Prussian Blue Staining Kit (Eosin)

P774832

Store at room temperature and store in the dark.

Introduction:

Hemosiderin is a hemoglobin-derived pigment composed of golden yellow or brownish-yellow granules. It is named "hemosiderin" due to its iron content and golden yellow color. When red blood cells are phagocytosed by macrophages, hemoglobin is decomposed by lysosomal enzymes into orange hematoidin (iron-free) and hemosiderin (iron-containing). 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 involves separating ferric ions (Fe^{3+}) from proteins using dilute hydrochloric acid in a potassium ferrocyanide solution. The ferric ions then react with 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 offers excellent stability, can be stored long-term, rarely forms precipitates, and has a wide range of applications. Eosin is used as the counterstain for this staining solution.

Usage method:

(1) Paraffin Section Staining

a) Fix tissues in 10% neutral formalin and perform conventional dehydration and embedding.
b) Cut sections at 4 μm 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 20–30 minutes.
e) Thoroughly rinse with distilled water for 5–10 minutes.
f) Transfer sections to eosin staining solution and lightly stain nuclei for 15–30 seconds.
g) Rinse with tap 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, dewaxing, clearing, and mounting steps are the same as those for paraffin sections.

(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, dewaxing, clearing, and mounting steps are the same as those for paraffin sections.

Result:
Hemosiderin or ferric iron (Fe^{3+}): Blue-violet
Nucleus, other tissues: Red

Matters needing attention:

1. Dewaxing of sections should be as thorough as possible. Tissues are typically fixed with 10% neutral formalin, as long-term fixation with ordinary formalin may cause tissue damage. Avoid using acidic fixatives, 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. For frozen sections and cell staining, it is advisable to optimize experimental conditions based on specific circumstances.