

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.

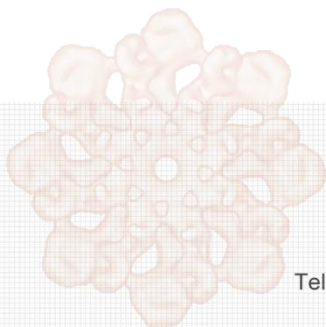
- b) Clear in xylene twice for 5 min each.
- c) Mount with neutral balsam.
- d) Observe under the microscope. The nucleus is blue.

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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