

## Movat-Russell Modified Pentachrome Stain Kit - Technical Memo

### KIT INCLUDES:

Solution A: Alcian Blue Stain 1%, Aqueous	250 ml
Solution B: Ammonium Hydroxide 28-30%, ACS	50 ml
Solution C: Hematoxylin 10%, Alcoholic	100 ml
Solution D: Ferric Chloride 10%, Aqueous	100 ml
Solution E: Iodine, Verhoeff, Aqueous	100 ml
Solution F: Ferric Chloride 2%, Aqueous	250 ml
Solution G: Sodium Thiosulfate 5%, Aqueous	250 ml
Solution H: Crocein Scarlet 7B Stain, Aqueous	250 ml
Solution I: Acid Fuchsin Stain, Aqueous	100 ml
Solution J: Phosphotungstic Acid 5%, Aqueous	500 ml
Solution K: Orange G Stain 1%, Aqueous	250 ml
Solution L: Acetic Acid 0.5%, Aqueous	500 ml

### Part 9150A

**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:

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 Movat-Russell Modified Pentachrome Stain Kit provides a single staining procedure that demonstrates five connective tissue elements; mucin, fibrin, elastic fibers, muscle, and collagen, along with nuclei.

### METHOD:

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

**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.

### STAINING PROCEDURE:

1. 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 #1 and #2 (page 2).
2. Stain in Solution A: Alcian Blue Stain 1%, Aqueous for 20 minutes.
3. Wash in running tap water for 5 minutes.
4. Prepare fresh Alkaline Alcohol Solution; combine and mix well.
  - a. Solution B: Ammonium Hydroxide 28-30% 5 ml
  - b. Alcohol, Ethyl Denatured, 95% (10842) 45 ml
5. Place slides in fresh Alkaline Alcohol Solution for 30 minutes.
6. Wash in running tap water for 10 minutes followed by a distilled water rinse.
  - a. See Procedure Note #3 (page 2).
7. Prepare fresh Hematoxylin Working Stain Solution just before use in the order given; combine and mix well.
  - a. Solution C: Hematoxylin 10%, Alcoholic 10 ml
  - b. Alcohol, Ethyl Denatured, 100% (10841) 10 ml
  - c. Solution D: Ferric Chloride 10%, Aqueous 10 ml
  - d. Solution E: Iodine, Verhoeff, Aqueous 10 ml

8. Stain in fresh Hematoxylin Working Stain Solution for 15 minutes.
  - a. Discard after successful differentiation in Step #10.
9. Rinse in several changes of distilled water.
10. Differentiate one slide at a time in Solution F: Ferric Chloride 2%, Aqueous until elastic fibers contrast sharply with the background; approximately 5-10 dips.
  - a. See Procedure Note #4 (page 2).
11. Rinse in distilled water.
12. Place in Solution G: Sodium Thiosulfate 5%, Aqueous for 1 minute.
13. Wash in running tap water for 5 minutes; rinse in distilled water.
14. Prepare Crocein Scarlet-Acid Fuchsin Solution:
  - a. Solution H: Crocein Scarlet 7B Stain, Aqueous 40 ml
  - b. Solution I: Acid Fuchsin Stain, Aqueous 10 ml
15. Stain in Crocein Scarlet-Acid Fuchsin Solution for 1 minute.
16. Rinse in several changes of distilled water.
17. Rinse in Solution L: Acetic Acid 0.5%, Aqueous for 30 seconds.
18. Place slides in Solution J: Phosphotungstic Acid 5%, Aqueous; two changes of 5 minutes each.
19. Rinse in Solution L: Acetic Acid 0.5%, Aqueous.
20. Stain in Solution K: Orange G Stain 1%, Aqueous for 15 minutes.
21. Dehydrate through three changes of 100% ethyl alcohol, 10 dips each. Clear in three changes of xylene, 10 dips each; coverslip with compatible mounting medium.
  - a. Do not use 95% ethyl alcohol in the dehydration step.

### RESULTS:

Nuclei and elastic fibers	Black
Collagen and reticular fibers	Yellow
Ground substance and mucin	Blue
Fibrinoid, fibrin	Intense red
Muscle	Red

**PROCEDURE NOTES:**

1. Drain staining rack/slides after each step to prevent solution carry over.
2. Do not allow sections to dry out at any point during staining procedure.
3. It is important to completely remove Alkaline Alcohol Solution with running tap water. Failure to do so will inhibit the subsequent staining steps.
4. Do not over-differentiate in Solution F: Ferric Chloride 2%, Aqueous. If the background is completely colorless, the section may be over-differentiated. Over-differentiated sections may be restained in Hematoxylin Working Stain Solution (Step #8) provided sections have not been treated with an alcohol/dehydration step.
5. 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. 172-174.
2. Movat, Henry, "Demonstration of All Connective Tissue Elements in a Single Section". *AMA Archives of Pathology*. 1955; 60 (3): 289–295.
3. Russell H. K. Jr. "A Modification of Movat's Pentachrome Stain". *AMA Archives of Pathology*. 1972; 94 (2): 187–191.
4. Modifications developed by Newcomer Supply Laboratory.