Modified Hematoxylin-Eosin (HE) Stain Kit

Introduction

Hematoxylin and Eosin (H&E) staining is one of the most widely used techniques in histopathology. Hematoxylin, a basic dye, stains nuclear chromatin and ribosomes a purplish-blue, while Eosin, an acidic dye, stains the cytoplasm and extracellular matrix pink to red.

This kit provides ready-to-use working solutions. It incorporates a differentiated-resistant hematoxylin to replace conventional formulas, allowing for flexible adjustment of nuclear staining intensity and clarity. Alcohol-based Eosin is used instead of water-based Eosin, offering superior solution stability and more durable staining results. Furthermore, a specialized enhanced bluing agent ensures high-contrast, vivid nuclear coloration.

Components and Storage

Components \ Size	100 mL	Storage
Reagent (A): Hematoxylin dye solution	100 mL	Room temperature protected from light
Reagent (B): Differentiation Solution	100 mL	Room temperature protected from light
Reagent (C): Bluing Solution	100 mL	Room temperature protected from light
Reagent (D): Eosin staining solution	100 mL	Room temperature protected from light
Shipping: Blue ice	**Shelf life: 12 months**	

Protocol

1. For Paraffin Sections

   1) Collect tissue samples, fix, embed in paraffin, and section.

   2) Deparaffinize in xylene twice for 5 min each.

   3) Hydrate through absolute ethanol twice for 5 min each.

   4) Hydrate through 95%, 85%, and 75% ethanol for 2 min each.

   5) Rinse in distilled water for 2 min.

   6) Stain with Hematoxylin dye solution for 3-10 min (adjust time based on results and requirements), rinse with tap water for 5-10 sec.

7) Differentiate in Differentiation Solution for 1-5 sec, rinse with tap water for 20-30 sec.

8) Bluing: Blue with Bluing Solution for 10 sec-1 min, rinse with tap water for 20-30 sec.

9) Stain with Eosin staining solution for 30 sec-2 min (adjust time as needed), rinse with tap water for 1-5 sec.

10) Dehydrate through 75%, 85%, 95%, and 100% ethanol for 2-3 sec each.

11) Rinse in 100% ethanol for 1 min.

12) Clear in xylene twice for 1 min each.

13) Mount with neutral balsam and observe under microscope.

2. For Frozen Sections

1) Warm frozen sections to room temperature, fix directly for 3-5 min, and wash with water for 3-5 min.

Note: Frozen sections derived from previously fixed tissues do not require fixation again. However, frozen sections obtained from unfixed tissues should be fixed with pre-chilled 4% paraformaldehyde at 2-8°C prior to staining.

2) Stain with Hematoxylin dye solution for 1-2 min.

3) Subsequent staining steps are identical to those for paraffin sections.

3. Staining Results

Nucleus	Blue
Cytoplasm and fibers	Red

Note

1. Ensure complete deparaffinization of sections. Frequently replace the ethanol series with fresh solutions.
2. 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.
3. For your safety and health, please wear lab coats and gloves during the experiment.
4. For research use only. Not to be used in clinical diagnostic or clinical trials.

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