# Modified Lillie-Mayer Hematoxylin Staining Solution

Cat #: A-CSH255

Size: 100mL, 500mL

Storage: 10-30 °C, Avoid exposure to light (1 year)

## Product Description

Hematoxylin and Eosin (HE) staining, also known as Hematoxylin-Eosin staining, is the most commonly used staining method in pathology and histology. Hematoxylin, an alkaline natural dye, stains the cell nuclei. The main component of chromatin in the cell nucleus is DNA. In the double helix structure of DNA, the phosphate groups on the two nucleotide chains are oriented outward, resulting in a negative charge on the outer side of the DNA double helix. This acidic nature of DNA allows it to readily bind to the positively charged basic dye, hematoxylin, through ionic or hydrogen bonding, resulting in staining.

The modified Lillie-Mayer hematoxylin staining solution is non-toxic, free from oxidation film, and provides deep and subtle staining of the chromatin in cell nuclei. It is often used as a substitute for Harris hematoxylin staining solution in clinical practice. It provides clear staining of cell nuclei without staining the cytoplasm and fibrous components. It is considered a progressive staining method, which means that differentiation with hydrochloric acid-ethanol is not necessary after staining. The staining time is approximately 5 to 8 minutes, or 3 to 5 minutes if fully oxidized. It is commonly used for counterstaining cell nuclei in special stains such as glycogen staining, enzyme histochemistry, and immunohistochemistry. It is especially useful for restaining cell nuclei when acid treatment is not possible after special staining. In special staining, it is often combined with a solution of Azure B to prevent fading of the cell nuclei stain by subsequent acidic dyes.

## Usage

### I. Paraffin Section Staining

#### (a) Deparaffinization

1. Deparaffinize the sections in water.

2. Treat with xylene twice for 5 to 10 minutes each time.

3. (Optional) Treat with absolute ethanol twice for 3 to 5 minutes each time.

4. Treat with 95% ethanol for 3 to 5 minutes.

5. Treat with 90% ethanol for 3 to 5 minutes.

6. Treat with 80% ethanol for 3 to 5 minutes.

7. Rinse with tap water or distilled water for 1 to 3 minutes.

**(b) Staining**

1. Stain with modified Lillie-Mayer hematoxylin staining solution for 5 to 8 minutes.

2. Rinse with tap water or distilled water for 5 to 10 seconds.

3. (Optional) Differentiate with hydrochloric acid-ethanol for 2 to 5 seconds.

4. Rinse with tap water for 20 to 30 seconds.

5. (Optional) Counterstain with bluing solution for 20 to 40 seconds.

6. Rinse with tap water for 30 to 60 seconds.

7. Stain with eosin staining solution for 3 to 5 minutes.

8. Rinse with tap water for 1 to 5 seconds.

**(c) Dehydration, Clearing, and Mounting**

1. Treat with 80% ethanol for 10 to 20 seconds.

2. Treat with 90% ethanol for 10 to 20 seconds.

3. Treat with 95% ethanol twice for 1 to 2 minutes each time.

4. Treat with absolute ethanol twice for 2 to 3 minutes each time.

5. Treat with xylene three times for 2 to 3 minutes each time.

6. Mount with neutral resin.

## II. Frozen Section Staining

(a) Fixation with Ether-Ethanol mixture for 5 to 10 seconds.

(b) Rinse with tap water for 2 to 5 seconds.

(c) Drop staining with modified Lillie-Mayer hematoxylin staining solution for 1 to 2 minutes (can be heated to 50° C if desired).

(d) Rinse with tap water for 2 to 5 seconds.

(e) (Optional) Differentiate with hydrochloric acid-ethanol for 2 to 5 seconds.

(f) Rinse with tap water for 2 to 5 seconds.

(g) (Optional) Counterstain with bluing solution for 2 to 5 seconds.

(h) Rinse with tap water for 5 to 10 seconds.

(i) Stain with eosin staining solution for 2 to 5 seconds.

(j) Rinse with tap water for 1 to 2 seconds.

(k) Treat with 80% ethanol for 1 to 2 seconds.

(l) Treat with 95% ethanol for 1 to 2 seconds.

(m) Treat with absolute ethanol for 2 to 5 seconds.

(n) Treat with phenol xylene (1:3) for 2 to 5 seconds.

(o) Treat with xylene three times for 2 to 5 seconds each time.

(p) Mount with neutral resin.

### III. Cell Staining

(a) Fix with 4% paraformaldehyde for 10 to 20 minutes.

(b) Rinse with tap water twice for 2 minutes each time.

(c) Rinse with distilled water twice for 2 minutes each time.

(d) Follow the staining, deparaffinization, clearing, and mounting steps as in paraffin section staining, with appropriate reduction in staining time. Staining Results:

## Staining Results

| | |
|---|---|
| Cell Nucleus | Blue |
| Cytoplasm and Fibers | Red |

## Notes

1. Ensure thorough deparaffinization of the sections.

2. Replace the series of ethanol frequently with fresh solutions.

3. Differentiation time with hydrochloric acid-ethanol should be adjusted based on section thickness, tissue type, and solution freshness. Additionally, sufficient rinsing time with tap water is necessary to thoroughly wash off the acid.

4. The fixative mixture of ether-ethanol is prepared by mixing equal amounts of ether and 95% ethanol, with the addition of a suitable amount of acetic acid. Store in a tightly closed container.

5. Keep the staining time for frozen sections as short as possible.

6. A bluing solution commonly consists of 0.2-1% ammonia water or Scott's bluing solution or 0.1-1% lithium carbonate solution.

7. For your safety and health, please wear lab coat and disposable gloves during the procedure.

## Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.