

## Reticulum, Gordon & Sweets Stain Kit - Technical Memo

### KIT INCLUDES:

|   |        |                   |
|---|--------|-------------------|
| Solution A: Potassium Permanganate 1%, Aqueous    | 250 ml | <b>Part 9168A</b> |
| Solution B: Oxalic Acid 1%, Aqueous               | 250 ml |                   |
| Solution C: Ferric Ammonium Sulfate 2.5%, Aqueous | 250 ml |                   |
| Solution D: Silver Nitrate 10%, Aqueous           | 50 ml  |                   |
| Solution E: Ammonium Hydroxide 28-30%, ACS        | 50 ml  |                   |
| Solution F: Sodium Hydroxide 3%, Aqueous          | 50 ml  |                   |
| Solution G: Formalin 10%, Aqueous                 | 250 ml |                   |
| Solution H: Gold Chloride 0.2%, Aqueous           | 250 ml |                   |
| Solution I: Sodium Thiosulfate 5%, Aqueous        | 250 ml |                   |
| Solution J: Nuclear Fast Red Stain, Kernechtrot   | 250 ml |                   |

**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](http://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                               |

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

### APPLICATION:

Newcomer Supply Reticulum, Gordon & Sweets Stain Kit procedure is a silver staining method for demonstration of reticular fibers; regarded as specialized connective tissue fibers. This stain is useful in the differential diagnosis of certain types of tumors.

### METHOD:

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

**Technique:** Paraffin sections cut at 5 microns

**Solutions:** All solutions 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.

### STAINING PROCEDURE:

1. All glassware/plasticware must be acid cleaned prior to use.
  - a. See Procedure Notes #1 and #2 (page 2).
2. 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.
  - a. See Procedure Notes #3 and #4 (page 2).
3. Oxidize sections in Solution A: Potassium Permanganate 1%, Aqueous for 5 minutes.
4. Wash in running tap water for 2 minutes; rinse in distilled water.
5. Bleach in Solution B: Oxalic Acid 1%, Aqueous for 2 minutes or until sections are colorless.
6. Wash in running tap water for 2 minutes; rinse in distilled water.
7. Sensitize sections in Solution C: Ferric Ammonium Sulfate 2.5%, Aqueous between 15 to 20 minutes.
8. Rinse slides in several changes of distilled water.

9. Prepare Ammoniacal Silver Working Solution:
  - a. Place 5 ml of Solution D: Silver Nitrate 10%, Aqueous in a flask; add Solution E: Ammonium Hydroxide 28-30%, ACS drop by drop, swirling continuously until formed precipitate completely dissolves. Do not add any excess Ammonium Hydroxide.
  - b. Add 5 ml of Solution F: Sodium Hydroxide 3%, Aqueous.
  - c. Re-dissolve formed precipitate with Solution E: Ammonium Hydroxide 28-30%, ACS until a faint cloudiness remains.
  - d. If proceeded too far and no cloudiness remains, add Solution D: Silver Nitrate 10%, Aqueous drop by drop, until one drop causes the solution to become permanently cloudy. A faint cloudiness is the optimum.
  - e. Bring solution total volume to 50 ml with distilled water; filter.
10. Impregnate sections in filtered Ammoniacal Silver Working Solution for 2 minutes.
11. Rinse slides well in running distilled water for 1 minute.
  - a. See Procedure Note #5 (page 2).
12. Reduce in Solution G: Formalin 10%, Aqueous for 2 minutes.
13. Rinse in running tap water for 3 minutes.
14. Tone in Solution H: Gold Chloride 0.2%, Aqueous for 10 minutes.
15. Rinse well in distilled water.
16. Place slides in Solution I: Sodium Thiosulfate 5%, Aqueous for 1 minute.
17. Wash well in tap water for 2 minutes; rinse in distilled water.
18. Counterstain with Solution J: Nuclear Fast Red Stain, Kernechtrot for 5 minutes.
  - a. Shake solution well before use; do not filter.
19. Rinse well in distilled water.
  - a. See Procedure Note #6 (page 2).
20. Quickly 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:

|                  |       |
|------------------|-------|
| Reticular fibers | Black |
| Background       | Red   |

#### **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 silver solutions to prevent precipitation of silver salts. No metals of any kind should come in contact with silver solutions.
3. Drain staining rack/slides after each step to prevent solution carry over.
4. Do not allow sections to dry out at any point during staining procedure.
5. This rinse step is critical for good reticulum demonstration. If rinsing is insufficient, excessive background staining may occur.
6. Wash well after Nuclear Fast Red Stain, Kernechtrot to avoid cloudiness in dehydration steps.
7. If using a xylene substitute, closely follow the manufacturer's recommendations for deparaffinization and clearing steps.

#### **REFERENCES:**

1. Bancroft, John D., and Marilyn Gamble. *Theory and Practice of Histological Techniques*. 6th ed. Oxford: Churchill Livingstone Elsevier, 2008. 155-156.
2. Carson, Freida L., and Christa Hladik. *Histotechnology: A Self-Instructional Text*. 3rd ed. Chicago, Ill.: American Society of Clinical Pathologists, 2009. 177-179
3. Gordon, Harold, and Henry Sweets. "A Simple Method for the Silver Impregnation of Reticulum." *American Journal of Pathology* 12.4 (1936): 545-552.
4. Modifications developed by Newcomer Supply Laboratory.