Hematoxylin and Eosin (H&E) Staining Kit, Aqueous

Catalog No.: RA20141

Basic Information

Product name	Hematoxylin and Eosin (H&E) Staining Kit, Aqueous
Sizes	100 mL, 500 mL
Storage	RT
Shipping	RT
Validity	12 months

Product Introduction

Hematoxylin and eosin (H&E) staining is the most fundamental staining method in routine histopathology, widely used in pathology, teaching, and scientific research. Hematoxylin is a natural alkaline dye extracted from logwood (Haematoxylon campechianum), appearing as light yellow-brown crystals. After oxidation, it forms hematein, which, together with a mordant (commonly trivalent aluminum or iron salts), stains cell nuclei. H&E staining is used to observe the morphological structure of normal and pathological tissues, and to identify abnormal substances or specific components in cells and tissues. Special staining, enzyme histochemistry, and immunohistochemistry are all performed based on the observation of H&E-stained sections. In H&E-stained sections, nuclei appear blue and cytoplasm appears red, providing a clear contrast for easy observation and analysis.

EnkiLife Hematoxylin and Eosin Staining Solution uses high-purity imported hematoxylin and oxidizing agents, free of harmful substances such as mercuric oxide and methanol. It provides excellent nuclear staining with minimal precipitation and metal film formation. It is widely applicable to human, animal, veterinary, and aquatic samples, and can be used for paraffin-embedded sections, frozen sections, and cytological smears. Both hematoxylin and eosin solutions can be reused.

Staining Principle

1. Nuclear staining principle: Hematoxylin is a natural basic dye that stains cell nuclei. The main component of chromatin in the nucleus is DNA. In the DNA double helix, the phosphate groups on the two nucleotide chains face outward, giving the outer surface of the DNA a negative charge and an acidic character. This allows it to easily bind with positively charged basic dyes such as hematoxylin via ionic or hydrogen bonds. Hematoxylin appears blue in alkaline solution, so the nucleus is stained blue.

2. Cytoplasmic staining principle: Eosin is a synthetic acid dye that stains cytoplasm under certain conditions. The main component of cytoplasm is protein, which is an amphoteric compound. The staining of cytoplasm is closely related to the pH of the staining solution. When the pH of the staining solution is below the isoelectric point of cytoplasmic proteins (4.7–5.0), the proteins ionize in a basic form, giving the cytoplasm a positive charge, allowing it to be stained by negatively charged acid dyes. Eosin dissociates in water to form negatively charged anions, which bind to positively charged cytoplasmic proteins, staining the cytoplasm red.
3. Differentiation: After staining, certain solutions are used to remove excess dye bound to the tissue, a process known as differentiation. The solution used is called a differentiating solution. In H&E staining, 0.5–1% hydrochloric acid in ethanol is commonly used as the differentiating solution. The acid destroys the quinoid structure of hematoxylin, causing the dye to dissociate from the tissue and fade. After hematoxylin staining, most tissues must be differentiated with hydrochloric acid ethanol to remove excess hematoxylin from the nuclei and cytoplasm before eosin staining, ensuring clear contrast between nuclear and cytoplasmic staining.
4. Bluing: After differentiation, hematoxylin exists in a red ionic state under acidic conditions. Under alkaline conditions, it exists in a blue ionic state. After differentiation with acidic ethanol, tissue sections appear red or pink. Washing with water removes the acid and stops differentiation. Weakly alkaline water (or bluing solution) is then used to convert the hematoxylin-stained nuclei to blue. This process is called bluing or blue conversion. Washing with tap water (especially warm water) can also achieve bluing, but takes longer.

Product Components

Components	2x 100mL	2x 500mL
Reagent (A): Hematoxylin Staining Solution	100 mL	500 mL
Reagent (B): Eosin Staining Solution (Aqueous)	100 mL	500 mL

Materials Required (Not Supplied)

1. Xylene or dewaxing/transparent reagent, hydrochloric acid ethanol differentiating solution, graded ethanol series, tap or distilled water.
2. Bluing solution (dilute ammonia water, lithium carbonate solution, etc.), ether-ethanol fixative, 4% paraformaldehyde, neutral balsam.

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Experimental procedure

(I) Paraffin Section Staining

1. Dewaxing and rehydration
   ① Xylene or dewaxing/transparent reagent, 2 changes, 5–10 min each.
   ② (Optional) Absolute ethanol, 2 changes, 3–5 min each.
   ③ 95% ethanol: 3–5 min.
   ④ 90% ethanol: 3–5 min.
   ⑤ 80% ethanol: 3–5 min.
   ⑥ Rinse with tap or distilled water (warm water at 30–40 °C may be used): 1–3 min.
2. Staining
   ① Stain with hematoxylin staining solution: 3–8 min.
   ② Rinse with tap or distilled water: 5–10 s.
   ③ (Optional) Differentiate with hydrochloric acid ethanol: 2–5 s.
   ④ Rinse with tap water: 20–30 s.
   ⑤ Blue with bluing solution or warm water: 20–40 s.
   ⑥ Rinse with tap water: 30–60 s.
   ⑦ Stain with eosin staining solution (aqueous): 20–120 s.
   ⑧ Rinse with tap water: 30–60 s.
3. Dehydration, clearing and mounting
   ① 80% ethanol: 10–20 s.
   ② 90% ethanol: 10–20 s.
   ③ 95% ethanol, 2 changes, 1–2 min each.
   ④ Absolute ethanol, 2 changes, 2–3 min each.
   ⑤ Clear with xylene or dewaxing/transparent reagent, 3 changes, 2–3 min each.
   ⑥ Mount with neutral balsam.

(II) Frozen Section Staining

1. Fix with ether-ethanol mixed fixative: 5–10 s.
2. Rinse with tap water: 2–5 s.
3. Drop hematoxylin staining solution and stain for 1–5 min (may be heated to 50 °C).
4. Rinse with tap water: 2–5 s.
5. (Optional) Differentiate with hydrochloric acid ethanol: 2–5 s.
6. Rinse with tap water: 2–5 s.
7. Blue with bluing solution or warm water: 2–5 s.

8. Rinse with tap water: 5–10 s.
9. Stain with eosin staining solution (aqueous): 2–20 s.
10. Rinse with tap water: 5–10 s.
11. 80% ethanol: 1–2 s.
12. 95% ethanol: 1–2 s.
13. Absolute ethanol: 2–5 s.
14. Phenol-xylene (1:3): 2–5 s.
15. Clear with xylene or dewaxing/transparent reagent, 3 changes, 2–5 s each.
16. Mount with neutral balsam.

(III) Cell Smear Staining

1. Fix 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. Proceed with staining, dehydration, clearing and mounting as for paraffin sections, but shorten incubation times accordingly.

Staining Results

Component	Color
Nuclei	Blue
Keratin, red blood cells	Bright orange-red
Cytoplasm, muscle fibers, collagen fibers, thyroid colloid	Various shades of red

Notes

1. Dewax sections thoroughly; if temperature is low, incubate at 60–70 °C in an oven.
2. Replace graded ethanol solutions regularly.
3. Differentiation time with hydrochloric acid ethanol should be adjusted according to section thickness, tissue type and age; ensure adequate washing with tap water after differentiation to remove all acid.
4. Ether-ethanol mixed fixative is prepared by mixing equal volumes of ether and 95% ethanol, adding a small amount of acetic acid, and storing in a sealed container.
5. Keep staining times as short as possible for frozen sections.

6. Common bluing solutions include 0.2–1% ammonia water, Scott’ s tap water substitute, or 0.1–1% lithium carbonate solution.
7. For your safety and health, wear a lab coat and disposable gloves during operation.

This product is for research use only!