Hematoxylin and Eosin Staining Kit

Introduction

Hematoxylin and Eosin Staining, also known as H&E staining, is one of the most widely used staining technique in histopathology. Hematoxylin is a kind of natural dye extracted from the logwood tree. When oxidized, hematoxylin forms the hematein, which is the actual dye used in H&E staining. Hematein can form colored complexes with mordant (mainly iron or aluminum salts). The colored complexes are positively charged and easily bind to the anionic (negatively charged) nuclei, causing the nuclei to be blue in color.

Eosin is an acid dye that binds to alkaline or basic structures, mainly proteins in the cell cytoplasm, staining the cytoplasm vary shades of red and pink.

Hematoxylin staining gives intense blue nuclei providing optimal contrast to the eosin stained cytoplasm, which can readily differentiate cellular components. H&E staining is widely used in many areas, such as paraffin section, frozen section and smear staining. Reagents in this kit can be used directly.

Components and Storage

Components	K1142-100 mL	K1142-500 mL
Hematoxylin Stain Solution	100 mL	500 mL
Eosin Staining Solution	100 mL	500 mL
Store the components at room temperature protecting from light. Stable for at least 1 year.		

Protocol

1. Sample pretreatment

1) Paraffin section

① Soak the sections in the xylene for 2 times (5-10 min/per time) to remove the wax

② Absolute ethanol treatment for 2 times (5 min/per time)

③ 90% ethanol treatment for 3 min

④ 80% ethanol treatment for 3 min

⑤ 70% ethanol treatment for 3 min

⑥ Rinse with tap water or distilled water for 2 min

2) Frozen section

① Fixative treatment for more than 10 min

② Rinse with distilled water for 2 min

3) Cell smear

① 4% paraformaldehyde fixation for 10-20 min

② Rinse with tap water for 2 min

③ Rinse with distilled water for 2 min

2. Sample staining

1) Hematoxylin stain solution treatment for 3-8 min

*Note: The time of hematoxylin staining will depend on the experimental needs.

2) Rinse with tap water for 5-10 s

3) (Optional step) Dip in acid alcohol for 2-5 s.

4) Rinse with tap water for 20-30 s

5) Dip in ammonia water for 20-40 s until the sections become blue

6) 80% ethanol treatment for 30-60 s

7) Eosin staining solution treatment for 30 s-3 min

*Note: Similarly, the time of eosin staining will depend on the experimental needs.

3. dehydration, clearing, sealing

1) 80% ethanol treatment for 10-20 s

2) 90% ethanol treatment for 10-20 s

3) 95% ethanol treatment for 2 times (1-2 min/per time)

4) Absolute ethanol treatment for 2 times (1-2 min/per time)

5) Dip in xylene for 3 times (2-3 min/per time)

6) Mount with neutral balsam mounting medium

4. Microscopy

Nuclei: Blue

Cytoplasm, muscle, collagen: Varying shades of pink

Red blood cells: Orange-red

Note

1. Paraffin sections should be dewaxed as clean as possible, and the ethanol solution used in the experiment should be changed frequently.
2. The staining time of frozen sections should be as short as possible.
3. The differentiation step after staining is optional, but the nuclei will be clearer after differentiation. The length of differentiation time depends on the condition of the section, and make ensure that the acid can be thoroughly washed off after differentiation.
4. The time for hematoxylin staining should not be too long, and excessive staining of hematoxylin will interfere the cytoplasmic staining results.
5. For bluing, ammonia water can be replaced by Scott's solution or 0.1-1% lithium carbonate solution.
6. For the first time using this kit, it is recommended to take 1-2 samples for preliminary test.
7. For your safety and health, please wear lab coats and gloves during the experiment.

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