

Iron Stain Kit

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Intended Use

For In Vitro Diagnostic Use

Summary and Explanation

The Iron Stain Kit is intended for use in the detection of ferric iron in tissues, blood smears, or bone marrow smears. Ferric iron is normally found in small amounts in bone marrow and the spleen. Abnormally large deposits may be seen in hemochromatosis and hemosiderosis. This product is based on the Prussian Blue reaction in which ionic iron reacts with acid ferrocyanide producing a blue color.

Tissue Sections

Iron: Bright Blue

Nuclei: Red

Background: Pink

Bone or Blood Smears

Sideroblasts: These are nucleated erythrocytes containing at least one small blue granule. If the blue granules surround the nucleus, the cell is a ringed sideroblast.

Siderocytes: These are non-nucleated erythrocytes containing at least one blue granule.

Reticuloendothelial Iron: Usually seen as blue particles on the marrow smear or as blue particles in the cytoplasm or phagocytic cells.

Control Tissue

Tissues fixed in 10% formalin are suitable for use prior to paraffin embedding. Consult references (Kiernan, 1981; Sheehan & Hrapchak, 1980) for further details on specimen preparation.

1. Cut sections, usually 3 to 5 μ m and pick the sections up on glass slides.
2. Bake the slides for at least 30 minutes at approximately 70°C.
3. Allow to cool.

Recommended Positive Control

1. Spleen
2. Bone Marrow

Reagents Provided

Kit Contents	Volume	Storage
Potassium Ferrocyanide Solution	500 mL	15-30°C
Hydrochloric Acid Solution (2%)	500 mL	15-30°C
Nuclear Fast Red Solution	125 mL	15-30°C

Storage and Handling

Do not use product after the expiration date printed on vial. If reagents are stored under conditions other than those specified here, they must be verified by the user. Diluted reagents should be used promptly.

Note

1. Use acid-washed or bleach-washed glassware.
2. Rinse all glassware with distilled water prior to use.
3. Do not use metal forceps to transfer slide during staining procedure.

Staining Procedure

1. Deparaffinize sections if necessary and hydrate to distilled water.
2. Mix equal volumes of Potassium Ferrocyanide Solution and Hydrochloric Acid Solution to make a working Iron Stain Solution. Use once and discard.
3. Incubate slide in working Iron Stain Solution for 3 minutes.
4. Rinse thoroughly in distilled water.
5. Stain slide in Nuclear Fast Red Solution for 5 minutes.
6. Rinse in 4 changes of distilled water.
7. Dehydrate in 95% alcohol followed by absolute alcohol.
8. Clear, and mount in synthetic resin.

Limitations of the Procedure

1. Histological staining is a multiple step diagnostic process that requires specialized training in the selection of the appropriate reagents, tissue selections, fixation, processing, preparation of the slide, and interpretation of the staining results.
2. Tissue staining is dependent on the handling and processing of the tissue prior to staining.
3. Improper fixation, freezing, thawing, washing, drying, heating, sectioning, or contamination with other tissues or fluids may produce artifacts or false negative results.
4. The clinical interpretation of any positive staining, or its absence, must be evaluated within the context of clinical history, morphology and other histopathological criteria. It is the responsibility of a qualified pathologist to be familiar with the special stain and methods used to produce the slide.
5. Staining must be performed in a certified licensed laboratory under the supervision of a pathologist who is responsible for reviewing the stained slides and assuring the adequacy of positive and negative controls.

Precautions

1. Consult local and/or state authorities with regard to recommended method of disposal.
2. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions.
3. Avoid microbial contamination of reagents. Contamination could produce erroneous results.
4. This reagent may cause irritation. Avoid contact with eyes and mucous membranes.
5. If reagent contacts these areas, rinse with copious amounts of water.
6. Do not ingest or inhale any reagents.

Troubleshooting

If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem is suspected, contact Diagnostic BioSystems Technical Support at (925) 484-3350, extension 2 or techsupport@dbiosys.com.



References

- I. Sheenan, D.C., Hrapchak, B.B. Theory and Practice of Histotechnology, 2nd Edition. Battelle Press, Columbus, OH. Page 217. 1980.
- II. Clark, G., et al., Staining Procedures. 4th Edition. Williams & Wilkins. Page 202-203. 1981.
- III. Carson, F.L., Histotechnology; A Self-Instructional Text, ASCP Press, Chicago, IL. Pages 214-215. 1990.