Hematoxylin Harris

Reagent for strong, regressive staining in histopathology

MenidiMedica
Biotechnology Applications

REF 70430-1000: 1 lt,
70430-5000: 5 lt.

24 months, storage at RT

Introduction

MenidiMedica Biotech Hematoxylin Harris is a widely recognized hematoxylin formulation employed in histopathology to achieve highly precise nuclear cell staining. In routine histology staining, such as hematoxylin and eosin (HE) staining, Hematoxylin according to Harris is utilized using a regressive method. Hematoxylin is derived from logwood (Haematoxylon campechianum L.). It undergoes oxidation to become hematein, which then forms bonds with metal ions known as mordants. Hematein undergoes this transformation to develop into an indelible nuclear color. Subsequently, the positively charged hematein-mordant complex attaches to the negatively charged phosphate ions present in the DNA's nucleus, resulting in the characteristic blue coloration. The original Hematoxylin according to Harris formula utilizes mercury oxide for oxidation. However, MenidiMedica Biotech version of Hematoxylin according to Harris does not contain mercury oxide due to its toxicity concerns. Instead, it employs environmentally friendly sodium iodate for this purpose. Hematoxylin Harris excels in staining the nuclear membrane, nucleoplasm, and nucleolus with exceptional precision.

Product description

Reagent for regressive nuclear staining in histopathology. Contains optimally oxidized hematoxylin with sodium iodate, aluminum ions and antioxidants.

Other slides and reagents that may be used in staining:

- Fixatives such as MenidiMedica Biotech neutral buffered formaldehyde solutions (Formaldehyde NB 10%)
- Dehydrating/rehydrating agent, such as MenidiMedica Biotech alcohol solutions
- Clearing agents, such as xylene or a substitute agent on the aliphatic hydrocarbons basis
- Infiltration and fitting agent, such as granulated paraffin
- High-quality glass slides for use in histopathology and cytology
- Differentiation agent, such as MenidiMedica Biotech Acid alcohol
- Bluing agents, such as MenidiMedica Biotech Scott's solution or Bluing reagent
- Covering agents for microscopic sections and mounting cover glass, such as MenidiMedica Biotech Eukitt
- Cover glass, dimensions range from 18x18mm to 24x60mm
- Counterstaining reagents, such as MenidiMedica Biotech eosin solutions

Preparing histological sections for staining

- Fix the tissue sample tightly (10% NB Formaldehyde), rinse with water and dehydrate through series of ascending alcohol solutions (Histanol 100).
- Clear the sample with intermedium; in xylene or in a xylene substitute.
- Infiltrate and fit the sample in paraffin
- Cut the paraffin block to 4-6 µm slices and place them on a glass slide

Hematoxylin and eosin (HE) staining procedure, progressive

- Deparaffinize the section in xylene or in a xylene substitute - 3 exchanges, 2 min each
- Rehydrate using 100% alcohol (Histanol 100) - 2 exchanges, 5 and 3 min
- Rehydrate using 95% alcohol - 2 min
- Rehydrate in distilled water - 2 min
- Stain using Hematoxylin Harris - 3-5 minutes

Note: In the case of subsidence in the solution or a formation of metallic glow on the surface, reagent should be filtrated before use.

- Immerse the section in distilled or demineralized water until dye is no longer being released from the section
- Make nuclei turn blue using Scott's solution or Bluing reagent - 1 min

Note: Finish the process of bluing after the nuclei turn blue If no Scott's solution or Bluing reagent is available, rinse the sections under tap water for 3-5 minutes.

- Stain with one of eosin contrast solutions until the section is optimally stained - 15 seconds - 2 minutes

Note: Staining the sections in eosin alcoholic solutions causes intensive eosinophil color to show much faster (in under 15 seconds' time). Recommended exposure time for eosin aqueous solutions is 90 seconds to 2 minutes

- Rinse under tap water - 2 min
- Dehydrate using 95% alcohol - 2 exchanges, 10-15 dips
- Dehydrate using 100% alcohol (Histanol 100) - 3 exchanges, 10-15 dips
- Clear the section in xylene or in a xylene substitute

Immediately after clearing apply an appropriate mount medium for covering/mounting on the section. If xylene was used, use one of mounting xylene-based media. If xylene substitute was used, use the appropriate covering agent for this case. Cover the section with a cover glass.

Hematoxylin and eosin (HE) staining procedure, regressive

- Deparaffinize the section in xylene or in a xylene substitute - 3 exchanges, 2 min each
- Rehydrate using 100% alcohol (Histanol 100) - 2 exchanges, 5 and 3 min
- Rehydrate using 95% alcohol - 2 min
- Rehydrate in distilled water - 2 min
- Stain using Hematoxylin Harris - 4-8 minutes

Note: In the case of subsidence in the solution or a formation of metallic glow on the surface, reagent should be filtrated before use.

- Immerse the section in distilled water until dye is no longer being released from the section
- Differentiate using Acid alcohol - 3-10 dips

Note: This step removes excessive hematoxylin from the nucleus and cytoplasm. Discoloration of the nuclei can occur if the section is treated with the differentiation agent for too long.

- Rinse in distilled water
- Make nuclei turn blue using Scott's solution or Bluing reagent - 1 min

Note: Finish the process of bluing after the nuclei turn blue If no Scott's solution or Bluing reagent is available, rinse the sections under tap water for 3-5 minutes.

- Immerse the sections in distilled/demineralized water.
- If alcoholic eosin solution is used, immerse the sections in 95% alcohol. Skip this step if aqueous eosin solution is used.
- Stain with one of eosin contrast solutions until the section is optimally stained - 15 seconds - 2 minutes

Note: Staining the sections in eosin alcoholic solutions causes intensive eosinophil color to show much faster (in under 15 seconds' time). Recommended exposure time for eosin aqueous solutions is 90 seconds to 2 minutes

- Rinse under tap water - 2 min
- Dehydrate using 95% alcohol - 2 exchanges, 10-15 dips
- Dehydrate using 100% alcohol (Histanol 100) - 3 exchanges, 10-15 dips
- Clear the section in xylene or in a xylene substitute - 2 exchanges, 2 min each

Immediately after clearing apply an appropriate mount medium for covering/mounting on the section. If xylene was used, use one of mounting xylene-based media. If xylene substitute was used, use the appropriate covering agent for this case. Cover the section with a cover glass.

Result

Nuclei - blue

Cytoplasm, collagen, muscle fibers, erythrocytes - hues of pink

Note

Time periods of staining processes are not entirely standardized and they approximately correspond to clinical and laboratory practical experience. Intensity of staining depends on the period of immersion in the dye. Real staining protocol depends on personal requests and priorities.

Preparing the sample and diagnostics

Use only appropriate instruments for collecting and preparing the samples. Process the samples with modern technology and mark them clearly. Follow the manufacturer's instructions for handling. In order to avoid mistakes, the staining procedure and diagnostics should only be conducted by authorized and qualified personnel. Use only microscope according to standards of the medical diagnostic laboratory.

Safety at work and environmental protection

Handle the product in accordance with safety at work and environmental protection guidelines. Used solutions and out of date solutions should be disposed of as special waste in accordance with national guidelines. Chemicals used in this procedure could pose danger to human health. Tested tissue specimens are potentially infectious. Necessary safety measures for protecting human health should be taken in accordance with good laboratory practice. Act in accordance with signs and warnings notices printed on the product's label, as well as in MenidiMedica Biotech material safety data sheet.

Storing, stability and expiry date

Keep Hematoxylin Harris in a tightly closed original package at temperature between +15°C and +25°C. Keep in dry places, do not freeze and avoid exposing to direct sunlight. Date of manufacture and expiry date are printed on the product's label.

References

1. Baker, J.R. (1962): Experiments on the action of mordants. 2. Aluminium-hematein. Q.J.Microsc. Sci. p103 493-517.
2. Conn, J. (1977): Biological Stains, 9th ed., Baltimore: Williams and Wilkens Co.
3. Harris, H.F. (1898): A new method of “ripening” haematoxylin. Microsc. Bull. (Philadelphia) Dec. 47.
4. Harris, H.F. (1900): On the rapid conversion of haematoxylin into haematein in staining reactions. J. Appl. Microsc. p3 777-780

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