Prussian Blue Staining Kit (Nuclear Fast Red Method)

Cat #: A-CSH296

Size: 2×50mL, 2×100mL

Storage: Room temperature, avoid light (12 months)

Product Composition

Component		2×50mL	2×100mL	Store at
Reagent (A): Perls staining solution	A1: Perls staining solution A	25ml	50ml	10~30°C
	A2: Perls staining solution B	25ml	50mL	10~30°C
Before use, take the equal amount of A1 and A2 as Perls staining solution, which should not be prepared in advance.				
Reagent (B): Nuclear solid staining solution		50ml	100ml	10~30°C, and were protected from light

Product Description

Hemosiderin is an iron-containing pigment derived from hemoglobin. When there is bleeding in the tissue, red blood cells that escape from the blood vessels are engulfed by macrophages and degraded in their lysosomes. Hemoglobin is broken down into non-iron orange-colored hemosiderin and iron-containing hemosiderin. The Prussian blue reaction, also known as Prussian blue staining, is a method to detect hemosiderin. It involves treating the tissue with potassium ferrocyanide and dilute acid, resulting in the formation of a blue color. This staining is commonly observed in macrophages or the stroma and is based on the principle that the potassium ferrocyanide solution separates the trivalent iron ions from the protein, which then react with potassium ferrocyanide to form an insoluble blue compound called ferric ferrocyanide.

Prussian blue staining is used to visualize various hemorrhagic lesions within local tissues, commonly observed in macrophages, and can effectively differentiate hemosiderin from other pigments. The staining solution has good stability, does not easily produce precipitates, and has a wide range of applications. It can be restained. The restaining

solution for this staining method uses nuclear fast red, which is the most classic and commonly used restaining solution.

Usage

(I) Paraffin section staining:

1. Fix the tissue in 10% neutral formalin and perform routine dehydration and embedding.

2. Cut sections with a thickness of 4μm and dewax them in water.

3. Wash the sections with distilled water for 1 minute.

4. Place the sections in the Perls staining solution (see note 2) and incubate for 15-30 minutes.

5. Thoroughly rinse the sections with distilled water for 2-5 minutes.

6. Stain the sections with nuclear fast red for 5-10 minutes to lightly stain the cell nuclei.

7. Wash the sections with distilled water for 1-5 seconds.

8. Perform routine dehydration, clearing, and seal with neutral gum.

(II) Frozen section staining:

1. No dewaxing is required. Rinse the sections quickly with distilled water for 2-3 minutes.

2. Follow the staining and sealing steps for paraffin sections, with corresponding shorter times.

(III) Cell staining:

1. Fix the cells with 4% paraformaldehyde for 10-20 minutes.

2. Rinse twice with tap water for 2 minutes each time.

3. Wash twice with distilled water for 2 minutes each time.

4. Follow the staining and sealing steps for paraffin sections, but extend the operation time accordingly.

Negative control:

Dewax the same sections in water. Incubate in 5% oxalic acid for 2-6 hours, followed by Perls stain. Follow the remaining steps as mentioned above. The result should be negative.

Staining Results

Presiderin or triiron	blue
Nuclei, and other tissues	red

Notes

1. The dewaxing of sections should be as clean as possible. Tissue fixation is commonly done using 10% neutral formalin. Prolonged fixation with regular formalin can damage the tissue. Avoid using acidic fixatives, as well as chromate treatment, as they can hinder iron preservation.

2. The containers used throughout the entire process should be clean, and metal iron products should be avoided. When washing sections and containers, use distilled water, as regular water contains iron. When staining with Perls staining solution, adjust the staining time according to the sample conditions.

3. It is important to use the same positive control section for all sections. Choosing a suitable control is crucial. Autopsy lung tissue is a good control, as it contains a significant number of iron-positive macrophages (heart failure cells).

4. For frozen section staining and cell staining, it is best to experiment with specific conditions based on the circumstances.

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.