**Gallyas Silver Stain**

From George Edwards, 2021

Optimized for NeuroScience Associates multibrain sections

**NOTE:** This protocol also works well with paraffin-imbedded sections; however, incubation time in reducing solution may need to be shortened.

Temperature can influence the rate of stain development. Incubations at RT can develop quickly, and if left too long will cause the sections to have a blackish-brown background. The reaction can be better controlled using chilled reducing solution.

If needed to conserve solution, slides can be incubated in all reagents while laid flat and washed with tap water upright in histology dishes.

**Solutions:**

**5% Periodic Acid:** 50 g periodic acid in 1 L dH2O

**1% Silver Nitrate:** 2 g silver nitrate in 200 ml dH2O (protect from light)

**Silver Iodide solution** (*protect from light)***:** 12 g sodium hydroxide + 150 ml dH2O // 30 g potassium iodide // 10.5 ml 1% silver nitrate // dH2O up to 300 ml (fresh)

**0.5% Acetic acid:** 5 ml glacial acetic acid in 1 L dH2O

**Developer Working Solution** (*protect from light; make fresh before use)***:** 200 ml A + 100 ml B + 100 ml C

Developer A: 50 g anhydrous sodium carbonate in 1 L dH2O

Developer B (*protect from light)*: 1.9 g ammonium nitrate // 2 g silver nitrate // 10 g tungstosilicic acid in 1 L dH2O

Developer C (*protect from light)*: 1.9 g ammonium nitrate // 2 g silver nitrate // 10 g tungstosilicic acid // 7.6 ml 37% formaldehyde in 1 L dH2O

**Method:**

1. Mount sections on slide and dry at RT O/N.
2. IF USING NSA MULTIBRAIN SECTIONS (Not required for regular free-floating tissue): Bond tissue to the slide by incubation in 95% EtOH (minimum 1 min), followed by 95% EtOH/10% formalin (9:1 mix, for final formaldehyde content of 3.7%) (5 min), and rinse in 95% EtOH. Note: The first 95% EtOH step may not be necessary – may be able to go straight into EtOH/formalin solution.
3. Dehydrate to xylene: 2x 100% EtOH, 2x xylene (2-3 min ea)
4. Rehydrate to 70% EtOH: 2x 100% EtOH, 1x 90% EtOH, 1x 80%, 2x 70% EtOH (2-3 min ea)
5. Wash in gentle running lukewarm tap water (2-5 min)
6. Use lipid pen for barrier to surround tissue (PAP pen liquid blocker, Fisher #NC9827128)
7. Incubate 0.25% potassium permanganate at RT (15 min)
8. Wash in gentle running lukewarm tap water (1 min)
9. Incubate in 2% oxalic acid at RT (2 min)
10. Wash in gentle running lukewarm tap water (1 min)
11. Incubate in 5% periodic acid at RT (7.5 min)
12. Wash in gentle running lukewarm tap water (1 min)
13. Incubate in silver iodide solution at RT (1-2 min, *protect from light)*
14. Move slides to 0.5% acetic acid (2 x 5 min)
15. Wash in gentle running lukewarm tap water (1 min)
16. Incubate slides in developer working solution (30-45 min, or ~15 min for paraffin sections, human tissue may be quicker, *protect from light*). Monitor under microscope for development.
17. Incubate in 0.5% acetic acid to stop the reaction (2 x 5 min)
18. Counterstain with hematoxylin or nuclear fast red (1-2 min, *if necessary)*
19. Wash in gentle running lukewarm tap water (1 min)
20. Dehydrate to xylene
21. Mount with DPX/Permount