Cole's Hematoxylin Solution (For Conventional Stain)

Introduction

Hematoxylin is a basic natural dye that stains cell nuclei. The primary component of nuclear chromatin is DNA; within the double helix structure of DNA, phosphate groups on the two nucleotide chains face outward, causing the exterior of the DNA double helix to carry a negative charge and exhibit acidic properties. Consequently, it readily binds to the positively charged hematoxylin basic dye via ionic or hydrogen bonds, resulting in staining.

This solution utilizes a modified version of the classic formula and contains no toxic or harmful components such as mercury or methanol. As a gentle progressive stain, it requires no acid differentiation and is ideal for IF/IHC. It allows flexible sequencing (before or after immunostaining) and stains nuclei blue with a simple, rapid protocol.

Storage

Store at room temperature, stable for 1 year.

Protocol

1. Sample Preparation

    1) For Paraffin Sections: Deparaffinize in xylene twice for 5-10 min each. Rehydrate through a graded ethanol series (100%, 95%, 85%, 75%) for 3 min per step. Finally, rinse in distilled water for 2 min.

    2) For Frozen Sections: Warm to room temperature and rinse in distilled water for 2 min.

    3) For Cultured Cells: Fix with 4% paraformaldehyde for more than 10 min, then rinse with distilled water twice for 2 min each.

2. Hematoxylin Staining

    1) Stain with Cole's Hematoxylin Solution (For Conventional Stain) for 5-10 min.

    2) Rinse with distilled water to remove excess stain.

    3) Rinse in tap water for 10 min to allow bluing, or treat with bluing solution for 3-5 min.

3. Dehydration, Clearing, Mounting or Additional Staining

    1) Dehydration, Clearing, Mounting

        a) Dehydrate in 95% ethanol twice for 2 min each.

b) Clear in xylene twice for 5 min each.

c) Mount with neutral balsam.

d) Observe under the microscope. The nucleus is blue.

2) Additional Staining (e.g., Immunofluorescence)

a) After hematoxylin staining, rinse with 70% ethanol twice for 2 min each.

b) Equilibrate in PBS, saline, TBS, or TBST for 5 min.

c) Proceed with immunofluorescence or other fluorescent staining.

Note

1. Light staining is recommended during the staining process. Usually, it is only necessary to distinguish the cell nucleus. Excessive color intensity may affect the cytoplasmic color.
2. For your safety and health, please wear lab coats and gloves during the experiment.
3. For research use only. Not to be used in clinical diagnostic or clinical trials.

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