Movat-Russell Modified Pentachrome Stain Kit - Technical Memo

KIT INCLUDES:

- Part 9150A
  - 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 Thiocyanate 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

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:

- 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.
   - 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.
   - Solution B: Ammonium Hydroxide 28-30% 5 ml
   - 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.
   - See Procedure Note #3 (page 2).
7. Prepare fresh Hematoxylin Working Stain Solution just before use in the order given; combine and mix well.
   - Solution C: Hematoxylin 10%, Alcoholic 10 ml
   - Alcohol, Ethyl Denatured, 100% (10841) 10 ml
   - Solution D: Ferric Chloride 10%, Aqueous 10 ml
   - Solution E: Iodine, Verhoeff, Aqueous 10 ml
8. Stain in fresh Hematoxylin Working Stain Solution for 15 minutes.
   - 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.
   - See Procedure Note #4 (page 2).
11. Rinse in distilled water.
12. Place in Solution G: Sodium Thiocyanate 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:
   - Solution H: Crocein Scarlet 7B Stain, Aqueous 40 ml
   - 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.
   - 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

SUPPORT/WARRANTY: For assistance regarding this product contact Newcomer Supply at 800-383-7799 or newly@newcomersupply.com. The information presented in this technical memo is to the best of our knowledge accurate. No warranty is expressed or implied. The user is responsible for determining the suitability of this product for their use and upon receipt assumes all liability for its use and responsibility for compliance with any laws or regulations. Please refer to www.newcomersupply.com for complete warranty information. Newcomer Supply, Inc. Active September 2014
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:

4. Modifications developed by Newcomer Supply Laboratory.