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Part 4620
Revised January 2020

Reticulum Control Slides – Technical Memo

CONTROL SLIDES: **Part 4620A** 10 Slide/Set **Part 4620B** 98 Slide/Set

PRODUCT SPECIFICATIONS:
Tissue: Positive staining liver.
Fixation: Formalin 10%, Phosphate Buffered (Part 1090).
Section/Glass: Paraffin sections cut at 4 microns on Superfrost™ Plus slides.
Quality Control Stain: Gordon & Sweets Reticulum quality control stained slide(s) included.
Reactivity: Guaranteed product specific reactivity for one year from date of receipt. Revalidate after one year to verify continued reactivity.
Storage: 15-30°C in a light deprived and humidity controlled environment.
Intended Use: To verify histological techniques and reagent reactivity.

Before using unstained control slides, review the enclosed stained slide(s) to ensure that this tissue source is acceptable for testing needs.

CONTROL SLIDE VALIDATION:

With Reticulum, Gordon and Sweets Stain Kit:	**Part 9168A**	**Individual Stain Solution**
Solution A: Potassium Permanganate 1%, Aqueous	250 ml	Part 13393
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	Part 13806
Solution E: Ammonium Hydroxide 28-30%, ACS	50 ml	Part 1006
Solution F: Sodium Hydroxide 3%, Aqueous	50 ml	
Solution G: Formalin 10%, Aqueous	250 ml	
Solution H: Gold Chloride 0.2%, Aqueous	250 ml	Part 11286
Solution I: Sodium Thiosulfate 5%, Aqueous	250 ml	Part 1389
Solution J: Nuclear Fast Red Stain, Kernechtrot	250 ml	Part 1255

APPLICATION:

Newcomer Supply Reticulum Control Slides are for the positive histochemical staining of reticulum fibers; regarded as specialized connective tissue fibers.

PRESTAINING PREPARATION:

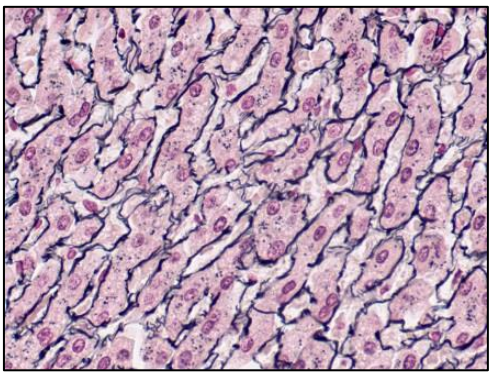
1. Heat dry sections in oven according to your laboratory protocol.
2. All glassware/plasticware must be acid cleaned prior to use.
   a. *See Procedure Notes #1 and #2 (page 2).*
3. Prepare Ammoniacal Silver Working Solution. Save for Step #11.
   a. *Place 5 ml of Solution D: Silver Nitrate 10%, Aqueous in a flask.*
   b. *Add Solution E: Ammonium Hydroxide 28-30%, ACS drop by drop, continuously swirling until formed precipitate completely dissolves. Do not add any excess Ammonium Hydroxide.*
   c. *Add 5 ml of Solution F: Sodium Hydroxide 3%, Aqueous.*
   d. *Re-dissolve formed precipitate with Solution E: Ammonium Hydroxide 28-30%, ACS until a faint cloudiness remains.*
   e. *If proceeded too far and no cloudiness remains, add Solution D: Silver Nitrate 10%, Aqueous drop by drop, until one drop causes solution to become permanently cloudy. Faint cloudiness is the optimum.*
   f. *Bring solution volume to 50 ml with distilled water; filter.*

STAINING PROCEDURE:

4. 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).*
5. Oxidize in Solution A: Potassium Permanganate 1%, Aqueous for 5 minutes.
6. Wash in running tap water for 2 minutes; rinse in distilled water.
7. Bleach in Solution B: Oxalic Acid 1%, Aqueous for 2 minutes or until sections are colorless.
8. Wash in running tap water for 2 minutes; rinse in distilled water.
9. Sensitize in Solution C: Ferric Ammonium Sulfate 2.5%, Aqueous; 15 to 20 minutes.
10. Rinse in several changes of distilled water.
11. Impregnate sections in filtered Ammoniacal Silver Working Solution (Step #3) for 2 minutes.
12. Rinse well in running distilled water for 1 minute.
    a. *See Procedure Note #5 (page 2).*
13. Reduce in Solution G: Formalin 10%, Aqueous for 2 minutes.
14. Rinse in running tap water for 3 minutes.
15. Check control slide microscopically for sufficient black reticular fiber development.
    a. *See Procedure Note #6 (page 2).*
16. Tone in Solution H: Gold Chloride 0.2%, Aqueous for 10 minutes.
17. Rinse well in distilled water.
18. Place in Solution I: Sodium Thiosulfate 5%, Aqueous for 1 minute.
19. Wash well in tap water for 2 minutes; rinse in distilled water.
20. Counterstain with Solution J: Nuclear Fast Red Stain, Kernechtrot for 5 minutes.
    a. *Shake solution well before use; do not filter.*
21. Rinse well in distilled water.
    a. *See Procedure Note #7 (page 2).*
22. 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.
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 slides after each step to prevent solution carry over.
4. Do not allow sections to dry out at any point during procedure.
5. This rinse step is critical for good reticulum demonstration. If rinsing is insufficient, excessive background staining may occur.
6. If black reticular fibers are not evident or are lightly/poorly stained, return all slides to Ammoniacal Silver Working Solution (Step #11) and repeat Steps 11-14 with the same timings.
7. Wash well after Nuclear Fast Red Stain, Kernechtrot to avoid cloudiness in dehydration steps.
8. If using a xylene substitute, closely follow the manufacturer's recommendations for deparaffinization and clearing steps.

REFERENCES:

1. Carson, Freida L., and Christa Hladik. *Histotechnology: A Self-Instructional Text*. 3rd ed. Chicago, Ill.: American Society of Clinical Pathologists, 2009. 177-179.
2. Gordon, Harold, and Henry Sweets. "A Simple Method for the Silver Impregnation of Reticulum." *American Journal of Pathology* 12.4 (1936): 545-552.
3. Modifications developed by Newcomer Supply Laboratory.