Hematoxylin Staining Solution (For IHC Use Only)

H1507743

Storage: 2-8℃. Store in the dark.

Introduction:

The combined staining of Hematoxylin and Eosin, 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 binds to the positively charged basic hematoxylin dye through ionic bonds or hydrogen bonds and is thus stained.

Hematoxylin Staining Solution (For IHC Use Only) is a type of alum hematoxylin. It has a low hematoxylin content, no oxide film formation, and stains cell nuclei very clearly without staining cytoplasmic or fibrous components. It is a progressive stain, so no hydrochloric acid-ethanol differentiation is required after staining, and the staining time is approximately 3-5 minutes. This reagent is often used for counterstaining cell nuclei after special staining (such as glycogen staining), enzyme histochemistry, and immunohistochemistry (IHC) staining. It is particularly suitable for counterstaining cell nuclei when acid treatment cannot be performed after special staining. In such cases, the staining time is shorter (usually 5-10 minutes), and bluing can be performed immediately after staining without differentiation. In special staining, it can be used in combination with Celestine Blue B to prevent the stained cell nuclei from fading due to subsequent acidic dyes. This reagent is for research use only and not intended for clinical diagnosis or other purposes.

Staining Principle:

1. Principle of Cell Nucleus Staining:

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 binds to the positively charged basic hematoxylin dye through ionic bonds or hydrogen bonds and is thus stained. Hematoxylin appears blue in an alkaline solution, so the cell nuclei are stained blue.

2. Principle of Cytoplasm Staining:

Eosin is a synthetic 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 value of the staining solution is below the isoelectric point (4.7-5.0) of cytoplasmic proteins, the cytoplasmic proteins ionize in a basic form, making the cytoplasm positively charged. It can then be stained

by the negatively charged acidic eosin dye. Eosin dissociates into negatively charged anions in water, which combine with the positively charged cations of cytoplasmic proteins to stain the cytoplasm red.

3. Differentiation:

After staining, the process of removing excess dye bound to 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. Acid can destroy the quinoid structure of hematoxylin, causing the separation of tissue and pigment and thus fading. After most tissues are stained with hematoxylin, they must be differentiated with 1% hydrochloric acid-ethanol to remove excess hematoxylin dye bound to cell nuclei and hematoxylin dye adsorbed by cytoplasm. Only then can eosin staining be performed to ensure clear differentiation between the staining of cell nuclei and cytoplasm.

4. Bluing:

After differentiation, hematoxylin exists in a red ionic state (red color) under acidic conditions and in a blue ionic state (blue color) under alkaline conditions. Tissue sections appear red or pink after differentiation with acidic ethanol. Immediately rinse the sections with water to remove the acid and stop differentiation, then use weakly alkaline water to make the hematoxylin-stained cell nuclei appear blue. This process is called bluing. Additionally, rinsing with tap water can also blue the cell nuclei, but it takes a longer time.

Self-Prepared Materials:

1. Hydrochloric acid-ethanol differentiating solution, a series of ethanol solutions, environment-friendly wax-immersing, dewaxing, and clearing solution.
2. Bluing solution, such as dilute ammonia water, lithium carbonate solution, etc.

Operating Steps (for reference only):

1. Adjust the staining volume appropriately according to the specific experimental requirements and the tissues or cells to be stained.
2. No hydrochloric acid-ethanol differentiation is required. The staining time is 3-5 minutes, and generally should be controlled within 10 minutes. For frozen sections, the staining time should be as short as possible.

Staining Results:

Cell nuclei: Blue.

Cytoplasm, muscle fibers, collagen fibers, etc.: Red (with varying shades).

Keratin, red blood cells, etc.: Bright orange-red.

Precautions:

1. Tissue sections must be thoroughly dewaxed. The series of ethanol solutions should be replaced with new ones regularly.
2. The differentiation time with hydrochloric acid-ethanol depends on the thickness of the

sections, tissue type, and the freshness of the solution. In addition, the rinsing time with tap water after differentiation should be sufficient to completely remove the acid.

3. This product can be used for staining ordinary tissue sections as well as immunohistochemical staining. If used for ordinary tissue section staining, it can be stored at room temperature. However, the staining intensity of the reagent will increase over time, so the staining time needs to be adjusted. Storage at 4°C is generally recommended.
4. For your safety and health, please wear a lab coat and disposable gloves during operation.