

## Prussian Blue Staining Kit (Nuclear Fast Red)

**P774831**

**Store at room temperature and store in the dark.**

### Introduction:

Hemosiderin is a hemoglobin-derived pigment. When tissue hemorrhage occurs, red blood cells extravasated from blood vessels are phagocytosed by macrophages and degraded by their lysosomes. Hemoglobin is decomposed into iron-free orange hematoidin and iron-containing hemosiderin. Perls' Prussian blue reaction, also known as hemosiderin staining, produces a blue color after treatment with potassium ferrocyanide and dilute acid. This reaction is commonly observed within phagocytes or the interstitium. The staining principle lies in that the potassium ferrocyanide solution allows ferric ions ( $Fe^{3+}$ ) to be separated from proteins by dilute hydrochloric acid. Ferric ions then react with potassium ferrocyanide to form an insoluble blue compound—ferric ferrocyanide.

Prussian blue staining is used to visualize various hemorrhagic lesions in local tissues, typically within phagocytes. It effectively differentiates hemosiderin from other pigments. The staining solution features good stability, rarely forms precipitates, has a wide application range, and allows counterstaining. Nuclear fast red, the most classic and commonly used counterstain, is employed for this staining solution.

### Component

Component	2×50 mL	2×100mL	Storage
Perls Staining Solution A	25mL	50mL	RT.
Perls Staining Solution B	25mL	50mL	RT.
Nuclear Fast Red Staining Solution	50mL	100mL	RT. Store in the dark

### Usage method:

Before use, mix equal amounts of Perls Staining Solution A and Perls Staining Solution B to prepare the Perls staining solution. It is not recommended to prepare it in advance.

#### (1) Paraffin Section Staining

- a) Fix tissues in 10% neutral formalin and perform conventional dehydration and embedding.
- b) Cut sections at 4  $\mu$ m thickness and dewax them to water using standard procedures.
- c) Rinse sections with distilled water for 1 minute.
- d) Immerse sections in Perls staining solution (see Note 2) and incubate for 15–30 minutes.
- e) Thoroughly rinse with distilled water for 2–5 minutes.

- f) Transfer sections to nuclear fast red staining solution and lightly stain nuclei for 5–10 minutes.
- g) Rinse with distilled water for 1–5 seconds.
- h) Dehydrate and clear the sections conventionally, then mount with neutral gum.

## (2) Frozen Section Staining

- a) Skip dewaxing; directly rinse sections rapidly with distilled water for 2–3 minutes.
- b) Staining and mounting steps are the same as those for paraffin sections, though times may be correspondingly reduced.

## (3) Cell Staining

- a) Fix cells with 4% paraformaldehyde for 10–20 minutes.
- b) Rinse twice with tap water, 2 minutes each time.
- c) Rinse twice with distilled water, 2 minutes each time.
- d) Staining and mounting steps are the same as those for paraffin sections, but processing times should be correspondingly extended.

### Negative Control

Dewax identical sections to water. Incubate in 5% oxalic acid for 2–6 hours, then perform Perls staining. Follow the remaining steps as above. The result should be negative.

Result:

Hemosiderin or ferric iron ( $\text{Fe}^{3+}$ ): Blue-violet

Nucleus, other tissues: Red

### **Matters needing attention:**

1. Dewaxing of sections should be as thorough as possible. Tissues are usually fixed with 10% neutral formalin, as long-term fixation with ordinary formalin may cause tissue damage. Acidic fixatives should be avoided, and chromate treatment can also interfere with iron preservation.
2. Containers must be clean throughout the entire operation. Avoid using metal iron products, and rinse sections and containers with distilled water, as ordinary water contains iron. When performing Perls staining, the staining time should be adjusted according to the characteristics of the sample.
3. All sections should use the same positive control section, and selecting a suitable control is crucial. Autopsy lung tissue is an excellent control, as it contains a substantial number of iron-positive macrophages (heart failure cells).
4. For frozen sections and cell staining, it is advisable to optimize experimental conditions based on specific circumstances.