

Silver Methenamine Stain

Modified Yajima's Methenamine Silver Stain

Use formalin or methacarn- fixed paraffin embedded **2 μ m** sections. If the tissue is human and fixed with methacarn ,possibly immerse in Thiosemicarbazide. Use a water bath at a stable 63°C for the silver incubation step. Rinse all glassware with dH₂O before use and use only very clean glassware for the silver stain step (acid wash if necessary).

1. Turn on water baths and deparaffinize/ rehydrate slides up to dH₂O
2. Immerse in **1% Periodic Acid** for 15 minutes, prepare silver methenamine solution

Silver Methenamine Solution

(rectangular glass jars hold 45 mls solution and 14 slides each)

3% Methenamine	25.0 ml
5% Silver Nitrate Solution	2.5 ml

** Mix well, and then add**

5% Sodium Borate	2.5 ml
DH ₂ O	<u>20.0 ml</u>
Total	45.5 ml

3. Wash with distilled water for 5 minutes
4. Immerse in **0.5% Thiosemicarbazide** for 5 minutes
Place the coplin jar with silver methenamine solution in the preheated water bath.
5. Wash with distilled water for 5 minutes
6. Add **0.5 ml of 2% gelatin** to silver solution and incubate slides in the **Silver Solution**. Check the slides every 3 minutes or so beginning after 5 minutes incubation. Slide should be rinsed in cold distilled water and checked under the scope. When the color is dark brown and well developed, move slides to dH₂O, empty the silver solution from the coplin jar and rinse with hot distilled water.
7. Rinse with room temperature dH₂O
8. Immerse in **0.25 % Gold Chloride Solution** for 1 minute
9. Rinse in two dH₂O washes
10. Incubate in **3% Sodium Thiosulfate** for 2 minutes
11. Rinse in tap water for 10 minutes
12. Stain with **Hematoxylin** for 2 minutes
 - Rinse in three changes of tap water.
 - Blue with five dips of Ammonia Water (or one minute in Tris, pH 7.8)
 - Rinse in two buckets of dH₂O
 - Rinse in one bucket of 70% ethanol
13. Counterstain in **2% eosin** for 4 minutes, dehydrate, clear and mount

Stock Solutions for Silver Stain:**1% Periodic Acid (Waste: rinse down the sink)**

Distilled water **200ml** + Periodic Acid **2g**
Store at room temperature

0.5% Thiosemicarbazide (Waste: collect for EH&S disposal)

Distilled water **200mls** + Thiosemicarbazide **1g**
Store at room temperature.

Silver Methenamine Solution (Waste: collect for EH&S disposal)**3% Stock Methenamine Solution**

Distilled water **500ml** + Methenamine (hexamethylene tetramine) **15g**
Store at 4C

5% Stock Silver Nitrate

Distilled water **50ml** + Silver Nitrate **2.5g**
Store at 4C in foil wrapped bottle.

5% Stock Sodium Borate

Distilled water **50ml** + Sodium Borate (tetrahydrate) **2.5g**

Dissolves slowly, stir > 2 hrs

Store at room temperature. This is a saturated solution – OK to use if crystals are visible.

0.25% Gold Chloride Solution (Waste: collect for EH&S disposal)

Gold Chloride Solution **1g** + Distilled water **400ml**
Store in the dark at 4°C. Reuse solution for up to six months.

3% Sodium Thiosulfate (Waste: rinse down the sink)

Distilled water **300ml** + Sodium Thiosulfate **9g**
Store at room temperature.

Hematoxylin (Waste: collect for EH&S disposal)

Purchased Harris hematoxylin – always filter before use.

Eosin (Waste: collect for EH&S disposal)

Stock	Eosin 1g		
	Distilled water	100 ml	
	Mix then add	400 ml	95% Ethyl Alcohol
Working	Eosin stock	100 ml	
	80% Ethyl Alcohol	100 ml	
	Glacial Acetic Acid	2 ml	