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**INTENDED USE**

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Sigma-Aldrich Iron Stain is for use in the histologic staining of iron in blood or bone marrow films. Iron Stain reagents are for "In Vitro Diagnostic Use".

Iron Stain is based on the well known Prussian blue reaction<sup>1</sup> in which ionic iron reacts with acid ferrocyanide producing a blue color. Both the standard procedures and a procedure for rapid staining in the microwave<sup>2-4</sup> are described.

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**REAGENTS**

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**POTASSIUM FERROCYANIDE SOLUTION**, Catalog No. HT20-1  
Potassium ferrocyanide, 4% w/v.

**HYDROCHLORIC ACID SOLUTION**, Catalog No. HT20-2  
Hydrochloric acid, 1.2 mmol/l.

**PARAROSANILINE SOLUTION**, Catalog No. HT20-3  
Pararosaniline hydrochloride, 1% w/v, in methanol with a stabilizer.

**STORAGE AND STABILITY:**

Store reagents at room temperature (18–26°C). Reagents are stable until expiration date shown on the labels.

**PREPARATION:**

**WORKING IRON STAIN SOLUTION** is prepared by mixing equal volumes of Potassium Ferrocyanide Solution, Catalog No. HT20-1, and Hydrochloric Acid Solution, Catalog No. HT20-2. Use once and then discard.

**WORKING PARAROSANILINE SOLUTION** is prepared by adding 1 ml of Pararosaniline Solution, Catalog No. HT20-3, to 50 ml water. Prepare fresh daily. Use once and discard.

**PRECAUTIONS:**

Normal precautions exercised in handling laboratory reagents should be followed. Dispose of waste observing all local, state, provincial or national regulations. Refer to Material Safety Data Sheet for any updated risk, hazard or safety information.

Iron TISSUE-TROL control slides are paraffin embedded human tissue containing iron and should be considered potentially infectious.

**US Risks and Safety Statements**

**Caution:** Avoid contact and inhalation of Potassium Ferrocyanide Solution. Target organ: Blood.

Hydrochloric Acid Solution is TOXIC. Toxic by inhalation. Causes burns. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves, and eye/face protection. In case of accident of if you feel unwell, seek medical advice immediately (show the label where possible).

Pararosaniline Solution is FLAMMABLE and TOXIC. May cause cancer. Toxic by inhalation, in contact with skin and if swallowed. Toxic: danger of very serious irreversible effects through inhalation, in contact with skin and if swallowed. Irritating to eyes and skin. Keep container tightly closed. Avoid exposure - obtain special instructions before use. Keep away from sources of ignition - no smoking. Avoid contact with skin. Take precautionary measures against static discharges. Wear suitable protective clothing, gloves, and eye/face protection. In case of accident of if you feel unwell, seek medical advice immediately (show the label where possible).

Reagent Alcohol is FLAMMABLE and an IRRITANT. Irritating to eyes, respiratory system and skin. Keep container tightly closed. Keep away from sources of ignition - no smoking. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing. Target organs: Nerves and liver.

Methanol is FLAMMABLE and TOXIC. Toxic by inhalation, in contact with skin and if swallowed. Toxic: danger of very serious irreversible effects through inhalation, in contact with skin and if swallowed. Irritating to eyes and skin. Keep container tightly closed. Keep away from sources of ignition - no smoking. Take precautionary measures against static discharges. Avoid contact with skin. Wear suitable protective clothing and gloves. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

Xylene is FLAMMABLE and HARMFUL. Possible risk of impaired fertility. May cause harm to the unborn child. Harmful by inhalation and in contact with skin. Irritating to respiratory system and skin. Risk of serious damage to eyes. Keep away from sources of ignition - no smoking. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves and eye/face protection. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

**EU Risks and Safety Statements**

Pararosaniline Solution is HIGHLY FLAMMABLE and TOXIC. May cause cancer. Highly flammable. Toxic by inhalation, in contact with skin and if swallowed. Toxic: danger of very serious irreversible effects through inhalation, in contact with skin and if swallowed. Restricted to professional users. Attention - Avoid exposure - obtain special instructions before use. Keep away from sources of ignition - no smoking. Wear suitable protective clothing and gloves. In case of accident of if you feel unwell, seek medical advice immediately (show the label where possible).

Reagent Alcohol is HIGHLY FLAMMABLE and an IRRITANT. Highly Flammable. Irritating to eyes, respiratory system and skin. Keep container tightly closed. Keep away from sources of ignition - no smoking. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing.

Methanol is HIGHLY FLAMMABLE and TOXIC. Highly flammable. Toxic by inhalation, in contact with skin and if swallowed. Toxic: danger of very serious irreversible effects through inhalation, in contact with skin and if swallowed. Keep container tightly closed. Keep away from sources of ignition - no smoking. Wear suitable protective clothing and gloves. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

Xylene is HARMFUL. Flammable. Harmful by inhalation and in contact with skin. Irritating to skin. Avoid contact with eyes.

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**PROCEDURE:**

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**SPECIMEN COLLECTION:**

It is recommended that specimen collection be carried out in accordance with NCCLS document M29-A2. No known test method can offer complete assurance that blood samples or tissue will not transmit infection. Therefore, all blood derivatives or tissue specimens should be considered potentially infectious.

Any well prepared tissue section cut 5–6 microns and fixed in neutral buffered formalin is satisfactory for iron staining. Blood or bone marrow films are prepared in the usual fashion, air dried at least 30 minutes and fixed in absolute methanol for 7 minutes.<sup>5</sup>

**SPECIAL MATERIALS REQUIRED BUT NOT PROVIDED:**

Positive control slides, such as Sigma Iron Tissue-Trol™, Catalog No. I 3387, should be included in each run

Ethanol, absolute, or Reagent Alcohol

Methanol, Acetone Free, (NOTE: required for blood/bone marrow films only)

**FOR MICROWAVE PROCEDURE ONLY:**

ACCUMATE™ H2100 Microwave Oven, Catalog Nos. A 9084 (110 v), or A 9209 (220 v) Staining Jars, Catalog No. S 5641

**NOTES:**

If the Sigma-Aldrich H2100 Microwave Oven is used, please see the Owner's Manual for instructions.

Iron deposits may be removed by the use of acid containing fixatives.<sup>7</sup>

The data obtained from this procedure serves only as an aid to diagnosis and should be reviewed in conjunction with other clinical diagnostic tests or information.

**PROCEDURE:****PROCEDURE FOR TISSUE SECTIONS:**

1. Deparaffinize and hydrate tissue to deionized water.
2. Place slides in Working Iron Stain Solution for **10 minutes**.
3. Rinse in deionized water.
4. Stain in Working Pararosaniline Solution for **3–5 minutes**.
5. Rinse in deionized water.
6. Rapidly dehydrate through alcohol and xylene and mount.

**PROCEDURE FOR BLOOD OR BONE MARROW FILMS:**

1. Place fixed films in Working Iron Staining Solution for **10 minutes**.
2. Rinse in deionized water.
3. Counterstain **5 minutes** in Working Pararosaniline Solution.
4. Rinse in deionized water and air dry.

**ACCUMATE™ H2100 MICROWAVE PROCEDURE:**

1. Deparaffinize slides and hydrate to deionized water. If staining peripheral blood or bone marrow smears fix in methanol for **7 minutes**, then hydrate to deionized water.
2. Place slides in **40 ml** of Working Iron Staining Solution contained in plastic Coplin jar. Loosely cover with lid before placing in oven, or use Coplin jar lids with holes drilled into them.
3. Microwave on **400 watts** for **30 seconds**. Discard solution after use.
4. Rinse in deionized water.
5. Place slides in **40 ml** of Working Pararosaniline Solution contained in plastic Coplin jar.
6. Microwave on **800 watts** for **10 seconds**. Let incubate for **2 minutes**. Use once and discard.
7. Rinse in tissue sections in deionized water, dehydrate quickly through alcohols, clear in xylene and mount. Blood or bone marrow smears should be rinsed in deionized water and air dried.

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**PERFORMANCE CHARACTERISTICS**

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**TISSUE SECTIONS**

Iron Pigment — Bright Blue  
Nuclei — Red  
Cytoplasm — Light Pink

NOTE: Heavy deposits appear dark blue.

**BLOOD OR BONE MARROW FILMS**

**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 film or as blue particles in the cytoplasm or phagocytic cells.

If observed results vary from expected results, please contact Sigma-Aldrich Technical Service for assistance.

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## REFERENCES

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1. Theory and Practice of Histotechnology, 2nd ed. DC Sheehan, BB Hrapchak, Editors, CV Mosby, St. Louis (MO) 1980 p 217
2. Leong AS-Y, Milios J: Rapid immunoperoxidase staining of lymphocyte antigens using microwave irradiation. J Pathol 148:183, 1986
3. Brinn NT: Rapid metallic histologic staining using the microwave oven. J Histotechnol 6:125, 1983
4. Valle S: Special stains in the microwave oven. J Histotechnol 9:237, 1986
5. Carson: A Self Instructional Text. ASCP Press, Chicago (IL), 1990
6. Atlas of Cytochemistry and Immunocytochemistry of Hematologic Neoplasms. T Sun, C-Y Li, LT Yam, Editors, ASCP, Chicago (IL), 1985, p 205
7. Theory and Practice of Histological Techniques, 5th Edition JD Bancroft, M Gamble, Editors Churchill Livingstone, New York 2002 p 244

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