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

Protocol

*All glassware must be acid washed. Use plastic forceps and gloves when handeling 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:

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

Solution A 200ml
Solution B 100ml added dropwise at first
Solution C 100ml added dropwise at first
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 ture 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