluorescent Nissl stains, fluorescent markers of amyloid plaques and immunofluorescent tracers. Cr/F-J is visualized under blue light; Ethidium Bromide is visualized under green light or blue light.

This kit contains an optional Ethidium Bromide (EB) counterstain. Ethidium Bromide will stain the cytoplasm and nuclei of neurons a fluorescent red color under green light excitation. The stain will be of high contrast, resolution and brightness and will also be very resistant to fading.

The kit also includes an optional Ruthenium Red stain that greatly increases the contrast of the Cr/F-J staining by reducing the background fluorescence. Our testing has shown that the Ruthenium Red stain is not compatible with Ethidium Bromide.

Kit includes: 10x Potassium Chromate 40ml, 10x Fluoro-Jade C 40ml, 10x Ethidium Bromide 40ml, 10x Ruthenium Red 40ml. Stock solutions should be diluted 1 part stock solution to 9 parts diluent (Example: 10ml stock solution in 90ml distilled water).

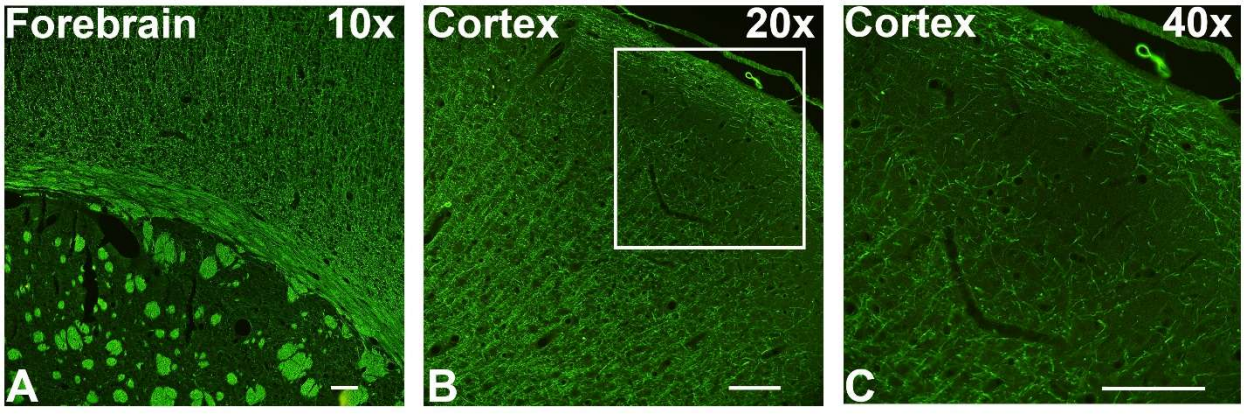


Fig. 1. Examples of Cr/F-J staining. A survey view of a coronal section of the rat forebrain reveals large myelinated tracts including the corpus callosum and the fascicles of the striatum (A). Fine fibers can be seen throughout the cortex (B). At high magnification (small square in B), both

radial fibers and fine parallel myelinated fibers in layer I are shown (C). Blue light excitation. Mag bars = 100 µm.

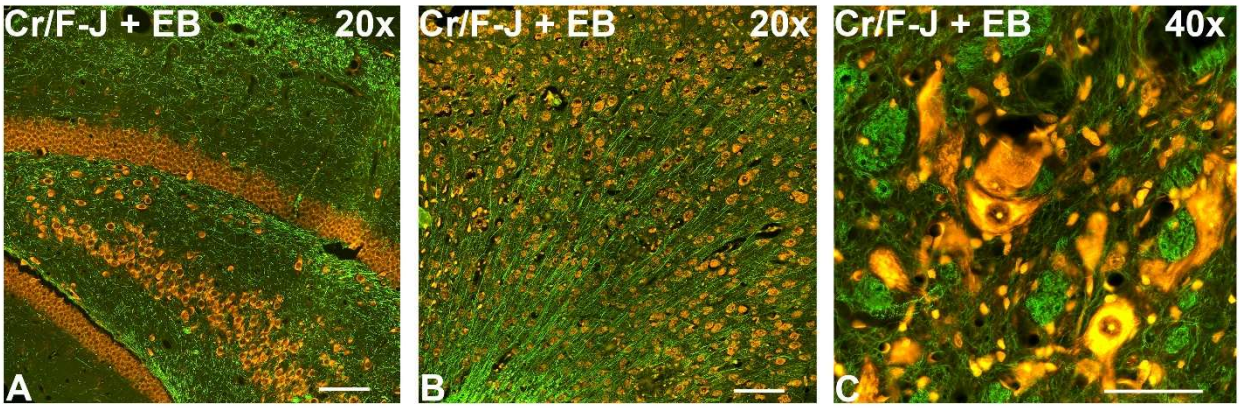


Fig. 2. Examples of double labeling with a fluorescent Nissl stain. Combined Cr/F-J and Ethidium Bromide method for the simultaneous localization of neuronal cell bodies (red) and myelinated fibers (green) is shown. The dentate gyrus of the hippocampus shows fine individual myelinated fibers in relationship to granule and polymorphic neurons (A). In the cingulate cortex, individual radial myelinated fibers can be seen (B). Both myelinated fibers of the medial forebrain bundle and neurons within the facial nucleus in the ventral brain stem can be seen (C). Combined blue/green light excitation. Mag bars = 100 µm.

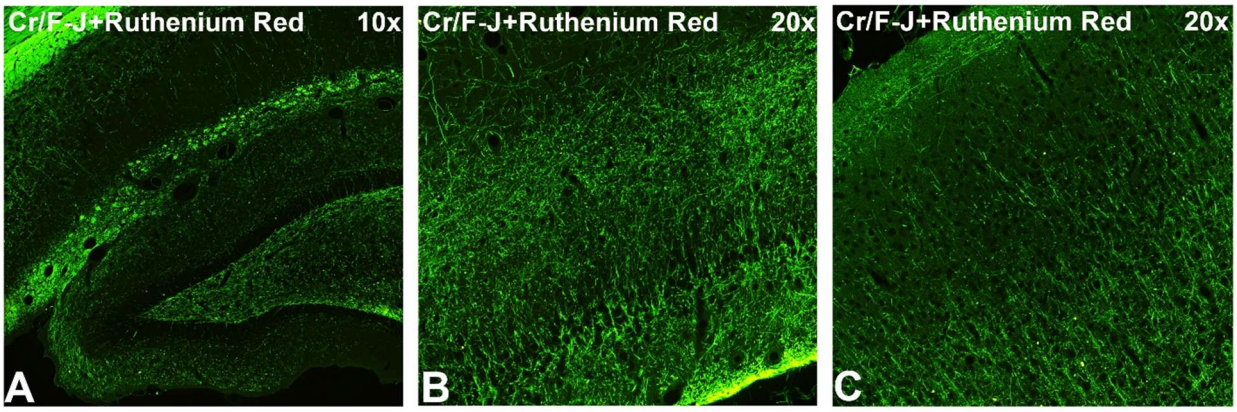


Fig. 3. Examples of pretreatment with Ruthenium Red to reduce background fluorescence and thus increase the contrast of the stain. A survey view (A) and higher magnification view (B) of the hippocampus reveals both myelinated tracts and individual fibers. Staining of the distal portion of the parietal cortex reveals both fine individual radiating myelinated fibers as well as parallel fibers in layer I (C). This treatment increases contrast but is not compatible with the subsequent use of counterstains.

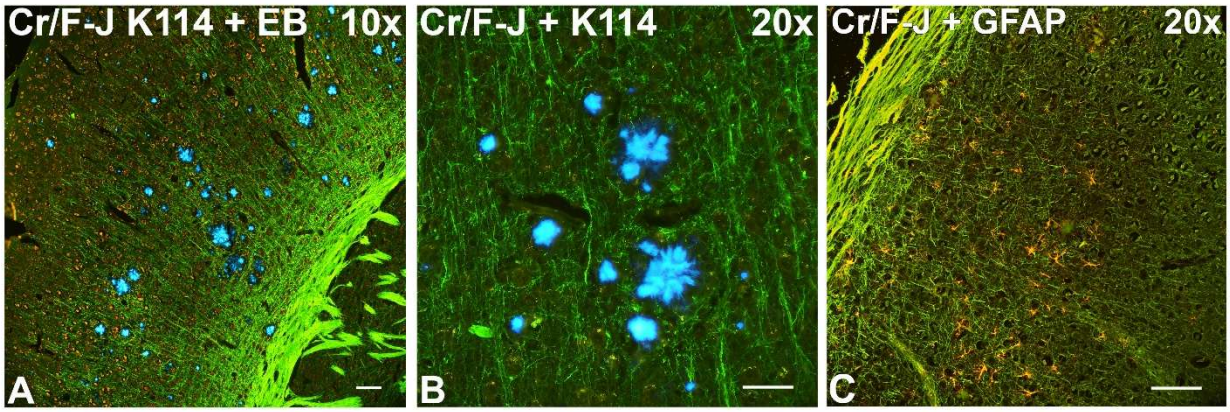


Fig. 4. Examples of pathological myelin in APP/PS1 mice. Myelin changes in this mouse model of Alzheimer's disease are seen following combined labeling with Cr/ F-J and K114 or Amylo-Glo (A-G) (K114 is used in alcoholic solutions while AG dissolves in aqueous solutions). A survey view of the cortex reveals the distribution of amyloid plaques (blue), Ethidium Bromide positive neurons (red) and myelinated fibers (green) (A). The cortex at higher magnification reveals blue K114 labeled amyloid plaques amongst green myelinated fibers (B). Multiple labeling with immunofluorescent methods include double labeling with combined Cr/F-J and anti-GFAP immunofluorescent methods which results in red fluorescent astrocytes localized relative to green fluorescent myelinated fibers in the APP/PS1 mouse cortex (C). Mag bars = 100 µm.

Equipment and Reagents needed: Staining dishes or Coplin jars · Slide warmer · Coverslips · Distilled water · Ethanol · Xylene · DPX mounting media.

Protocol

1. **Deparaffinize:** Place slide with paraffin embedded tissue section on a hot plate or slide warmer set at 60°C. Once the paraffin on the slide has melted put the slide in two changes of 100% xylene for 10 minutes per change. Then transfer the slide through two changes of 100% ethanol for 5 minutes per change. Then place the slide in 70% ethanol for 5 minutes. Lastly, transfer the slide to 100% distilled water for 5 minutes.

2. **Chromate staining:** To prepare the chromate stain add 1 part chromate stock solution to 9 parts distilled water and place into a laboratory oven set at 60°C and allow it to equilibrate. Transfer the slide from the distilled water to the chromate solution in the oven for 30 minutes. Then rinse the slide in warm distilled water for 10 minutes, followed by another 5 minutes in distilled water at room temperature.

3. **Fluoro-Jade C stain:** To prepare the Fluoro-Jade C stain add 1 part Fluoro-Jade C stock solution to 9 parts 50% ethanol. Transfer the slide to 50% ethanol for 3 minutes. Then transfer the slide to the F-C in 50% ethanol for 40 minutes. Then rinse the slide two times

in 50% ethanol for 3 minutes each.

4. **Optional Ruthenium Red stain:** The Ruthenium Red stain should be done before the chromate solution. To prepare the Ruthenium Red stain add 1 part Ruthenium Red stock to 9 parts distilled water. Transfer the deparaffinized slide from distilled water to the Ruthenium Red stain for 90 seconds. Rinse slide two times in distilled water for 5 minutes each. Then proceed with the chromate stain instructions listed above.

5. **Optional Ethidium Bromide stain:** If you did not use the Ruthenium Red stain you can counterstain the slide with Ethidium Bromide after the Fluoro-Jade C stain. To prepare the Ethidium Bromide stain add 1 part Ethidium Bromide stock solution to 9 parts distilled water. After rinsing the slide in 50% ethanol transfer it to distilled water for 3 minutes, then transfer the slide to the Ethidium Bromide stain for 5 minutes. Then rinse two times in distilled water for 3 minutes each.

6. **Differentiation/solvent dehydration & coverslipping:** After the FJC or EB stain start the differentiation/solvent dehydration process. This will help increase the contrast and reduce the FJ-C background fluorescence as well as prepare the slide for coverslipping. Place the slide in a Copland jar of 70% reagent ethanol for 5 minutes. Then transfer it to two changes of 100% ethanol for 5 minutes each. Lastly, transfer the slide to two changes of 100% xylene for 5 minutes each and cover-slip with DPX nonfluorescent mounting media.

Notes: Protect Ruthenium Red from sunlight, the solution should appear as a dark magenta/ reddish color. A degraded solution will appear brown in color.

The chromate solution can be stored in the laboratory oven at 60°C. If precipitate occurs, make a new solution.

Reference & Link to the full Cr/F-J article:

Schmued L, Schmued C. Chromate/Fluoro-Jade: A high resolution and contrast fluorescent staining method for localizing myelinated fibers in paraffin embedded brain tissue sections. J Neurosci Methods. 2026 Nov;435:110845.

https://www.sciencedirect.com/science/article/pii/S0165027026001755?via%3Dihub#sec0010