aladdin®

Hematoxylin Staining Solution (Gill No.3)

G774772

Storage： Store at room template for 12 months, store in the dark.

Introduction:

Hematoxylin and Eosin staining, abbreviated as HE staining, is the most commonly used staining method in pathology and histology.

Hematoxylin is a basic natural dye that can stain cell nuclei. The main component of chromatin in the cell nucleus is DNA. In the double-helix structure of DNA, the phosphate groups on the two nucleotide chains face outward, making the outer side of the DNA double helix negatively charged and acidic. It easily combines with the positively charged basic hematoxylin dye through ionic bonds or hydrogen bonds, thus being stained.

Hematoxylin Staining Solution (Gill No. 3), also known as Gill Ⅲ Solution, is a semi-oxidized hematoxylin staining solution. Its hematoxylin concentration is twice that of Gill No. 1 Hematoxylin Staining Solution. It belongs to regressive staining, so hydrochloric acid-ethanol differentiation is required after staining. It is particularly suitable for staining paraffin sections, with the staining time for paraffin sections being longer than 15 minutes. It is rarely used for staining slides prepared for clinical diagnosis. A drawback of this staining solution is that even the adhered gelatin and even the glass slides themselves will be stained.

Principles of Staining

1. Principle of Cell Nucleus Staining:

Hematoxylin is a basic natural dye that can stain cell nuclei. The main component of chromatin in the nucleus is DNA. In the DNA double-helix structure, the phosphate groups on the two nucleotide chains face outward, making the outer side of the DNA double helix negatively charged and acidic. This allows it to easily bind to the positively charged basic hematoxylin dye via ionic bonds or hydrogen bonds, resulting in staining. Hematoxylin appears blue in alkaline solutions, so the cell nuclei are stained blue.

2. Principle of Cytoplasm Staining:

Eosin is a chemically synthesized acidic dye that can stain 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 value of the staining solution. When the pH of the staining solution is below the isoelectric point (4.7 – 5.0) of cytoplasmic proteins, the cytoplasmic proteins undergo basic ionization, making the cytoplasm positively charged. This enables it to be stained by negatively charged acidic dyes. Eosin dissociates in water into negatively charged anions, which bind to the positively charged cations of cytoplasmic proteins, staining the cytoplasm red.

3. Differentiation:

After staining, the process of removing excess bound dyes from tissues using specific solutions is called differentiation, and the solution used is called a differentiating solution. In HE staining, 1% hydrochloric acid-ethanol is commonly used as the differentiating solution. Acids can disrupt the quinoid structure of hematoxylin, causing the separation of tissues from the dye and thus decolorization. Most tissues, after hematoxylin staining, must undergo differentiation with 1% hydrochloric acid-ethanol to remove excess hematoxylin bound to cell nuclei and hematoxylin adsorbed by cytoplasm. Only after this step is eosin staining performed can clear distinction between nuclear and cytoplasmic staining be ensured.

4. Bluing (or Re-bluing):

After differentiation, hematoxylin exists in a red ionic state under acidic conditions, appearing red; under alkaline conditions, it exists in a blue ionic state, appearing blue. Tissue sections appear red or pink after differentiation with acidic ethanol. To terminate differentiation, the acid on the tissue sections is immediately removed with water. Then, weakly alkaline water is used to make the hematoxylin-stained cell nuclei appear blue — this process is called bluing or re-bluing. Additionally, rinsing with tap water can also achieve nuclear bluing, but it requires a longer time.

Usage method:

1. Operate according to the specific requirements of the experiment.
2. Hydrochloric acid-ethanol differentiation is required after staining.

Precautions:

1. Dewaxing of sections should be as thorough as possible.
2. Serial ethanol solutions should be replaced with fresh ones regularly.
3. The staining time for frozen sections should be as short as possible.
4. For bluing solutions, 0.2–1% ammonia water, Scott's bluing accelerator, or 0.1–1% lithium carbonate solution is commonly used.
5. For your safety and health, please wear a lab coat and disposable gloves during operation.
6. This product is for scientific research use only and is strictly prohibited for other purposes.