Prussian Blue Staining Solution (Neutral Red Method)

Cat #: A-CSH311

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, the equal amount of A1A2 is Perls staining solution, which should not be prepared in advance.				
Reagent (B): neutral red staining solution		50ml	100ml	10~30°C, and were protected from light

Product Description

Hemosiderin is a pigment derived from hemoglobin and appears as golden-yellow or brown-yellow granules. It is called hemosiderin because of its iron content and golden color. When red blood cells are engulfed by macrophages, hemoglobin is broken down by lysosomal enzymes into non-iron-containing orange-colored hematoidin and iron-containing hemosiderin. The Perls' Prussian blue reaction, also known as hemosiderin staining, involves the production of a blue color after treatment with potassium ferrocyanide and dilute acid. It is commonly observed in phagocytes or in the interstitium. The staining principle is that the potassium ferrocyanide solution separates trivalent iron ions from proteins, and these ions react with potassium ferrocyanide to form an insoluble blue compound, ferric ferrocyanide.

Prussian blue staining is used to demonstrate various hemorrhagic lesions within local tissues, commonly found in phagocytes. It can effectively differentiate hemosiderin from other pigments. The staining solution has good stability,

can be stored for a long time, does not easily produce precipitates, and has a wide range of applications. It can be restained, and the restaining solution uses neutral red.

Usage

(1) 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 through routine procedures.
3. Rinse the sections with distilled water for 1 minute.
4. Immerse the sections in Perls stain (see notes 2) for 15-30 minutes.
5. Thoroughly rinse the sections with distilled water for 2-5 minutes.
6. Stain the cell nuclei lightly with neutral red staining solution for 5-10 minutes.
7. Rinse with tap water for 1-5 seconds.
8. Perform routine dehydration, clearing, and seal with neutral mounting medium.

(2) Frozen section staining:

1. No dewaxing is required. Rinse quickly with distilled water for 2-3 minutes.
2. Stain the sections. Perform the sealing step as in paraffin section staining, but the time can be shortened accordingly.

(3) Cell staining:

1. Fix the cells in 4% paraformaldehyde for 10-20 minutes.
2. Rinse twice with tap water for 2 minutes each.
3. Rinse twice with distilled water for 2 minutes each.
4. Stain the cells. Perform the sealing step as in paraffin section staining, but the operating time should be extended accordingly.

Negative control (optional):

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

Staining Results

Presiderin or triiron	blue
Nuclei, and other tissues	red

Notes

1. Dewaxing of sections should be done as thoroughly as possible. Tissue fixation is commonly done with 10% neutral formalin, but long-term fixation with regular formalin can cause tissue damage. Avoid using acidic fixatives, as chromic acid treatment can also interfere with iron preservation.

2. Throughout the entire procedure, containers should be kept clean, and it is advisable to avoid using metal iron products. When washing sections and containers, distilled water should be used to avoid the presence of iron in regular water. During Perls stain staining, the coloring time should be adjusted according to the sample conditions.

3. All sections should be accompanied by the same positive control section, and selecting an appropriate 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 particular circumstances.

Disclaimer

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