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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.

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

EQUIPMENT AND REAGENTS NEEDED

Gelatin coated microscope slides
Staining dishes/Coplin jars
Cover slips
DPX mounting media
Slide warmer
Convection oven
Distilled water
Ethanol
Xylene

Preparation of Gelatin Coated Slides:

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 kit can be stored unopened for up to 12 months at 2-8ºC after date of receipt. We recommend that you store the kit components protected from light. After opening the kit the components can be stored up to an additional 6 months at 2-8ºC. Diluted solutions can be stored up to one month at 2-8ºC. We recommend using aseptic techniques when handling the reagents to avoid contamination.

INSTRUCTIONS: (non-paraffin embedded sections)

1. Mount brain tissue sections on gelatin coated slides and dry at 50-60°C on a slide warmer for at least 30 minutes for good adhesion.
2. Mix 9 parts 70% ethanol to 1 part Solution A (sodium hydroxide), to 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. 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.
6. Rinse slides for 2 minutes in distilled water.
7. 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.
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°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 fluorescent, 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.

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. 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.

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).

Question: the staining is present, but has low contrast. (high background).
Answer: reduce dye concentration or increase time in $KMnO_4$.

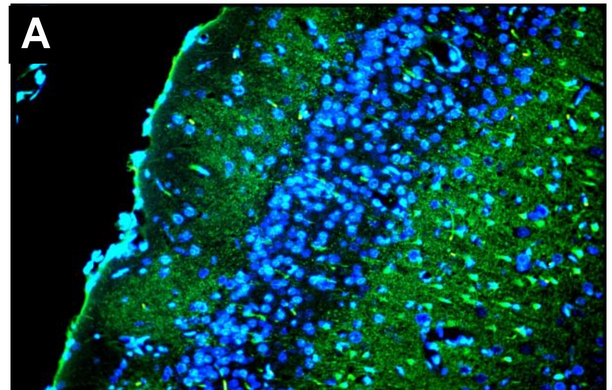
Question: the staining is present, but faint.
Answer: increase the FJ-C concentration or reduce time in $KMnO_4$.

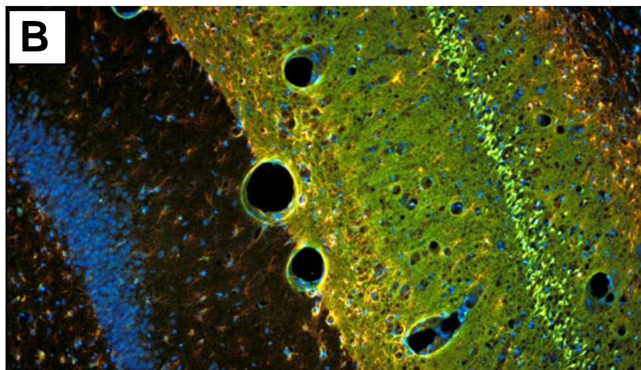
Question: the stain is present after final rinse, but lost following coverslipping.
Answer: air dry slides rather than ethanol dehydration and avoid mounting media that contain polar solvents (eg. water, ethanol, glycerin)

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. Fixative may consist of 10% formalin or 4% paraformaldhyde dissolved in either neutral phosphate buffer or physiological saline.

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