CAT Hematoxylin

Modified Lillie-Mayer's Method 901-CATHE-081117



Catalog Number: CATHE-M, MM, GL **Description:** 500, 1000 ml, 1 gal

Intended Use:

For In Vitro Diagnostic Use

CAT Hematoxylin is a modified Lillie-Mayer Hematoxylin staining reagent intended for visualization of cellular nuclei in tissue sections and cellular preparations. The clinical interpretation of any staining or its absence should be complemented by morphological studies and proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

Summary & Explanation:

Biocare's CAT Hematoxylin is intended for use in the histologic demonstration of nuclear staining. Hematoxylin solutions mordanted with aluminum are routinely used in the histopathology laboratory for nuclear staining. This staining technique is used to make the critical distinction between a normal nucleus and an abnormal one. The traditional formulas for nuclear demonstration using an aluminum mordant are those of Delafield, Harris, Ehrich, Lillie and Mayer. All use aluminum alum (ammonium aluminum sulfate) as the mordant salt. Since the aluminum salts are not in themselves oxidizers, it is necessary to expose the hematoxylin solution to air or chemicals to affect the conversion of hematoxylin to hematein. The aforementioned formulas are also used in special-staining procedures as counterstains for nuclear demonstration. The addition of acid to alum hematoxylin solutions is believed to increase the selectivity of the stain for the nuclei and to counteract the rapid oxidizing effects of chemical oxidizing agents. This latter function enables the solution to maintain some hematoxylin in equilibrium with the hematein to ensure a better stain. Glycerol tends to stabilize the system against over-oxidation and aids in preventing rapid evaporation.

Known Applications:

Any well-fixed tissue. Tissue can be frozen, paraffin embedded or celloidin embedded

Supplied As:

Ready-to-use

Materials and Reagents Needed But Not Provided:

Microscope slides, positively charged Desert Chamber* (Drying oven)

Positive and negative tissue controls

Xylene (Could be substituted with xylene substitute*)

Ethanol or reagent alcohol

Decloaking Chamber* (Pressure cooker)

Deionized or distilled water

Wash buffer*(TBS/PBS)

Pretreatment reagents*

Enzyme digestion*

Avidin-Biotin Blocking Kit*(Labeled streptavidin kits only)

Peroxidase block*

Protein block*

Primary antibody*

Negative control reagents*

Detection kits*

Detection components*

Chromogens*

Bluing reagent*

Mounting medium*

* Biocare Medical Products: Refer to a Biocare Medical catalog for further information regarding catalog numbers and ordering information. Certain reagents listed above are based on specific application and detection system used.

Storage and Stability:

Store at room temperature. Do not use after expiration date printed on vial. If reagents are stored under conditions other than those specified in the package insert, they must be verified by the user.

Protocol Recommendations:

- 1. Deparaffinize and hydrate to water.
- 2. If sections are Zenker-fixed, remove the mercuric chloride crystals with iodine and clear with sodium thiosulfate (hypo). Rinse in running water.
- 3. Rinse in distilled water.
- 4. CAT Hematoxyin for 4-8 minutes.

(*For IHC counterstain stain 30 seconds to 1 minute)

- 5. Wash in tap water until clear (generally 30 seconds to 1 minute).
- 6. Blue in Tacha's Bluing Solution 30 seconds to 1 minute.
- 7. Wash in tap water 3-4 minutes.
- 8. Place in 95% alcohol for 30 seconds.
- 9. Counterstain with Edgar Degas Eosin 1-3 minutes.
- (*Alternately, counterstain with Rubens Eosin-Phloxine 30-40 seconds)
- 10. Dehydrate through graded alcohols.
- 11. Clear in clearing agents.
- 12. Mount with resinous mounting media.

Technical Notes:

1. Filter product with a 0.4 micron filter if particulate is seen.

Protocol Notes:

Biocare Medical uses only stains that are certified by the Biological Stain Commission. CAT Hematoxylin is specially formulated to eliminate the necessity for differentiation of the section.

NOTE: The biggest objection to Mayer's hematoxylin as used in the past, has been that stained slides often fade after 1-3 years. This problem can be eliminated, however, when slides are washed, after hematoxylin, in running water for a minimum of 3-4 minutes.

NOTE: Biocare's research studies show pH of tap and D.I.water for rinsing slides can have an effect on staining. pH can change from acidic to basic seasonally in different regions of the country. It has been further reported, that there have been changes in tap water from the morning to the afternoon in some states. Long rinsing times after bluing may differentiate out Hematoxylin. We have modified our rinse time to 3-4 minutes, instead of the previous 7-10 minutes.

Limitations:

The protocols for a specific application can vary. These include, but are not limited to: fixation, heat-retrieval method, incubation times, tissue section thickness and detection kit used. Due to the superior sensitivity of these unique reagents, the recommended incubation times and titers listed are not applicable to other detection systems, as results may vary. The data sheet recommendations and protocols are based on exclusive use of Biocare products. Ultimately, it is the responsibility of the investigator to determine optimal conditions. The clinical interpretation of any positive or negative staining should be evaluated within the context of clinical presentation, morphology and other histopathological criteria by a qualified pathologist. The clinical interpretation of any positive or negative staining should be complemented by morphological studies using proper positive and negative internal and external controls as well as other diagnostic tests



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Quality Control:

Refer to CLSI Quality Standards for Design and Implementation of Immunohistochemistry Assays; Approved Guideline-Second edition (I/LA28-A2) CLSI Wayne, PA USA (www.clsi.org). 2011

Precautions:

- 1. Specimens, before and after fixation, and all materials exposed to them should be handled as if capable of transmitting infection and disposed of with proper precautions. Never pipette reagents by mouth and avoid contacting the skin and mucous membranes with reagents and specimens. If reagents or specimens come in contact with sensitive areas, wash with copious amounts of water.
- 2. Microbial contamination of reagents may result in an increase in nonspecific staining.
- 3. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change.
- 4. Do not use reagent after the expiration date printed on the vial.
- 5. The SDS is available upon request and is located at http://biocare.net.

Warnings:

- 1. Contains glacial acetic acid (use in a well ventilated area).
- 2. Wear appropriate personal protective equipment and clothing.
- 3. Avoid contact with skin and eves.
- 4. Seek medical attention if swallowed.

Troubleshooting:

Follow the antibody specific protocol recommendations according to data sheet provided. If atypical results occur, contact Biocare's Technical Support at 1-800-542-2002.



The Netherlands