

Gomori Prussian Blue Iron Stain - Technical Memo

SOLUTIONS:

	500 ml	1 Liter
Hydrochloric Acid 20%, Aqueous	Part 12087A	Part 12087B
Potassium Ferrocyanide 10%, Aqueous	Part 13392A	

Additionally Needed:

Iron Control Slides	Part 4320	or	Iron, Animal Control Slides	Part 4321
Xylene, ACS	Part 1445			
Alcohol, Ethyl Denatured, 100%	Part 10841			
Alcohol, Ethyl Denatured, 95%	Part 10842			
Nuclear Fast Red Stain, Kernechtrot	Part 1255			
Hydrochloric Acid 5%, Aqueous	Part 12086 (for acid cleaning glassware)			

For storage requirements and expiration date refer to individual product labels.

APPLICATION:

Newcomer Supply Gomori Prussian Blue Iron Stain is used to detect loosely bound ferric iron in tissue sections, bone marrow smears and blood smears. This histochemical reaction is sensitive enough to demonstrate even minute amounts of iron deposits in blood cells, bone marrow, spleen and liver.

METHOD:

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

- a. Chromate fixatives should be avoided
- b. Fix smears per laboratory protocol

Technique: Paraffin sections cut at 5 microns or prepared smears

Solutions: All solutions are manufactured by Newcomer Supply, Inc.

All Newcomer Supply stain procedures are designed to be used with Coplin jars filled to 40 ml following the staining procedure provided below.

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

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SOLUTIONS:

	500 ml	1 Liter
Hydrochloric Acid 20%, Aqueous	Part 12087A	Part 12087B
Potassium Ferrocyanide 10%, Aqueous	Part 13392A	

Additionally Needed:

Iron Control Slides	Part 4320	or	Iron, Animal Control Slides	Part 4321
Xylene, ACS	Part 1445			
Alcohol, Ethyl Denatured, 100%	Part 10841			
Alcohol, Ethyl Denatured, 95%	Part 10842			
Nuclear Fast Red Stain, Kernechtrot	Part 1255			
Hydrochloric Acid 5%, Aqueous	Part 12086 (for acid cleaning glassware)			

For storage requirements and expiration date refer to individual product labels.

APPLICATION:

Newcomer Supply Perls' Prussian Blue Iron Stain is used for the demonstration of ferric iron in tissue sections, bone marrow smears and blood smears. By exposure to the combined solution of hydrochloric acid and potassium ferrocyanide, ferric iron is released from hemosiderin and forms the insoluble compound Prussian blue.

METHOD:

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

- a. *Chromate fixatives should be avoided*
- b. *Fix smears per laboratory protocol*

Technique: Paraffin sections cut at 5 microns or prepared smears

Solutions: All solutions are manufactured by Newcomer Supply, Inc.

All Newcomer Supply stain procedures are designed to be used with Coplin jars filled to 40 ml following the staining procedure provided below.

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. Prepare stock Hydrochloric Acid 2%, Aqueous:

a. Hydrochloric Acid 20%, Aqueous	10 ml
b. Distilled Water	90 ml
c. <i>Store stock solution at 2-8°C for up to 6 months.</i>	
3. Prepare stock Potassium Ferrocyanide 2%, Aqueous:

a. Potassium Ferrocyanide 10%, Aqueous	20 ml
b. Distilled Water	80 ml
c. <i>Store stock solution at 2-8°C for up to 6 months.</i>	
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 #2 and #3.*
5. Prepare fresh Working Perls' Ferrocyanide Solution directly before use; combine and mix well.

a. Hydrochloric Acid 2%, Aqueous	20 ml
b. Potassium Ferrocyanide 2%, Aqueous	20 ml
6. Place slides in fresh Working Perls' Ferrocyanide Solution for 20-30 minutes.
7. Rinse in three changes of tap water; rinse in distilled water.
8. Place slides in Nuclear Fast Red Stain, Kernechtrot for 5 minutes.
 - a. *Shake solution well before use; do not filter.*
9. Rinse well in distilled water.
 - a. *See Procedure Note #4.*
10. 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
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REFERENCES:

1. Culling, C. F. A. *Handbook of Histopathological and Histochemical Techniques: (including Museum Techniques)*. 3rd ed. London: Butterworth, 1974. 378.
2. Luna, Lee G. *Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology*. 3rd ed. New York: Blakiston Division, McGraw-Hill, 1968. 184.
3. McPherson, Richard and Matthew Pincus. *Henry's Clinical Diagnosis and Management by Laboratory Methods*. 22nd ed. Philadelphia: Elsevier Saunders, 2011. 532-533.
4. Suvarna, S. Kim., Christopher Layton, and John D. Bancroft. *Bancroft's Theory and Practice of Histological Techniques*. 7th ed. Oxford: Churchill Livingstone Elsevier, 2013. 241-242.
5. Modifications developed by Newcomer Supply Laboratory.