Prussian Blue Staining Kit (Nuclear Fast Red Counterstain Method)

Catalog No.: RA20122

Basic Information

Product name	Prussian Blue Staining Kit (Nuclear Fast Red Counterstain Method)
Sizes	50 mL
Storage	RT
Shipping	RT
Validity	12 months

Product Introduction

Hemosiderin is a hemoglobin-derived pigment that appears as golden or brownish-yellow granules. Due to its iron content and golden color, it is referred to as hemosiderin. When red blood cells are phagocytosed by macrophages, hemoglobin is broken down into iron-free orange chromogen and iron-containing hemosiderin under the action of lysosomal enzymes. The Prussian blue reaction, also known as hemosiderin staining, produces a blue color after treatment with potassium ferrocyanide and dilute acid. It is commonly observed in phagocytes or interstitial tissues. The staining principle is that potassium ferrocyanide solution, in the presence of dilute hydrochloric acid, dissociates trivalent iron from proteins. The trivalent iron then reacts with potassium ferrocyanide to form an insoluble blue compound—ferric ferrocyanide.

EnkiLife Prussian Blue Staining Solution (Nuclear Fast Red Counterstain Method) is used to visualize various hemorrhagic lesions in local tissues, commonly seen in phagocytes. It effectively distinguishes hemosiderin from other pigments. This staining solution offers good stability, long-term storage capability, minimal precipitation, and a wide range of applications. After the reaction, a red counterstain such as nuclear fast red, eosin, or neutral red may be used. Nuclear fast red is used as the counterstain in this kit, representing the most classic and commonly used method.

Product Components

Components		2x 50mL
Reagent (A): Perls Stain	A1: Perls Stain A	25 mL
	A2: Perls Stain B	25 mL

Prussian Blue Staining Kit (Nuclear Fast Red Counterstain Method)

Catalog No.: RA20122

Mix equal parts of A1 and A2 immediately before use. Do not prepare in advance.	
Reagent (B): Nuclear Fast Red Staining Solution	50 mL

Materials Required (Not Supplied)

1. Fixative: 10% neutral formalin, 4% paraformaldehyde, etc.
2. Graded ethanol series, xylene or dewaxing and clearing agent, neutral balsam, 5% oxalic acid.

Experimental procedure

(I) Paraffin Section Staining

1. Fix tissue in 10% neutral formalin, then perform routine dehydration and paraffin embedding.
2. Cut 4 μm thick sections. Deparaffinize using xylene or dewaxing agent, then rinse with distilled water for 1 min.
3. Immerse sections in Perls Stain (see Note 2) for 15–30 min.
4. Rinse thoroughly with distilled water for 2–5 min.
5. Counterstain nuclei lightly with Nuclear Fast Red Staining Solution for 5–10 min, then rinse with tap water for 1–5 s.
6. Dehydrate through graded ethanol, clear with xylene or clearing agent, and mount with neutral balsam.

(II) Frozen Section Staining

1. No dewaxing needed. Rinse quickly with distilled water for 2–3 min.
2. Follow the same staining and mounting steps as for paraffin sections, but reduce the incubation time accordingly.

(III) Cell Staining

1. Fix cells with 4% paraformaldehyde for 10–20 min.
2. Rinse with tap water twice, 2 min each.
3. Rinse with distilled water twice, 2 min each.
4. Follow the same staining and mounting steps as for paraffin sections, but extend the incubation time as needed.

Prussian Blue Staining Kit (Nuclear Fast Red Counterstain Method)

Catalog No.: RA20122

Staining Results

Component	Color
Hemosiderin or trivalent iron	Blue
Nuclei and other tissues	Red

Negative Control (Optional)

Take consecutive sections, dewax and hydrate to water; incubate in 5% oxalic acid for 2−6 h, then proceed with Perls Stain as above. The result should be negative.

Notes

1. Dewax sections thoroughly. Tissue fixation is typically done with 10% neutral formalin. Prolonged fixation with regular formalin may damage tissue. Avoid acidic fixatives; chromate treatment may also interfere with iron preservation.
2. Use clean containers throughout the procedure. Avoid iron-containing metal tools. Use distilled water for rinsing slides and containers, as tap water may contain iron. Adjust staining time with Perls Stain based on sample conditions.
3. Use the same positive control slide for all sections. Choosing an appropriate control is essential. Autopsy lung tissue is a good control, as it contains a significant number of iron-positive macrophages (heart failure cells).
4. For frozen sections and cell staining, optimize experimental conditions based on specific situations.
5. Use reagents promptly after opening to maintain optimal performance.
6. For your safety and health, wear a lab coat and disposable gloves during operation.

This product is for research use only!