

PAMS (Periodic Acid Methenamine Silver)

(Last update 11/17/2021)

Date: ___/___/___ Technician (Initials): _____

OBJECTIVE: To highlight carbohydrates that can be oxidized to aldehydes, including basement membranes and connective tissue components

RECOMMENDED CONTROL TISSUE: Glomeruli from a normal kidney

ID of Control Used: _____ **Type of Tissue:** _____

Reagent/Kit	Vendor	Catalog #	Was this a new Bottle (Y/N)? If Yes, check lot log.
Modified Grocott's Methenamine Silver (Periodic Acid) Stain Kit	Epredia/FisherScientific	87008	
Fast Green Stain Solution	Epredia/FisherScientific	88024	

SPECIAL INSTRUCTIONS: MUST USE CHEMICALLY CLEAN GLASSWARE!

REAGENT PREPARATION:

Working Methenamine Silver Solution (Prepare fresh each time, all components in kit):

- Wearing gloves, empty entire contents of 1 Methenamine-Borax capsule into 50 ml distilled or deionized water and dissolve
- Add 1 ml Silver Nitrate Solution
- Mix Well

PROTOCOL:

1. Deparaffinize and hydrate sections to deionized water
2. Oxidize sections in Periodic Acid for 5 minutes
3. Rinse sections well in MilliQ water (5-6 changes)
4. Place sections in coplin jar containing freshly prepared Working Methenamine Silver Solution. Apply lid loosely and place in 58-64 °C oven or water bath. Fill a second coplin jar with MilliQ water and place in oven. Check sections for staining intensity after 30 minutes (usually 1-2 hrs +). Basement membranes should be black and tissue a golden / brown. When checking under the microscope be sure to rinse sections in hot deionized water.
5. Return sections to Working Methenamine Silver Solution and continue checking every 5 minutes until sections appear golden brown and glomerular basement membranes are sharply defined.
6. Rinse sections in DiH₂O 5-6 times
7. Tone sections in Gold Chloride Solution for 30 seconds to 1 minute or until sections lose their golden-brown color and appear light gray.
8. Rinse sections 3-4X in MilliQ water
9. Place sections in Sodium Thiosulfate Solution for 1 minute
10. Rinse 3-4X in DiH₂O
11. Stain sections in Fast Green Stain Solution for 2 minutes to achieve desired intensity
12. Dehydrate sections in 95% alcohol for 30 seconds
13. Dehydrate sections in two changes of anhydrous alcohol for 1 minute each
14. Clear sections in three changes of xylene for 1 minute each

EXPECTED RESULTS: Basement Membranes – Black Background – Light green

CASE IDs/SLIDE NUMBERS: to be provided on back or on attached sheet