

Prussian Blue Reaction for the Demonstration of Iron

Catalog Number 24199

Please refer to the MSDS for chemical and safety information

Introduction

Prussian Blue or Perls' reaction is used to demonstrate ferric iron and ferritin. This is not a true staining technique rather, it is a histochemical reaction. The protein is split off by the hydrochloric acid, allowing the potassium ferrocyanide to combine with the ferric iron. This forms the ferric ferrocyanide or Prussian Blue.

Fixation

10% Neutral Buffered Formalin or alcohol can be used. Sections should be cut at 4 μ to 5 μ , or blood or bone marrow smears.

Deparaffinize

Slides should be deparaffinized through xylene or substitute to remove the paraffin from the section and descending grades of alcohol to water just prior to staining.

Staining Procedure: Routine Procedure for Room Temperature

1. Mix equal amounts of Solution A, 4% Potassium Ferrocyanide, Aqueous with Solution B, 4% Hydrochloric Acid, Aqueous, for the working solution. The standard room temperature staining will take 2 changes of the solution at 10 minutes each.
2. Rinse in several changes of distilled water.
3. Stain in Solution C, Nuclear Fast Red, for 2 to 5 minutes depending on the intensity of the counterstain required.
4. Rinse in running tap water for 1 minute. Dehydrate through ascending alcohols to xylene or substitute and coverslip with Poly Mount. The stain will be removed in the alcohols and should be dehydrated as quickly as possible.

Staining Procedure: Microwave Procedure

Step 1 should be done under a hood and the microwave must be vented or under the hood. The reaction of potassium ferrocyanide and acid (e.g. hydrochloric acid) will produce potentially dangerous hydrogen cyanide fumes! Larger amounts of stain can be made and heated by extending the times as indicated in the procedure. Heating in a Coplin jar will require 15 to 30 seconds to reach 60°C and 1 to 1 1/2 minutes for 250mL to reach 60°C in a larger staining dish on HIGH in a microwave or as directed by the manufacturer using a probe. Please calibrate your oven by checking this temperature with room temperature distilled water for accurate control.

1. Mix equal amounts of Solution A, 4% Potassium Ferrocyanide, Aqueous with Solution B, 4% Hydrochloric Acid, Aqueous, for the working solution. Heat solution in the microwave for 15 to 30 seconds in a Coplin jar or 1 to 1 1/2 minutes in a 250mL staining dish. Solution can be changed and reheated if the stain requires more intensity.
2. Rinse in several changes of distilled water.
3. Stain for 2 to 5 minutes in Solution C, Nuclear Fast Red. Do not heat the stain as it will precipitate.
4. Rinse in running tap water for 1 minute. Dehydrate through ascending alcohols to xylene or substitute and coverslip with Poly Mount. The stain will be removed in the alcohols and should be dehydrated as quickly as possible.

Results

Hemosiderin, some oxides and salts of iron bright blue.
Nuclei and cytoplasm pink to red

Ordering Information:

Cat. #	Description	Size
24199	Prussian Blue Iron Stain Kit Each kit contains: Solution A - 4% Potassium Ferrocyanide Aqueous Solution B - 4% Hydrochloric Acid Aqueous Solution C - 1% Nuclear Fast Red Aqueous	250ml 250ml 250ml
08381	Poly-Mount	120ml 940ml

To Order:

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In The U.S. FAX: 1-800-343-3291 • 215-343-0214

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