

**OLIVE VIEW-UCLA MEDICAL CENTER
DEPARTMENT OF PATHOLOGY
POLICY & PROCEDURE**

**NUMBER: 11897
VERSION: 1**

SUBJECT/TITLE: HIS 13 – PRUSSIAN BLUE STAIN FOR FERRIC IRON

POLICY: The Prussian Blue stain shall be performed at the request of the pathologist.
PURPOSE: To detect ferric iron in patient tissue samples

DEPARTMENTS: PATHOLOGY & LABORATORY SERVICES

DEFINITIONS:

PROCEDURE: FIXATION

- 10% Neutral Buffered Formalin
- Methyl Alcohol (for smear samples)

EQUIPMENT
Coplin jars, graduated cylinders, plastic forceps.

SLIDE PREPARATIONS

- Section paraffin sections at 4-5microns.
- Fix smears for 10 minutes in Methyl Alcohol, drain and place into distilled water.

QUALITY CONTROL
Positive Know Control for Iron.
Note: Excessive amounts of iron are not desirable in the control, as the reaction product is slightly soluble and may contaminate the incubating solution, giving a background stain in all sections. Coplin jars that have been used for iron hematoxylin solutions and not adequately cleaned may also contaminate the staining solution.

REAGENTS

- **Ferrocyanide Working Solution**
2% Aqueous Potassium Ferrocyanide.....25 ml
(Commercially prepared from Poly Scientific: S1872-16 oz)
- 2% Aqueous Hydrochloric Acid.....25 ml
(Commercially prepared from Poly Scientific: S2112-16 oz)

Note: Solution must be filtered.

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- **Nuclear Fast Red (Kernechtrot)**
Commercially prepared from Poly Scientific: S248-16oz

PROCEDURE

1. Deparaffinize and hydrate sections to distilled water utilizing non-metallic forceps.
2. Place in the freshly prepared Ferrocyanide Working Solution for 20 minutes.
3. Remove the slides and place into distilled water.
4. Wash thoroughly in distilled water.
5. Counterstain with Nuclear Fast Red for 3-5 minutes
6. Rinse in running tap water for at least 1 minute.
7. Rinse twice in distilled water
8. Dehydrate in 2 changes of 95% alcohol and 2 changes of absolute alcohol.
9. Clear in three changes of Xylene.
10. Coverslip with a synthetic mounting medium.

RESULTS

Hemosiderin (iron)..... bright blue
Nuclei and hemofuchsin bright red
Background pink

NOTES

1. If the excess Nuclear-Fast Red counterstain is not adequately removed before beginning the dehydration process, cloudy slides will result. If this occurs, return the sections to running water and wash well, and then re dehydrate and clear the sections.
2. Iron may be dissolved by some of the acidic fixatives and decalcification solutions.

References: Histotechnology: A Self-Instructional Text – third edition; Freida L. Carson and Crista Hladik	
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