

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 minute amounts of iron deposits in blood cells, bone marrow and spleen.

METHOD:

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

a. *Fix smears per laboratory protocol*

Technique: Paraffin sections cut at 4 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 provided staining procedure.

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. *Hydrochloric Acid 20%, Aqueous 20 ml*
 - 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 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

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Potassium Ferrocyanide 10%, Aqueous	Part 13392A	

Additionally Needed:

Iron Control Slides	Part 4320	or	Iron, Animal Control Slides	Part 4321
Xylene, ACS	Part 1445			
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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. *Fix smears per laboratory protocol*

Technique: Paraffin sections cut at 4 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 provided staining procedure.

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.*
3. Prepare stock Hydrochloric Acid 2%, Aqueous:
 - a. *Hydrochloric Acid 20%, Aqueous* 10 ml
 - b. *Distilled Water* 90 ml
 - c. *Store stock solution at 2°- 8°C for up to 6 months.*
4. Prepare stock Potassium Ferrocyanide 2%, Aqueous:
 - a. *Potassium Ferrocyanide 10%, Aqueous* 20 ml
 - b. *Distilled Water* 80 ml
 - c. *Store stock solution at 2°- 8°C for up to 6 months.*

STAINING PROCEDURE:

5. 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.*
6. 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
7. Place in Working Perls' Ferrocyanide Solution for 20-30 minutes.
8. Rinse in three changes of tap water; rinse in distilled water.
9. Place in Nuclear Fast Red Stain, Kernechtrot for 5 minutes.
 - a. *Shake solution well before use; do not filter.*
10. Rinse well in distilled water.
 - a. *See Procedure Note #4.*
11. 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. 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.