Heidenhain's Iron-Hematoxylin 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 uses ferric ammonium sulfate (iron alum) as both an oxidizer and differentiator, revealing distinct tissue structures based on differentiation levels. Initially, all components stain black or dark grayish-black. During differentiation with Heidenhain Differentiating Solution, staining fades at different rates depending on the tissue structure. The typical destaining sequence is: mitochondria, striated muscle, and finally nuclear chromatin.

Components and Storage

Components \ Size	100 mL	Storage
Reagent (A): Hematoxylin Differentiation	2 x 100 mL	Room temperature protected from light
Reagent (B): Heidenhain's Iron-Hematoxylin	100 mL	Room temperature protected from light
Reagent (C): Eosin staining solution	100 mL	Room temperature protected from light
Shipping: Blue ice	**Shelf life: 2 years**	

Protocol

1. Fix tissue, embed in paraffin, and cut sections (3-5 μm).
2. Deparaffinize in xylene twice for 5-10 min each.
3. Hydrate through absolute ethanol twice for 3-5 min each.
4. Hydrate through 95%, 90%, and 80% ethanol for 3-5 min each.
5. Rinse in distilled water for 1-3 min.
6. Mordantly stain in Hematoxylin Differentiation for 1 h, rinse with distilled water for 5-10 sec.
7. Stain with Heidenhain's Iron-Hematoxylin for 1 h, rinse with distilled water for 20-30 sec.
8. (Optional) Stain with Eosin staining solution for 2-4 min (adjust time as needed), rinse with tap water for 1-5 sec.

9. Differentiate with Hematoxylin Differentiation or a 1:1 dilution with distilled water, alternating with rinses in tap water. Monitor the degree of differentiation under a microscope. Finally, rinse with tap water for 10 min.
10. Dehydrate through 80% and 90% ethanol for 10-20 sec each, then treat with 95% ethanol twice for 1-2 min each.
11. Treat with absolute ethanol twice for 2-3 min each.
12. Clear in xylene three times for 2-3 min each.
13. Mount with neutral balsam and observe under a microscope.
14. Staining Results: Mitochondria, striated muscle, myelin, and chromatin appear grayish-black.

Note

1. Ensure complete deparaffinization of sections. Frequently replace the ethanol series with fresh solutions.
2. The staining time by Heidenhain Differentiation and Heidenhain's Iron-Hematoxylin depending on the fixative used. The reference time: 1 h for Formalin, Bouin, or Carnoy fixative; 3 h for Helly or Zenker's dichromate fixative; 24 h for Osmium tetroxide or Flemming's fixative.
3. Monitor the differentiation under a microscope until the target structures appear. If over-differentiation occurs, re-stain with hematoxylin for the same time and re-differentiate. Alternatively, dilute Heidenhain Differentiation with distilled water 2:1 before differentiation.
4. Thoroughly rinse slices after differentiation to remove all traces of the solution and prevent fading.
5. Cytoplasmic counterstaining (such as Eosin or Orange G) can enhance nuclear chromatin contrast, particularly effective for visualizing chromosomes or mitosis.
6. For your safety and health, please wear lab coats and gloves during the experiment.
7. For research use only. Not to be used in clinical diagnostic or clinical trials.

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