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Part 9136
Revised April 2020

Iron, Gomori Prussian Blue Stain Kit - Technical Memo

KIT INCLUDES:	**Part 9136A**	**Part 9136B**
Solution A: Hydrochloric Acid 20%, Aqueous	125 ml	250 ml
Solution B: Potassium Ferrocyanide 10%, Aqueous	125 ml	250 ml
Solution C: Nuclear Fast Red Stain, Kernechtrot	250 ml	500 ml

COMPLIMENTARY POSITIVE CONTROL SLIDES: Enclosed are two complimentary unstained positive control slides 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 Iron, Gomori Prussian Blue Stain Kit procedure is used to detect loosely bound ferric iron in tissue sections. This histochemical reaction is sensitive enough to demonstrate minute amounts of iron deposits in blood cells, bone marrow and spleen.

METHOD:

Fixation: Formalin 10%, Phosphate Buffered (Part 1090)
Technique: Paraffin sections cut at 4 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.

PRESTAINING PREPARATION:

1. If necessary, heat dry tissue sections/slides in oven.
2. Acid clean glassware prior to use to avoid residual iron staining.
   a. *See Procedure Note #1.*

STAINING PROCEDURE:

3. 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.*
4. Prepare fresh Ferrocyanide Working Solution directly before use; combine and mix well.
   a. *Solution A: Hydrochloric Acid 20%, Aqueous 20 ml*
   b. *Solution B: Potassium Ferrocyanide 10%, Aqueous 20 ml*
5. Place in fresh Ferrocyanide Working Solution for 20 minutes.
6. Rinse in three changes of tap water; rinse in distilled water.
7. Place in Solution C: Nuclear Fast Red Stain, Kernechtrot for 5 minutes.
   a. *Shake solution well before use; do not filter.*
8. Rinse well in distilled water.
   a. *See Procedure Note #4.*
9. 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:

Ferric iron deposits	Bright blue
Nuclei	Red
Cytoplasm	Pink

PROCEDURE NOTES:

1. Acid clean all glassware/plasticware (12086) and rinse thoroughly in several changes of distilled water.
2. Drain slides after each step to prevent solution carry over.
3. Do not allow sections to dry out at any point during 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. Luna, Lee G. *Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology*. 3rd ed. New York: Blakiston Division, McGraw-Hill, 1968. 179-184.
2. Sheehan, Dezna C., and Barbara B. Hrapchak. *Theory and Practice of Histotechnology*. 2nd ed. St. Louis: Mosby, 1980. 217-218.
3. Modifications developed by Newcomer Supply Laboratory.