**INTENDED USE**
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 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 are described.

**REAGENTS**

**POTASSIUM FERROCYANIDE SOLUTION**, Catalog No. HT201-250 ml
Potassium ferrocyanide, 4% w/v.

**HYDROCHLORIC ACID SOLUTION**, Catalog No. HT202-250 ml
Hydrochloric acid, 1.2 mmol/l.

**PARAROSANILINE SOLUTION**, Catalog No. HT203-30 ml
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 and Hydrochloric Acid Solution. Use once and then discard.

Working Pararosaniline Solution is prepared by adding 1 ml of Pararosaniline Solution 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 and product labeling 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.

**PROCEDURE**

**PROCEDURE FOR TISSUE SECTIONS:**

1. Deparaffinize and hydrate tissue to deionized water.
2. Place slides in Working Iron Staining Solution for 10 minutes.
3. Rinse in deionized water.
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.
4. Rinse in deionized water and air dry.

**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 dehydrate 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 tissue sections in deionized water, dehydrate quickly through alcohol, clear in xylene and mount. Blood or bone marrow smears should be rinsed in deionized water and air dried.

**PERFORMANCE CHARACTERISTICS**

<table>
<thead>
<tr>
<th>TISSUE SECTIONS</th>
<th>Iron Pigment</th>
<th>Nuclei</th>
<th>Cytoplasm</th>
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**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 of phagocytic cells.

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

**REFERENCES**

1. Theory and Practice of Histotechnology, 2nd ed. DC Sheehan, BB Harpach, Editors, CV Mosby, St. Louis (MO) 1980 p 217
6. Atlas of Cytochemistry and Immunochemistry of Hematologic Neoplasms. T Sun, C-Y Li, LT Yam, Editors, ASCP, Chicago (IL), 1985, p 205

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