

Steiner-Steiner Modified Silver Stain Kit - Technical Memo

KIT INCLUDES:

Solution A: Uranyl Nitrate 1%, Aqueous	250 ml	Part 9171A
Solution B: Silver Nitrate 1%, Aqueous	250 ml	
Solution C: Gum Mastic 2.5%, Alcoholic	175 ml x 2	
Ingredient D: Hydroquinone, Powder	5 grams	
Mini Sampling Spoon		

COMPLIMENTARY POSITIVE CONTROL SLIDES: Enclosed with this kit are two complimentary unstained positive control slides to be used for the initial verification of staining techniques and reagents. Verification must be documented by running one Newcomer Supply complimentary positive control slide along with your current positive control slide for the first run. Retain the second complimentary control slide for further troubleshooting, if needed.

Individual stain solutions and additional control slides may be available for purchase under separate part numbers at www.newcomersupply.com.

Additionally Needed:

Hydrochloric Acid 5%, Aqueous	Part 12086 (for acid cleaning glassware)
Xylene, ACS	Part 1445
Alcohol, Ethyl Denatured, 100%	Part 10841
Alcohol, Ethyl Denatured, 95%	Part 10842
Coplin Jar, Plastic	Part 5184 (for microwave modifications)

For storage requirements and expiration date refer to individual bottle labels.

APPLICATION:

Newcomer Supply Steiner-Steiner Modified Silver Stain Kit procedure, with included microwave modifications, is a silver technique effective for the demonstration of spirochetes, *Helicobacter pylori*, *Legionella pneumophila*, other nonfilamentous bacteria and fungus.

METHOD:

Fixation: Formalin 10%, Phosphate Buffered (Part 1090)

- Mercury fixatives will inhibit silver staining

Technique: Paraffin sections cut at 5 microns

Solutions: All solutions are manufactured by Newcomer Supply, Inc.

All Newcomer Supply Stain Kits are designed to be used with Coplin jars filled to 40 ml following the staining procedure provided below. Some solutions in the kit may contain extra volumes.

PRESTAINING PREPARATION:

- All glassware/plasticware must be acid cleaned prior to use.
 - See Procedure Notes #1 and #2 (page 2).
- Preheat Solution A: Uranyl Nitrate 1%, Aqueous to 60°C in a water bath. Save for Step #8.
- Preheat Solution B: Silver Nitrate 1%, Aqueous to 60°C in a water bath. Save for Step #10.
- Prepare Hydroquinone Solution; combine and mix well.
 - Ingredient D: Hydroquinone, Powder 0.5 gm
(or one rounded scoop with reusable mini sampling spoon)
 - Distilled Water 25 ml
- Prepare fresh Reducing Solution by combining in order listed.
 - Hydroquinone Solution (Step #4) 25 ml
 - Solution C: Gum Mastic 2.5%, Alcoholic 15 ml
 - Solution B: Silver Nitrate 1%, Aqueous 0.3 ml
 - Solution will turn milky white after addition of Gum Mastic.
 - Preheat solution in 45°C water bath. Save for Step #16.
- Do not preheat solutions if using Microwave Modifications.**

STAINING PROCEDURE:

- Deparaffinize sections thoroughly in three changes of xylene, 3 minutes each. Hydrate through two changes each of 100% and 95% ethyl alcohols, 10 dips each. Wash well with distilled water.
 - See Procedure Note #3 (page 2)
- Sensitize slides in preheated Solution A: Uranyl Nitrate 1%, Aqueous for 15 minutes in a 60°C water bath.
 - Agitate solution to evenly distribute heat.

Microwave Modification: See Procedure Note #4 (page 2).

- Place slides in a plastic Coplin jar containing Solution A: Uranyl Nitrate 1%, Aqueous and microwave at 60°C for 5 minutes.
- Rinse well in several changes of distilled water.
 - Place slides in preheated Solution B: Silver Nitrate 1%, Aqueous (Step #3) and incubate in a 60°C water bath for 30 minutes.
 - Agitate solution to evenly distribute heat.

Microwave Modification:

- Place slides in a plastic Coplin jar containing Solution B: Silver Nitrate 1%, Aqueous and microwave at 70°C for 5 minutes.
- Rinse well in several changes of distilled water.
 - Excessive rinsing may cause nucleus to pick up silver.
 - Dip 5 times in each of two changes of fresh 95% ethyl alcohol.
 - Dip 5 times in each of two changes of fresh 100% ethyl alcohol.
 - Place in Solution C: Gum Mastic 2.5%, Alcoholic for 5 minutes.
 - Air-dry for 5 minutes. Slides/sections will be milky white.
 - Place slides in preheated Reducing Solution (Step #5) and incubate in 45°C water bath for 10-30 minutes with frequent agitation; examine microscopically at 10 minutes.
 - Check staining progress at timed intervals.
 - Tissue will turn tan in color; continue to check staining progress at timed intervals.
 - Bacteria will be black when the tissue reaches a golden brown color.
 - Dip in warm distilled water before/after each examination.
- Microwave Modification:** See Procedure Note #5 (page 2)
- Heat slides in a plastic Coplin jar containing fresh Reducing Solution at 45°C for 30 seconds.
 - Remove from microwave. Continue to incubate slides in warm solution for an additional 2 minutes.
- Rinse well in several changes of distilled water.
 - Dehydrate in two changes each of 95% and 100% ethyl alcohol. Clear in three changes of xylene, 10 dips each; coverslip with compatible mounting medium.

RESULTS:

Spirochetes	Dark brown to black
<i>Helicobacter pylori</i>	Dark brown to black
<i>Legionella pneumophila</i>	Dark brown to black
Nonfilamentous bacteria and fungus	Dark brown to black
Background	Golden brown

PROCEDURE NOTES:

1. Acid clean all glassware/plasticware (12086) and rinse thoroughly in several changes of distilled water. Cleaning glassware with bleach is not equivalent to acid washing.
2. Plastic (5500), plastic-tipped or paraffin coated metal forceps must be used with any silver solution to prevent precipitation of silver salts. No metals of any kind should be in contact with any silver solution. Only glass thermometers should be used.
3. Drain staining rack/slides after each step to prevent solution carry over.
4. The suggested microwave procedure has been tested at Newcomer Supply using an "EB Sciences", 850 watt microwave oven with temperature probe and agitation tubes. This procedure is reproducible in our laboratory. It is nonetheless a guideline and techniques should be developed for your laboratory which meet the requirements of your situation. Microwave devices should be placed in a fume hood or vented into a fume hood, according to manufacturer's instructions, to prevent exposure to chemical vapors.
5. The Reducing Solution contains alcohol and will reduce its boiling point. To avoid boiling solution, adjust microwave times and power levels accordingly.
6. The use of some xylene substitutes have resulted in diminished spirochete staining. If using a xylene substitute exercise caution and closely follow the manufacturer's recommendation for deparaffinization and clearing steps.
7. Dispose of Uranyl Nitrate as hazardous waste and/or according to local and state environmental regulations. Refer to SDS for personal protective measures and handling information.

REFERENCES:

1. Garvey, Winsome. "Some Favorite Silver Stains." *The Journal of Histotechnology* 19.3 (1996): 269-278.
2. Luna, Lee G. *Histopathologic Methods and Color Atlas of Special Stains and Tissue Artifacts*. Gaithersburg, MD: American Histolabs, 1992. 218-219.
3. Steiner, Gabriel, and Grete Steiner. "New Simple Silver Stain for Demonstration of Bacteria, Spirochetes and Fungi in Sections of Paraffin Embedded Tissue Blocks." *Journal of Laboratory Clinical Medicine* 29 (1944). 868-871.
4. Swisher, Billie. "Modified Steiner Procedure for Microwave Staining of Spirochetes and Nonfilamentous Bacteria." *The Journal of Histotechnology* 10.4 (1987): 241-243.
5. Modifications developed by Newcomer Supply Laboratory.