NEWCOMER SUPPLY®
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Part 9110
Revised August 2016

Colloidal Iron, Müller-Mowry Stain Kit - Technical Memo

KIT INCLUDES:	**Part 9110A**
Solution A: Acetic Acid 12%, Aqueous	500 ml x 2
Solution B: Colloidal Iron Stock	75 ml
Solution C: Acetic Acid 40%, Aqueous	100 ml
Solution D: Potassium Ferrocyanide 2%, Aqueous	100 ml
Solution E: Hydrochloric Acid 2%, Aqueous	200 ml
Solution F: 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.

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 Colloidal Iron, Müller-Mowry Stain Kit procedure is used to demonstrate acid epithelial mucin (sialomucin, sulfomucin) and stromal (mesenchymal) mucin. This method is also excellent for the demonstration of the encapsulated yeast *Cryptococcus neoformans.*

METHOD:

Fixation: Formalin 10%, Phosphate Buffered (Part 1090)
- Chromate fixatives should be avoided

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. To avoid the possibility of residual background iron staining, acid clean glassware is recommended in the staining procedure.
   a. *See Procedure Note #1.*
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 #2 and #3.*
3. Prepare Colloidal Iron Working Solution; combine and mix well.
   a. *Solution B: Colloidal Iron Stock* — *10 ml*
   b. *Solution C: Acetic Acid 40%, Aqueous* — *12 ml*
   c. *Distilled Water* — *18 ml*
4. Place slides in Solution A: Acetic Acid 12%, Aqueous for 30 seconds. Drain Slides. Do not rinse.
5. Place slides in Colloidal Iron Working Solution for 1 hour.
6. Rinse in three changes of Solution A: Acetic Acid 12%, Aqueous, 3 minutes each.
7. Prepare fresh Ferrocyanide-Hydrochloric Acid Solution directly before use; combine and mix well.
   a. *Solution D: Potassium Ferrocyanide 2%, Aqueous* — *15 ml*
   b. *Solution E: Hydrochloric Acid 2%, Aqueous* — *30 ml*
8. Place slides in fresh Ferrocyanide-Hydrochloric Acid Solution for 20 minutes at room temperature.
9. Wash in running tap water for 5 minutes.
10. Counterstain in Solution F: Nuclear Fast Red Stain, Kernechtrot for 5 minutes.
    a. *Shake solution well before use; do not filter.*
11. Rinse well in running tap water for 1-5 minutes.
    a. *See Procedure Note #4.*
12. Dehydrate quickly 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:

Acid epithelial mucin	Blue
Stromal mucin	Blue
Capsule of *Cryptococcus neoformans*	Blue
Nuclei	Pink-red
Cytoplasm	Pale pink

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. Drain staining rack/slides after each step to prevent solution carry over.
3. Do not allow sections to dry out at any point during staining procedure.
4. Wash well after Nuclear Fast Red Stain, Kernechtrot to avoid cloudiness in dehydration steps.
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. 149-152.
2. Rekhtman, Natasha, and Justin Bishop. *Quick Reference Handbook for Surgical Pathologists*. Berlin: Springer, 2011. 69.
3. Sheehan, Dezna C., and Barbara B. Hrapchak. *Theory and Practice of Histotechnology*. 2nd ed. St. Louis: Mosby, 1980. 171-172.
4. Modifications developed by Newcomer Supply Laboratory.