

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

Catalog No: TR-100-FJT

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 works well with all tissue fixation preparations, but for the highest contrast and resolution, staining of degenerating neurons in formalin fixed brain tissue sections (frozen or paraffin embedded) placed upon gelatin coated slides is recommended.

MATERIALS PROVIDED

Sodium Hydroxide, Solution A (Dilute 1:10 prior to use) - 20 mL
Potassium Permanganate, Solution B (Dilute 1:10 prior to use) - 20 mL
Fluoro-Jade C, Solution C (Dilute 1:10 prior to use) - 20 mL
DAPI, Solution D (Add to diluted Fluoro-Jade C) - 20 mL

EQUIPMENT AND REAGENTS NEEDED

Gelatin coated tissue slides
Staining dishes/Copland 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 paraffin sections.

INSTRUCTIONS

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 Copland jar. Place slides in Copland jar and incubate slides for 5 minutes.
3. Transfer the slides to a new Copland jar containing a solution of freshly prepared 70% ethanol for 2 minutes.
4. Transfer slides to a new Copland 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 Copland 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 Copland jar in the dark, 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.

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 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 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)

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