

## Iron - Prussian Blue Reaction - Mallory's Method

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**[Abstract]** Purpose: To demonstrate ferric iron in tissue sections. Small amounts of iron are found normally in spleen and bone marrow. Excessive amounts are present in hemochromatosis, with deposits found in the liver and pancreas, hemosiderosis, with deposits in the liver, spleen, and lymph nodes.

Principle: The reaction occurs with the treatment of sections in acid solutions of ferrocyanides. Any ferric ion (+3) in the tissue combines with the ferrocyanide and results in the formation of a bright blue pigment called 'Prussian blue' or ferric ferrocyanide.

### **Materials and Reagents**

1. Control: A known positive control tissue (such as spleen)
2. Formalin
3. EtOH
4. Hydrochloric acid
5. Aluminum sulfate
6. HistoClear reagent
7. Mounting solution
8. Fixative (see Recipes)
9. 5 % potassium Ferrocyanide (see Recipes)
10. 5% hydrochloric acid (see Recipes)
11. Nuclear-fast red (Kernechtrot) (see Recipes)
12. Working solution (see Recipes)

### **Equipment**

1. Microwave oven
2. Acid-cleaned glassware
3. Non-metallic forceps.
4. Gloves, goggles and lab coat

5. Fume hood

**Procedure**

Safety: Wear gloves, goggles and lab coat. Avoid contact and inhalation. Potassium ferrocyanide; Low toxicity as long as it is not heated, it will release cyanide gas.

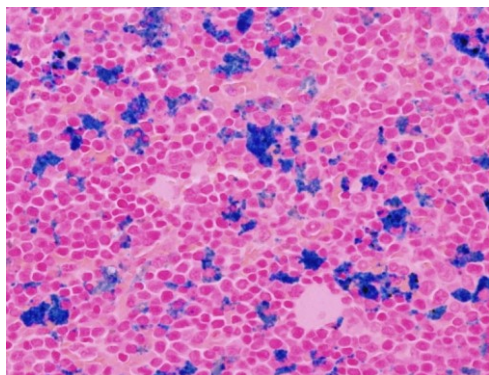
Hydrochloric acid; target organ effects on reproductive system and fetal tissue. Irritant to skin eyes and respiratory system.

1. Deparaffinize tissue slide and hydrate to distilled water (use HistoClear reagent 2x, 100% EtOH 2x, 95% EtOH 1x, 90% EtOH 1x, 80% EtOH 1x, 70% EtOH 1x, ddH<sub>2</sub>O 1x. leave 3-5 min in each solution).
2. Immediately transfer slides into working solution (avoid drying the tissue) and either use the microwave method (30 sec, then allow slides to stand in solution for 5 min in fume hood) or use the conventional method (room temperature for 30 min).
3. Rinse in distilled water.
4. Nuclear-fast red (incubate for 5 min at room temperature).
5. Wash in tap water (several washes).
6. Dehydrate, clear (reverse the order of step 1 above).
7. Add mounting solution and coverslip.
8. Results:
 

Iron (hemosiderin):	Blue
Nuclei:	Red
Background:	Pink

(Slide shows mouse spleen at 400x)

**Representative Data**



**Figure 1. Typical results from this experiment**

## Recipes

1. Fixative
  - 10% formalin
2. 5% potassium ferrocyanide
  - Potassium ferrocyanide 25.0 mg
  - Distilled water 500 ml
  - Mix well, pour into an acid-cleaned brown bottle
  - Stable for 6 months
  - Caution: Low toxicity if not heated.
3. 5% hydrochloric acid
  - Hydrochloric acid, conc. 25.0 ml
  - Distilled water 475.0 ml
  - Mix well, pour into brown bottle
  - Stable for 6 months
  - Caution: Corrosive, avoid contact and inhalation.
4. Nuclear-fast red (Kernechtrot)
  - Aluminum sulfate 25.0 mg
  - Distilled water 500 ml
  - Nuclear-fast red 0.5 mg
  - Dissolve the aluminum sulfate in distilled water, then the nuclearfast red, using heat. Cool, filter, and add a few grains of thymol as a preservative.
  - Label with date. Stable for 1 year
  - Caution: Irritant, avoid contact and inhalation
5. Working solution
  - 5% potassium ferrocyanide 25.0 ml
  - 5% hydrochloric acid 25.0 ml
  - Make fresh, discard after use
  - Caution: Avoid contact and inhalation.

## Acknowledgments

This protocol was adapted/modified from original versions as described in Luna (1968).

## **References**

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3. Crookham, J., Dapson, R. (1991). Hazardous chemicals in the histopathology laboratory. 2nd ed.; Anatech.