

Modified Harris' Hematoxylin Stain Solution

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 is an improvement upon the classic Harris hematoxylin staining method. It contains no mercury, is non-toxic, and does not form oxide films. It stains nuclear chromatin darkly and with fine detail. Clinically, it is often used as a substitute for the traditional Harris hematoxylin staining solution. The staining time is generally 5–8 minutes. Characterized by a high degree of hematoxylin oxidation and strong staining power, it tends to cause overstaining of the nucleus, cytoplasm, and fibers despite the short staining duration. Therefore, hydrochloric acid ethanol differentiation is required following staining, classifying it as a regressive stain.

Storage

Store at room temperature protected from light, stable for 1 year.

Protocol

1. Materials Required but Not Included

- 1) Hydrochloric acid ethanol differentiation solution
- 2) Bluing solution (such as dilute ammonia water, lithium carbonate solution, etc.)
- 3) Series of ethanol solutions
- 4) Eosin staining solution
- 5) 4% paraformaldehyde

2. Paraffin Section Staining

- 1) Slice Dewaxing to Water
 - a) Xylene treatment twice, 5-10 min each time.
 - b) Anhydrous ethanol treatment twice, 3-5 min each time.
 - c) 95% ethanol for 3 min, 85% ethanol for 3 min, 75% ethanol for 3 min.

d) Tap water or distilled water rinse for 1-3 min.

2) Staining

a) Stain with Modified Harris' Hematoxylin Stain Solution for 5-8 min.

b) Rinse with tap water or distilled water for 5-10 sec.

c) (Optional) Differentiate with hydrochloric acid ethanol for 2-5 sec, rinse with tap water for 20-30 sec.

d) (Optional) Return to blue with bluing solution for 3-5 min, rinse with tap water for 30-60 sec.

e) (Optional) Stain with eosin staining solution for 30 sec-2 min, rinse with distilled water for 1-5 sec.

3) Dehydration, Clearing, and Mounting

a) 75% ethanol for 3-5 sec, 85% ethanol for 3-5 sec, 95% ethanol for 3-5 sec. Anhydrous ethanol treatment twice, 1 min each time.

b) Xylene clearing twice, 1min each time.

c) Mount with neutral balsam.

3. Frozen Section Staining

1) Fix with acetone for 30 sec or 4°C pre-cooled 4% paraformaldehyde for 20 min.

2) Rinse with distilled water for 2 min.

3) Apply Modified Harris' Hematoxylin Stain Solution for 1-2 min (may be preheated at 50°C for 10 min).

4) The remaining operational steps are the same as those for paraffin section staining.

4. Cell Staining

1) Fix with 4% paraformaldehyde for 10-20 min.

2) Rinse with distilled water twice, 2 min each time.

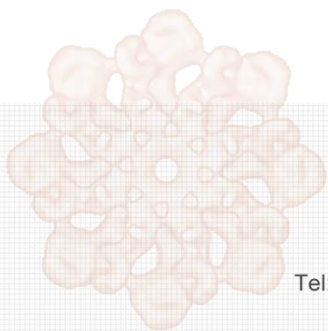
3) The steps for staining, dewaxing, clearing, and mounting are the same as those for frozen sections.

5. Staining Results

Nucleus	Blue
Cytoplasm, muscle fibers, collagen fibers, thyroid colloid, etc.	Red
Keratin, red blood cells	Orange-red

■ Note

1. Slices should be thoroughly dewaxed. The series of ethanol solutions should be frequently replaced with fresh ones.
2. The differentiation time in hydrochloric acid ethanol should be determined based on the thickness of the slice, tissue type, and whether the slide is new or old. Additionally, the rinsing time with tap water after differentiation should be sufficient to completely remove the acid.
3. Bluing solutions commonly used include 0.2-1% ammonia water, Scott's bluing solution, or 0.1-1% lithium carbonate solution.
4. For your safety and health, please wear lab coats and gloves during the experiment.
5. For research use only. Not to be used in clinical diagnostic or clinical trials.



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