PATHOLOGY CORE

Gallyas Stain Protocol

Stock Solutions

0.5% Acetic Acid

- Add 5ml of glacial acetic acid to 1L of dH2O

1% Silver Nitrate

- Add 2g of silver nitrate to 200ml of dH2O and stir until dissolved. Store in a dark container.

Developer A

- Dissolve 50g anhydrous sodium carbonate in 1000ml dH2O

Developer B

- Dissolve 1.9g ammonium nitrate
  2.0g silver nitrate
  10g Tungstosilicic Acid
  in 1000ml of dH2O

Developer C

- Dissolve 1.9g ammonium nitrate
  2.0g silver nitrate
  10g Tungstosilicic Acid
  7.6ml 37% Formaldehyde
  in 1000ml of dH20

5% Periodic Acid

- Dissolve 50g of periodic acid in 1000ml of dH2O

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Protocol

*All glassware must be acid washed. Use plastic forceps and gloves when handling all reagents. Don't use any metal in this protocol (staining racks, forceps, etc.)

1. Deparaffinize & hydrate: 2X Xylene 5min; 1min each 2X 100%EtOH; 1X 95%EtOH; 1X 80% EtOH; 1X 70% EtOH; dH2O

2. Place sections in 5% Perodic Acid 5min (3min for mouse sections)

3. Place in dH2O 2X for 5min

4. Prepare Silver Iodide solution:
    a. In 150ml dH2O add 12g of Sodium Hydroxide and stir until dissolved; then add 30g of Potassium Iodide and stir until dissolved; then add 10.5ml of 1% Silver Nitrate; then add dH2O up to a total volume of 300ml.

5. Place sections in Silver Iodide solution for 1min.

6. Place in 0.5% Acetic Acid for 5min (2X)

7. Rinse in dH2O

8. Prepare Developer
    a. Solution A 200ml
    b. Solution B 100ml added dropwise at first
    c. Solution C 100ml added dropwise at first
    d. Make sure you stir mixture as you add B and C. Also add with a pipette in a thin jet.

9. Place sections in developer until sections turn a pale brown/gray(5-10min). Check by eye only after placing in acetic acid. If needed to develop longer, place back in developer

10. Stop development in 0.5% acetic acid for 5min

11. Dehydrate 1 min each 1X 70%EtOH; 1X 80%EtOH; 2X 95%EtOH; 2X 100%EtOH; 5min each 2X Xylene

12. Cover slip with cytoseal