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Fluoro-Jade B Staining for Neuronal Cell Death

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[Abstract] Fluoro-Jade is a fluorescent derivative used for histological staining of degenerating neurons. This technique is simple and sensitive enough to label distal dendrites, axons, axon terminals as well as neuronal bodies. Fluoro-Jade has excitation and emission peak of 480 and 525 nanometer respectively. It can be visualized using a fluorescein/FITC filter. Some reports have demonstrated that Fluoro-Jade can also be useful to detect glial cell death (Anderson *et al.*, 2013; Damjanac *et al.*, 2007).

Materials and Reagents

1. Superfrost plus Microscope slide (Thermo Fisher Scientific, catalog number: 12-550-17)
2. Cover Glass (Thermo Fisher Scientific, catalog number: 12-545-88)
3. Tissue sample
4. Fluoro-Jade B (Merck Millipore Corporation, catalog number: AG310)
5. Paraformaldehyde (Electron Microscopy Science, catalog number: 19210)
6. Potassium permanganate ($KMnO_4$) (Sigma-Aldrich, catalog number: 223468)
7. DAPI (Life Technologies, catalog number: D3571)
   Note: Currently, it is "Thermo Fisher Scientific, Molecular Probes™, catalog number: D3571".
8. Glacial Acetic acid (CH_3CO_2H) (Sigma-Aldrich, catalog number: A9967)
9. Ethanol
10. Xylene (Sigma-Aldrich, catalog number: 534056)
11. Sodium hydroxide (NaOH) (Sigma-Aldrich, catalog number: S8045)
12. Sodium tetraborate decahydrate (Sigma-Aldrich, catalogue number: B9876)
13. Sodium chloride (NaCl) (Sigma-Aldrich, catalog number: S3014)
14. potassium phosphate dibasic (K_2HPO_4) (Sigma-Aldrich, catalog number: P3786)
15. potassium phosphate monobasic (KH_2PO_4) (Sigma-Aldrich, catalog number: P9791)
16. 4% paraformaldehyde (see Recipes)
17. KPBS (see Recipes)
18. 0.2% Fluoro-Jade (see Recipes)
19. Fluoro-Jade solution (see Recipes)
20. 0.2% DAPI (see Recipes)

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Equipment

1. Vacuum Desiccators (Thermo Fisher Scientific, catalog number: 08-642-5)
2. Tissue-Tek slide staining set (Electron Microscopy Science, catalog number: 62540-01)
3. 24 slide holder (Electron Microscopy Science, catalog number: 62543-06)
4. Orbital shaker
5. Timer
6. Slide Warmer
7. DPX mounting medium (a mixture of the polystyrene distyrene and the plasticizer dibutylphthalate) (Electron Microscopy Science, catalog number: 13512)

Procedure

1. Mount tissue sections (20-35 µm cut on microtome or cryostat) on Superfrost plus slide and let the slide dry overnight under vacuum.
2. Fix the tissue on slide warmer 30 min at 60 °C and/or 20 min in paraformaldehyde 4%.
3. Follow by 2 min in KPBS.

Note: All the following steps are done at room temperature.

4. Dehydrate in 50%-70%-100% Ethanol 2 min each.
5. Rehydrate by going back in 70%-50% Ethanol and KPBS, 2 min each.
6. Incubate in potassium permanganate 0.06% (dilute in water) 5 min at room temperature.
7. Rinse in water 1 min.
8. Incubate in Fluoro-Jade solution at room temperature for 10 min and gently shake on orbital shaker or by doing several dips during incubation (three dips of few seconds 3 times during incubation). Use opaque cup for the Fluoro-Jade incubation and keep the slide in shelters from light for the rest of the procedure.
   Note: Fluoro-Jade solution and potassium permanganate 0.06% must be prepared fresh.
9. Follow by three rinses of 1 min each in water.
10. Dry the slides overnight under vacuum at room temperature.
11. Clear the slide in Xylene (3 x 2 min).
12. Cover slip with DPX and dry 24-48 h under hood before microscope analysis.

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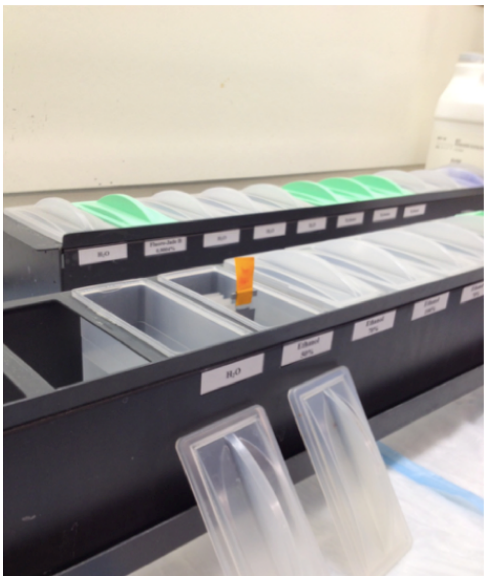

Figure 1. Example of the setup use for incubation and/or dipping of the slides in different solutions

Representative data

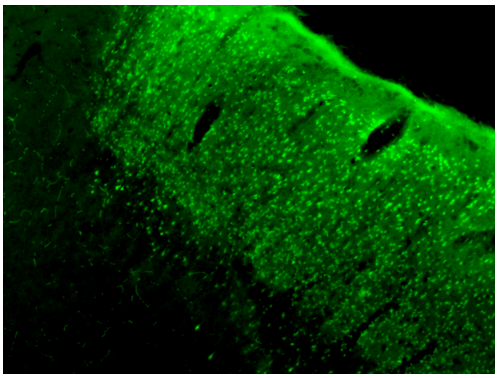

Figure 2. FJB-positive neurons in the mouse cerebral cortex following ischemic stroke

Notes

To ensure reproducibility between protocols, use the same method of tissue preparation.

Recipes

1. 4% paraformaldehyde
   Heat 700 ml distilled water at 65 °C
   Add 40 g paraformaldehyde
   5 ml NaOH

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When Paraformaldehyde is completely dissolved add 38 g sodium tetraborate

Complete at 1 liter with distilled water

2. KPBS

   3.81 g Potassium phosphate dibasic

   0.45 g Potassium phosphate monobasic

   8.1 g sodium chloride

   Complete to 1 liter with distilled water

3. 0.2% Fluoro-Jade

   Dilute 50 mg Fluoro-Jade B in 25 ml of sterile water and aliquot

   Keep this stock solution at -20 °C in shelters from light

4. Fluoro-Jade solution

   Fluoro-Jade B 0.0004%

   Acetic acid 0.1%

   DAPI 0.0001% in water

5. 0.2% DAPI

   Dilute 10 mg DAPI in 5 ml of sterile water and aliquot

   Keep this stock solution at 4 °C in shelters from light

Acknowledgments

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