biosensis® Ready-to-Dilute (RTD)™ Fluoro-Jade® C Staining Kit Protocol

Catalog No: TR-100-FJ

The biosensis® **RTD™** (Ready-to-Dilute) Fluoro-Jade® C staining kit is designed to stain for neuronal degeneration with a fluorescent Nissl counter stain. The reagents are all supplied in a liquid format and are ready-to-dilute. The stain is compatible with all common fixations, embedding, sectioning and staining procedures, although the use of slide mounted, formalin fixed, freeze cut tissue sections is most typical. The kit is shipped ambient, see storage instructions for details.

MATERIALS PROVIDED

Sodium Hydroxide, Solution A (Dilute 1:10 prior to use) - 40 mL
Potassium Permanganate, Solution B (Dilute 1:10 prior to use) - 40 mL
Fluoro-Jade C, Solution C (Dilute 1:10 prior to use) - 40 mL
DAPI, Solution D (Add to diluted Fluoro-Jade C) - 40 mL

Sufficient dye is provided to make 400 mL of 1X staining solution, or 8 50 mL Coplin jars of material; Depending on the size and source of tissue between 80 and 150 adult rat or mouse brain sections can easily be processed with one FJC kit in most cases. Actual number of slides stained is dependent upon the experiment and not guaranteed.

EQUIPMENT AND REAGENTS NEEDED

Gelatin coated or tissue treated microscope slides (i.e. Superfrost plus)
Staining dishes/Coplin jars
Cover slips
DPX mounting media or other permanent mounting medium. Traditional fluorescent mounting mediums are not recommended because of their high pH.
Slide warmer
Convection oven
Distilled water
Ethanol (100%) & 80% solutions (made with distilled water)
Xylene

Preparation of Gelatin Coated Slides (if using):

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°C. Drain excess gelatin on paper towel and transfer to paraffin-free convection oven overnight at 60°C. After overnight drying the slides are ready to be used to mount fresh cut or paraffin sections.

STORAGE CONDITIONS

The unopened kit can be stored for up to 6 months at 2-8ºC after the date of receipt. The kit and components should be stored protected from light. Diluted FJC dye solutions are not stable and should be used within 4 hours of making. The other diluted solutions can be reused and stored for up to 48 hours if refrigerated and protected from light. Best results require freshly diluted solutions. We recommend using aseptic techniques when handling the reagents to avoid bacterial growth and contamination. The FJC Ready to dilute kit is shipped ambient and stable at room temperature during transport. Refrigerate upon arrival, do not freeze.

INSTRUCTIONS: (non-paraffin embedded sections)

1. **Mounting:** Mount brain tissue sections on gelatin coated or treated slides and dry at 50-60°C on a slide warmer for at least 30 minutes to 1hr for good adhesion and drying. It is critical that the tissue adhere thoroughly to the slide for best results. If using already mounted and dried slides start at step 2.
2. **Basic ethanol treatment (Rehydration and permeabilization)**: Mix 9 parts 80% ethanol (made with distilled water) to 1 part Solution A (sodium hydroxide), in a beaker and pour into a Coplin jar. Place slides in Coplin jar and incubate slides for 5 minutes.
3. Transfer the slides to a new Coplin jar containing a solution of freshly prepared 70% ethanol for 2 minutes.
4. Transfer slides to a new Coplin jar containing distilled water for 2 minutes. Replace water after every 20 slides.
5. **Fluorescent background blocking & contrast optimization:** Mix 9 parts distilled water to 1 part Solution B (potassium permanganate) and place in clean Coplin jar. Add slides and incubate slides for up to 10 minutes. Rinse slides for 2 minutes in fresh distilled water, change water every 20 slides. Potassium permanganate is a powerful fluorescent quencher. Timings can be adjusted to optimize fluorescent background and signal.
6. **FJC Staining**; Mix 9 parts distilled water to 1 part Solution C (Fluoro-Jade C) and place in a Coplin jar in the dark or low light, 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. Keep FJ jar covered with foil to protect it from light. Diluted dye is not stable for more than 12 hours at 4C.
7. The slides are then rinsed for 1 minute in each of 3 distilled water rinses. Slides can be viewed immediately or dried; best photographs are typically with dried, permanently mounted slides.
8. The slides are dried on a slide warmer at 50-60°C for at least 5 minutes in the dark.
9. The dry slides are then cleared by brief (1-5 minutes) immersion in xylene, in the dark.
10. The slides are then coverslipped with a non-aqueous, low fluorescent, styrene based mounting media, such as DPX. Mounting medias containing water or glycerol are not compatible because their higher pH will cause FJC to come off targets.
11. Fluoro-Jade C labeled degenerating neurons are visualized with blue light excitation (like those used for visualizing fluorescein or FITC) while DAPI counter stained cell nuclei are visualized with ultra-violet (UV) illumination. The resulting slides are quite stable and require no special storage conditions or anti-quench agents. Furthermore, the potassium permanganate pretreatment further enhances the permanence of the preparation resulting in extremely slow fading, even under high magnification.

INSTRUCTIONS: (paraffin embedded sections)

FJ staining protocol with paraffin sections is nearly identical to that used non-treated sections, the only differences are in the initial steps in which the paraffin is removed in two changes of xylene, followed by two changes of alcohol and subsequent rehydration through gradated alcohols. The basic ethanol solution (step two in the above) is not needed as the xylene will also remove lipids.

1. Slides containing sections of brain tissue are dewaxed in xylene 2X, then immersed in 100% ethanol for 5 min 2X, then 70% alcohol for 2 min, and then rinsed with two 1-min changes of dd-H_20.
2. Transfer slides to a new Coplin jar containing distilled water for 2 minutes. This ensures that the slides are thoroughly hydrated. Replace water after every 20 slides.
3. Mix 9 parts distilled water to 1 part Solution B (potassium permanganate) and place in clean Coplin jar. Add slides and incubate slides for 10 minutes.
4. Rinse slides for 2 minutes in distilled water.

5. Mix 9 parts distilled water to 1 part Solution C (Fluoro-Jade C) and place in a Coplin jar in the dark or low light, 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. Keep FJ jar covered with foil to protect it from light.
6. The slides are then rinsed for 1 minute in each of 3 distilled water rinses.
7. The slides are dried on a slide warmer at 50-60°C for at least 5 minutes.
8. The dry slides are then cleared by brief (1-5 minutes) immersion in xylene.
9. The slides are then coverslipped with a non-aqueous, low fluorescent, styrene based mounting media, such as DPX. Mounting medias containing water or glycerol are not compatible.
10. Fluoro-Jade C labeled degenerating neurons are visualized with blue light excitation, while DAPI counter stained cell nuclei are visualized with ultra-violet illumination.

SPECIAL INSTRUCTIONS: DOUBLE LABELING WITH FLUORO-JADE

Double-labeling with antibodies or TUNEL staining can be performed with FJ but with modifications. We always recommend that FJ staining be performed last (IF *then* FJC). Also we recommend that labeling be performed on free-floating sections first, then sections transferred to gelatin slides for FJ staining. This will tend to preserve the structures better and "fix" the immune reagents on to the tissue in our experience, however some of our customers have been successful with slide mounted sections entirely so one should likely optimize for what works best in the particularly laboratory and skill level.

A red fluorescent Rhodamine (TRITC)-conjugated secondary antibody is suitable for multiple labeling with the FJ dyes. The loose immuno-fluorescent labeled tissue is mounted on gelled slides and air dried. Then the slides are rehydrated in water and transferred to the potassium permanganate pretreatment solution. Time in this solution may be reduced should it attenuate the immuno-fluorescent staining. Note: dead neurons may fail to express certain immunological epitopes upon cell death so one should always be sure that the particular marker is present in non-FJ treated sections.

For TUNEL labeled sections, omit the potassium permanganate pretreatment as can cause background in our experience. Also, the FJ staining time will need to be optimized for each section set. Staining time is typically much longer, maybe 30 minutes to an hour but trials will have to done to ensure that the background is not too high. We also recommend rinsing the sections 3X-4X in water, rather than just two times after long FJ staining incubations. Then proceed to dry and mount the slides as one normally would, remembering to use a non-aqueous, low fluorescent, styrene based mounting media, such as DPX for coverslipping.

COMMENTS ON ADDITIONAL AND ALTERNATIVE PROCEDURAL VARIANTS

1. Concerning the basic ethanol pretreatment (step 2), this step can be omitted when using paraffin processed tissue as the ethanol treatment is part of the paraffin removal procedure. Slides must be fully rehydrated prior to the subsequent staining steps.

2. Concerning the potassium permanganate pretreatment, it is necessary because it allows for 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 and sensitive epitopes.

3. Traditionally, the sections are air dried on a slide warmer, since ethanol dehydration can produce a more diffuse dye stain. 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.

TROUBLE SHOOTING

Question: the tissue wrinkles or fall off slides when processing.
Answer: use proper slide gelling procedure (see processing procedure described above); make sure tissue and slides are thoroughly dry and baked for best adhesion.

Question: the staining is present, but has low contrast. (high background).
Answer: reduce dye concentration or increase time in KMnO4.

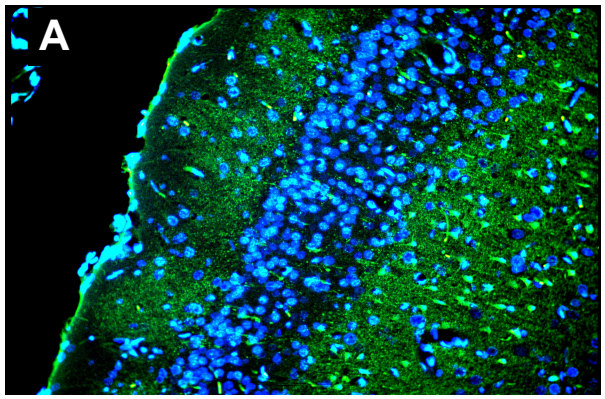
Question: the staining is present, but faint.
Answer: increase the FJ-C concentration or reduce time in KMnO4.

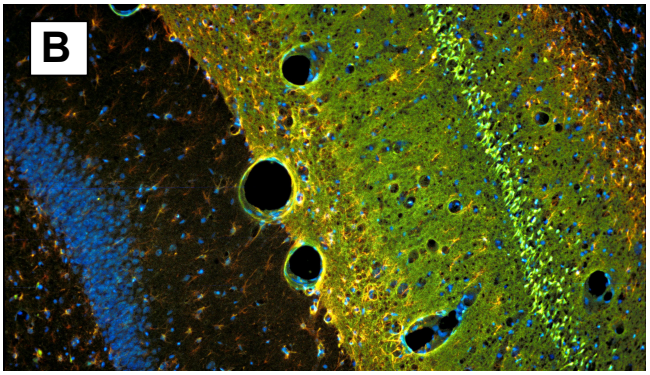
Question: the stain is present after final rinse, but lost following coverslipping.
Answer: air dry slides rather than ethanol dehydration and avoid mounting media that contain polar solvents (eg. water, ethanol, glycerin which and interfere with FJC binding).

Question: what if there is no staining.
Answer: may be due to absence of neurodegeneration – verify by running positive control (eg. kainic acid 10mg/kg i.p.) (Sigma). Kainic acid treated animals used with FJ work well, but only if the correct timings are used. KA positive control animals need to exhibit seizure activity for at least 1 full hour and must survive for at least 1 day before sacrifice, to optimize FJ-C labeling.

Sample Fluoro Jade Images:

(**A**) Double exposure using combined blue and ultraviolet epi-fluorescent illumination of the superficial layers of the cingulated rat cortex 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 denegerating pyramidal cells and DAPI-positive viable pyramidal cells. Photo is courtesy of Dr. Larry Schmued





(**B**) Triple exposure combining ultraviolet, blue and green light epi-fluorescent illumination (10X) of rat hippocampus 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. Photo is courtesy of Dr. Larry Schmued