Hematoxylin II

REF 790-2208

05277965001

IVD 250

INTENDED USE

Hematoxylin II is a modified Mayer's hematoxylin intended for laboratory use in staining cellular nuclei on slides containing cells from frozen tissue, or formalin fixed, paraffin-embedded tissue on a BenchMark IHC/ISH instrument. This reagent is intended as a counterstain to immunohistochemistry, and in situ hybridization applications.

This reagent is intended for in vitro diagnostic use.

SUMMARY AND EXPLANATION

Hematoxylin II stains a variety of tissue components including nuclei, mitochondria, mucin, hemoglobin, elastic fibers, and collagen. The dye component, hematein, is bound to the tissue indirectly through a mordant, aluminum sulfate.

PRINCIPLE OF THE PROCEDURE

In general, immunohistochemistry (IHC) allows the visualization of antigens via the sequential application of a specific antibody (primary antibody) to the antigen, a secondary antibody (link antibody) to the primary antibody, an enzyme complex and a chromogenic substrate with interposed washing steps. The enzymatic activation of the chromogen results in a visible reaction product at the antigen site.

Similarly, in situ hybridization (ISH) staining allows the visualization of target DNA or RNA sequences via the sequential hybridization of a labeled DNA or RNA probe to the target, a primary antibody against the labeled probe, and a secondary antibody to the primary antibody, an enzyme complex and a chromogenic substrate with interposed washing steps. The enzymatic activation of the chromogen results in a visible reaction product at the target site.

For both applications, at the end of incubation step, the instrument washes the slides to stop the reaction and remove unbound material. The specimen may then be counterstained with Hematoxylin II solution which is applied to the slide and mixed over the entire specimen area. Hematoxylin II stains nuclei blue through the binding of a mordant dye complex to nucleic acids and histone proteins of the heterochromatin. Coverslip may then be applied to the specimen. Results are interpreted using a light microscope and aid in the differential diagnosis of pathophysiological processes, which may or may not be associated with a particular antigen or nucleic acid target.

MATERIAL PROVIDED

Hematoxylin II contains sufficient reagent for 250 tests.

One 25 mL dispenser of Hematoxylin II (≤ 60%); contains glycol and acetic acid stabilizing solution

Reconstitution, Mixing, Dilution, Titration

No reconstitution, mixing, dilution, or titration is required. Further dilution may result in loss of staining specificity.

MATERIALS REQUIRED BUT NOT PROVIDED

Additional reagents including but not limited to VENTANA primary antibodies, probes, detection kits, and ancillary components, are not provided.

Not all products listed in the method sheet may be available in all geographies. Consult your local support representative.

1. General purpose laboratory equipment
2. Bluing Reagent (Cat. No. 760-2037 / 05266769001)
3. BenchMark IHC/ISH instrument

STORAGE AND STABILITY

Upon receipt and when not in use, store at 2-8°C. Do not freeze.

To ensure proper reagent delivery and stability of the product, replace the dispenser cap after every use and immediately place the dispenser in the refrigerator in an upright position.

This reagent is expiration dated. When properly stored, the reagent is stable to the date indicated on the label. Do not use reagent beyond the expiration date.

The signs indicating instability of this product are precipitation of the reagent with clearing of the solution. At the first sign of possible reagent instability, contact your local support representative.

Specimen Collection and Preparation for Analysis

Frozen Sections

Routinely processed, frozen tissues are also suitable for use with this reagent on a BenchMark IHC/ISH instrument. The recommended tissue fixation is 10 minutes in cold acetone. Variable results may occur as a result of prolonged fixation or special processes such as decalcification of bone marrow preparations.

Formalin Fixed, Paraffin Embedded Tissue

Routinely processed, formalin fixed, paraffin-embedded (FFPE) tissues are suitable for use with this reagent when used with VENTANA primary antibodies, detection kits, ancillary reagents, on a BenchMark IHC/ISH instrument. The recommended tissue fixative is 10% neutral buffered formalin.[1] Variable results may occur as a result of prolonged fixation or special processes such as decalcification of bone marrow preparations.

Each section should be cut the appropriate thickness and placed on a positively charged glass slide.

WARNINGS AND PRECAUTIONS

1. For in vitro diagnostic (IVD) use.
2. For professional use only.
3. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions. In the event of exposure, the health directives of the responsible authorities should be followed.[2,3]
4. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
5. Avoid microbial contamination of product as it may cause incorrect results.
6. For further information on the use of this device, refer to the BenchMark IHC/ISH instrument User Guide, and instructions for use of all necessary components located at navifyportal.roche.com.
7. Consult local and/or state authorities with regard to recommended method of disposal.
8. Product safety labeling primarily follows EU GHS guidance. Safety data sheet available for professional user on request.
9. To report suspected serious incidents related to this device, contact the local Roche representative and the competent authority of the Member State or Country in which the user is established.

This product contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Table 1. Hazard information.

Hazard	Code	Statement
Warning  	H315	Causes skin irritation.
	H319	Causes serious eye irritation.
	H373	May cause damage to organs through prolonged or repeated exposure.
	P260	Do not breathe mist or vapours.
	P264	Wash skin thoroughly after handling.
	P280	Wear protective gloves/ eye protection/ face protection.
	P314	Get medical advice/ attention if you feel unwell.
	P337 + P313	If eye irritation persists: Get medical advice/ attention.
	P501	Dispose of contents/ container to an approved waste disposal plant.

This product contains CAS # 107-21-1: ethane-1,2-diol.

EUH208: Contains sodium iodate. May produce an allergic reaction.

INSTRUCTIONS FOR USE

Refer to the appropriate method sheet for the recommended staining protocol and to the instrument User Guide for detailed instructions and additional protocol options.

The parameters for the automated procedures can be displayed, printed and edited according to the procedure in the instrument User Guide. Other operating parameters for the instrument have been preset at the factory.

Hematoxylin II is loaded onto the reagent tray on the BenchMark IHC/ISH instrument. Hematoxylin II is applied automatically as required for the selected procedure.

For more details on the proper use of this device, refer to the inline dispenser method sheet associated with P/N 790-2208.

STAINING INTERPRETATION / EXPECTED RESULTS

The automated IHC or ISH procedure causes a colored reaction product to precipitate at the antigen sites localized by the primary antibody, or the nucleic acid target localized by the probe. Refer to the appropriate detection kit method sheet for expected color reactions. Refer to the appropriate primary antibody or probe method sheet for expected patient sample results. Appropriate sample control results verify the reagents and system are working properly.

Application of Hematoxylin II using the BenchMark IHC/ISH instrument will result in blue staining of heterochromatin in cell nuclei. Depending on the incubation length and potency of the hematoxylin used, counterstaining will result in a pale to dark blue coloration of cell nuclei.

PERFORMANCE CHARACTERISTICS

ANALYTICAL PERFORMANCE

Hematoxylin II is used for counterstaining tissue samples for IHC, or ISH on BenchMark IHC/ISH instruments. Hematoxylin II was tested with a variety of VENTANA primary antibodies and probes at various incubation times with specific tissue types.

Within run and between day precision for Hematoxylin II resulted in blue staining of heterochromatin in cell nuclei in 100% of the cases.

LIMITATIONS

Excessive or incomplete counterstaining may compromise proper interpretation of results.

REFERENCES

1. Sheehan DC, Hrapchak BB. Theory and practice of histotechnology, 2nd Edition. The C.V. Mosby Company, St. Louis, 1980.
2. Occupational Safety and Health Standards: Occupational exposure to hazardous chemicals in laboratories. (29 CFR Part 1910.1450). Fed. Register.
3. Directive 2000/54/EC of the European Parliament and Council of 24 June 2020 on the protection of workers from risks related to exposure to biological agents at work.

NOTE: A point (period/stop) is always used in this document as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols

Ventana uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see elabdoc.roche.com/symbols for more information).

GTIN	Global Trade Item Number
Rx only	For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

REVISION HISTORY

Rev	Updates
F	Updates to Warnings and Precautions section. Updated to current template.

INTELLECTUAL PROPERTY

For USA: Rx only

CONTACT INFORMATION

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