

Methenamine Silver Trichrome Kit

Richard Allan Scientific

Date: ___/___/___

Positive Control: kidney

Type of Tissue: _____

Reagent	Vendor	Catalog #
Silver Nitrate Soln	Richard Allan Scientific	88036
Methenamine-Borax	Richard Allan Scientific	88023
Gold Chloride Soln	Richard Allan Scientific	88026
Sodium Thiosulfate Soln	Richard Allan Scientific	88025
Fast Green Staining Solution	Richard Allan Scientific	88024
Periodic Acid Soln 1%	Richard Allan Scientific	88027
Bouin's	Rowley	SO-306
Weigert's Iron Hematoxylin	Rowley	SO-465 (A) SO-466 (B)
Biebrich Scarlet	Rowley	F367-3
Phosphotungstic/ Phosphomolybdic Acid	Rowley	F367-4
Aniline Blue	Rowley	F367-5

*Working Methenamine Silver Solution- Methenamine-Borax (1 capsule)

Chemically cleaned glassware MUST be used (no metal). To clean glassware:

- 1) Perform all steps under chemical fume hood.
- 2) Place chromic-sulfuric acid solution (FisherScientific, SC88-500) in the glass pan and fill with desired glassware.
- 3) Let soak for at least 5 minutes.
- 4) Pour chromic acid back into bottle. (chromic-sulfuric acid solution may be reused unless it is green or expired).
- 5) Rinse glassware with DI water 6+ times. The first rinse water step must be collected as chemical waste.

PROTOCOL

1. Deparaffinize slides and bring to DH₂O
2. Oxidize in Periodic Acid Soln. 5 minutes
3. Wash in Milli Q H₂O 6X
4. Incubate slides in Working Methenamine Silver Solution (WMSS) in 60c. Check sections for staining intensity after 30 min. When checking microscopically, rinse sections in 60c milli Q H₂O. Return to WMSS until desired results.
5. Rinse sections in Milli Q H₂O 6X 6-8 changes
6. Tone slides in Gold Chloride Soln 10 sec or until sections lose their golden brown color and appear light gray.
7. Rinse sections in Milli Q H₂O 6X 6-8 changes
8. Place Sections in Sodium Thiosulfate Soln 1 minute
9. Rinse sections well in tap water for 1 min
10. Mordant tissue in preheated Bouin's solution @56°C 1 hour
11. Cool sections; Wash in running Tap H₂O until all yellow color is removed
12. Stain nuclei with Working (1A:1B) Weigert's iron Hematoxylin for 15 minutes
13. Wash in running Tap H₂O for 5 minutes
14. Rinse with DH₂O
15. Stain in Biebrich scarlet- Acid Fuchsin for 15 minutes
16. Rinse with DH₂O
17. Place sections in working Phosphotungstic / Phosphomolybdic Acid solution for 15 minutes (*discard used solution*)
18. Stain sections with Aniline Blue solution for 15 minutes
19. Rinse in DH₂O
20. QUICKLY Dehydrate in 95% and 100% ETOH
21. Clear in Xylene and Coverslip