Special Stain Kit
Modified Grocott’s Methenamine Silver Stain

Catalog No: 38016SS12

Intended Use: For In Vitro Diagnostic Use. For Laboratory Use.

The reagents in this kit are intended for "In Vitro" use only. The Modified Grocott’s Methenamine Silver Stain when used with appropriate histological protocols may be used for the demonstration of defined fungi and infectious agents such as Aspergillus sp., Pneumocystis carinii and Cryptococcus neoformans in formalin fixed, paraffin embedded tissue sections.

Probable Mode of Action
The mechanism of action of Modified Grocott’s Methenamine Silver Stain is based upon the capacity of aldehyde groups to reduce cationic silver (Ag+) to metallic silver. Chromic acid is used to generate aldehyde groups by the oxidation of 1-2-glycol groups within polysaccharide rich tissue components, e.g. glycogen, mucin, reticulum and fungal cell walls. When cationic silver is added to the section in the form of a methenamine-silver ion complex, the aldehyde groups reduce the silver ions to metallic silver. Sections are subsequently toned with Gold Chloride Solution to produce metallic gold which is more stable than metallic silver and produces superior contrast and clarity.

Because of the strong oxidizing potential of the Modified Chromic Acid Solution, many of the resultant aldehyde groups are further oxidized to carboxylic acid groups which are incapable of reducing silver. This capacity of the Modified Chromic Acid Solution has the advantage of reducing background reactions of collagen and basement membranes, and produces a strong impregnation with silver in only those structures that possess high levels of the reactive polysaccharide groups e.g. glycogen, mucin and fungal cell walls.

Reagents Provided
- Modified Chromic Acid Solution (Item No. 38016SS12A, 500 mL) (Please note: Exercise caution when handling the Modified Chromic Acid Solution.)
- Sodium Bisulfite Solution (Item No. 38016SS12C, 250 mL)
- Gold Chloride Solution (Item No. 38016SS12E, 500 mL)
- Silver Nitrate Solution (Item No. 38016SS12F, 500 mL)
- Sodium Thiosulfate Solution (Item No. 38016SS12F, 500 mL)
- Light Green SF (Item No. 38016SS12G, 500 mL)

The refrigerated Methenamine/Borax Solution (Item No. 38016SS12D, 250 mL) is not included. Item ordered and shipped separately.

Please see reverse side for hazardous ingredients and warning symbols.

Storage and Stability
The Methenamine/Borax Solution should be stored at 2–8 °C (36–46 °F). Store other components at room temperature 15–30 °C (59–86 °F). Do not use after the expiration date.

Specimen Preparation
Fixation
10% Neutral Buffered Formalin.

Sectioning
Following processing and paraffin embedding, cut sections at the standard thickness.

Staining Protocol (Conventional)
Note: Prior to staining, place 20 mL of the Methenamine/Borax Solution and 20 mL of the Silver Nitrate Solution into separate acid cleaned beakers. Heat the solutions to 50–55 °C and just prior to silver impregnation combine the two solutions in an acid washed Coplin jar.² Preheat a second Coplin jar with 40 mL of deionized water to 50–55 °C to be used as a rinse.

1. Deparaffinize tissue sections with xylene and rehydrate through graded alcohols to deionized water.
2. Place slides in Modified Chromic Acid Solution for 5 –10 minutes at room temperature. (Please note: Exercise caution when handling the Modified Chromic Acid Solution.)
3. Rinse slides in two changes of tap water.
4. Rinse slides in two changes of deionized water.
5. Place slides in Sodium Bisulfite Solution for 1 minute.
6. Rinse slides in running tap water for 30 seconds.
7. Rinse slides in two changes of deionized water.
8. Combine the prewarmed Silver Nitrate Solution and Methenamine/Borax Solution into a prewarmed acid cleaned Coplin jar.
9. Place slides in the Methenamine/Borax-Silver Nitrate Solution and incubate for 20–45 minutes at 50–55 °C. After 15–20 minutes, using non-metallic forceps, remove a control slide, dip in the prewarmed deionized water to rinse, and check microscopically for the completeness of silver deposition.
10. Rinse slides in 6 changes of deionized water.
11. Place slides in Gold Chloride Solution for 5 minutes.
12. Rinse slides in 3 changes of deionized water.
13. Place slides in Sodium Thiosulfate Solution for 2 minutes.
14. Rinse slides thoroughly in running tap water for 2 minutes.
15. Place slides in Light Green SF for 40 seconds.
16. Rinse slides briefly in deionized water.
17. Dehydrate slides in three changes of absolute alcohol.
18. Clear slides in two changes of xylene and mount in a xylene miscible medium.

Staining Protocol (Microwave)
Exercise caution when using the microwave oven to heat any solution or reagent. The microwave must be properly ventilated to prevent the accumulation of fumes in the laboratory. Microwave transparent Coplin jars and caps should be used during the staining process. The caps should be loosely attached to prevent splits. Caps with ventilation holes also may be used. All microwave ovens should be used in accordance with the manufacturer’s instructions. The procedures described here were performed using an Energy Beam Sciences H2250 laboratory microwave. Because of differences in microwave power and frequencies among different models, it may be necessary to adjust power levels or times to achieve optimal results.

1. Deparaffinize tissue sections with xylene and rehydrate through graded alcohols to deionized water.
2. Place slides in the Modified Chromic Acid Solution and microwave at 800 watts for 10 seconds. (Please note: Exercise caution when handling the Modified Chromic Acid Solution.)
3. Gently mix by swirling the Coplin jar and allow to stand for 1 minute.
4. Rinse slides in 8 changes of deionized water.
5. Place slides in Sodium Bisulfite Solution for 1 minute.
6. Rinse slides in 6 changes of deionized water.
7. Combine 20 mL of the Silver Nitrate Solution and 20 mL of the Methenamine/Borax Solution in an acid cleaned plastic Coplin jar.
8. Place slides in the solution and microwave at 600 watts for 45–50 seconds.
9. Gently mix by swirling the Coplin jar and allow to stand for 2 minutes.
10. Microwave solution for 10 seconds at 600 watts.
11. Gently agitate solution and using non-metallic forceps remove a control slide, dip in prewarmed deionized water to rinse and check microscopically for the completeness of silver impregnation. If necessary replace slides in the warm Methenamine/Borax Silver Nitrate Solution and swirl for 10–30 seconds.
12. If necessary, repeat steps 10 and 11 until a satisfactory level of silver deposition is observed.
13. Rinse slides in 6 changes of deionized water.
14. Place slides in Gold Chloride Solution for 5 minutes.
15. Rinse slides in 3 changes of deionized water.
16. Place slides in Sodium Thiosulfate Solution for 2 minutes.
17. Rinse slides thoroughly in running tap water for 2 minutes.
18. Place slides in Light Green SF for 40 seconds.
19. Rinse slides briefly in deionized water.
20. Dehydrate slides in 3 changes of absolute alcohol.
21. Clear slides in 2 changes of xylene and mount in a xylene miscible medium.

Technical Notes
(a) Solutions may be heated using a water bath or laboratory oven. Avoid over-heating as break-down of Methenamine is accelerated at temperatures above 50 °C.
(b) Distilled water may be substituted for deionized water at any step.

Expected Results
Fungi – Sharply delineated black
Inner parts of mycelia and hyphae – taupe to old rose
Mucin – taupe to dark grey
Background – green

Recommended Controls
Tissue known to contain fungal elements.

References
3. Koski, J.P. (1981) Silver methenamine borate (SMB): Cost reduction with technical models, it may be necessary to adjust power levels or times to achieve optimal results.

Date of Issue
November 2010
# Living up to Life

## Special Stain Kit

### Modified Grocott’s Methenamine Silver Stain

**Catalog No:** 38016SS12

<table>
<thead>
<tr>
<th>Modified Chromic Acid Solution (Item No. 38016SS12A, 500 mL)</th>
<th>Gold Chloride Solution (Item No. 38016SS12E, 500 mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hazardous Ingredient</strong></td>
<td><strong>Hazardous Ingredient</strong></td>
</tr>
<tr>
<td><strong>% wt.</strong></td>
<td><strong>% wt.</strong></td>
</tr>
<tr>
<td>Sulfuric Acid</td>
<td>Water</td>
</tr>
<tr>
<td>10-30</td>
<td>Gold chloride, trihydrate</td>
</tr>
<tr>
<td>Chromium trioxide</td>
<td></td>
</tr>
<tr>
<td>5-10</td>
<td></td>
</tr>
<tr>
<td>Potassium dichromate</td>
<td></td>
</tr>
<tr>
<td>5-10</td>
<td></td>
</tr>
<tr>
<td>Hazardous Ingredient % wt. CAS No.</td>
<td></td>
</tr>
<tr>
<td>Sodium Bisulfite Solution (Item No. 38016SS12B, 500 mL)</td>
<td></td>
</tr>
<tr>
<td><strong>% wt.</strong></td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>Water</td>
</tr>
<tr>
<td>60-100</td>
<td>Sodium bisulfite</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Silver Nitrate Solution (Item No. 38016SS12C, 250 mL)</th>
<th>Sodium Thiosulfate Solution (Item No. 38016SS12F, 500 mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hazardous Ingredient</strong></td>
<td><strong>Hazardous Ingredient</strong></td>
</tr>
<tr>
<td><strong>% wt.</strong></td>
<td><strong>% wt.</strong></td>
</tr>
<tr>
<td>Water</td>
<td>Water</td>
</tr>
<tr>
<td>60-100</td>
<td>Sodium thiosulfate</td>
</tr>
<tr>
<td>Silver nitrate</td>
<td></td>
</tr>
<tr>
<td>0.1-1</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Methenamine/Borax Solution (Item No. 38016SS12D, 250 mL)</th>
<th>Light Green SF (Item No. 38016SS12G, 500 mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hazardous Ingredient</strong></td>
<td><strong>Hazardous Ingredient</strong></td>
</tr>
<tr>
<td><strong>% wt.</strong></td>
<td><strong>% wt.</strong></td>
</tr>
<tr>
<td>Water</td>
<td>Water</td>
</tr>
<tr>
<td>60-100</td>
<td>Mixture, 3(2H)-isothiazolone, 5-chloro-2-methyl- with 5-chloro-2-methyl- with Mixture, 3(2H)-isothiazolone, 5-chloro-2-methyl- with</td>
</tr>
<tr>
<td></td>
<td>2-methyl-3(2H)-isothiazolone</td>
</tr>
<tr>
<td></td>
<td>Acetic acid</td>
</tr>
<tr>
<td></td>
<td>Light green SF</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Leica Biosystems Richmond, Inc.
5205 Route 12
Richmond, IL 60071
USA
1-800-225-3035

www.leica-microsystems.com

© Leica Microsystems GmbH • HRB 5187 • 95.10192 Rev A • RM 3816SS12