SIGMA-ALDRICH®

MAYER'S HEMATOXYLIN SOLUTION

(Procedure No. MHS)

INTENDED USE

Mayer's Hematoxylin Solution is commonly used after immunohistochemistry or cytochemistry staining as a nuclear counterstain. It may also be used for standard hematoxylin and eosin (H&E) staining, but it is more commonly used where acid alcohol differentiation, or exposure to alcohol, might destroy the stained cytoplasmic component.[3] Mayer's Hematoxylin Solution is formulated without alcohol, and as such will not dissolve out AEC (3-amino-9-ethylcarbazole), alkaline phosphatase/Fast Red chromogen or other soluble colored products. Mayer's Hematoxylin Solutions are for "In Vitro Diagnostic Use".

Hematoxylin, a common nuclear stain, is isolated from an extract of logwood (*Haematoxylin campechianun*).[1] The first successful biologic application of hematoxylin was described by Bohmer in 1865.[1] Mayer introduced his formulation in 1903.[2] Since then numerous formulations have appeared. Of these, Harris', Gill's, Mayer's and Weigert's have retained popularity. Before hematoxylin can be used as a nuclear stain, it must be oxidized to hematein and combined with a metallic ion (mordant). Most successful mordants have been salts of aluminum or iron.

Generally, hematoxylin solutions are classified as progressive or regressive based on dye concentration. Progressive stains (e.g., Mayer's hematoxylin) have a lower concentration of dye and selectively stain nuclear chromatin without staining cytoplasmic structures. The desired intensity is a function of time. If staining times are excessive, a progressive stain might act similarly to a regressive stain solution. Staining with progressive stains generally requires more time than staining with regressive stains. Regressive stains (e.g. Harris hematoxylin) color all stainable tissue components (nuclear and cytoplasmic) intensely. To arrive at the correct staining response, excess dye must be removed from the tissue section. After sufficient differentiation, a properly destained section will demonstrate nuclear staining, but will not stain cytoplasmic structures.

The final step in hematoxylin staining is the "blueing" of the tissue section. Initially tissue sections are colored either purple or a reddish purple. After exposure to alkaline solutions (warm tap water [if slightly alkaline], dilute ammonia water, Scott's tap water substitute, or lithium carbonate), the tissue section takes on the characteristic blue color of a hematoxylin stained slide.

REAGENT

MAYER'S HEMATOXYLIN SOLUTION, Catalog No. MHS
(MHS1-100ML / MHS16-500ML / MHS32-1L / MHS80-2.5L / MHS128-4L)
Certified hematoxylin (1.0 g/l), sodium iodate (0.2 g/l), aluminum ammonium sulfate·12 H_2O (50 g/l), chloral hydrate (50 g/l) and citric acid (1 g/l).

STORAGE AND STABILITY:

Store reagent at room temperature (18–26°C) protected from light. Reagent is stable until expiration date shown on the label. Do not return used solution to stock bottle.

DETERIORATION:

Discard if staining times becomes excessive or solution turns brown.

PREPARATION:

Filter Mayer's Hematoxylin Solution before each use. Solution is then ready to use.

PRECAUTIONS:

Normal precautions exercised in handling laboratory reagents should be followed. Dispose of waste observing all local, state, provincial or national regulations. Refer to Material Safety Data Sheet and product labeling for any updated risk, hazard or safety information.

PROCEDURE

SPECIMEN COLLECTION:

It is recommended that specimen collection be carried out in accordance with CLSI document M29-A3. No known test method can offer complete assurance that blood samples or tissue will not transmit infection. Therefore, all blood derivatives or tissue specimens should be considered potentially infectious.

Standard histology texts provide necessary details for specimen collection and storage.[4,5]

SPECIAL MATERIALS REQUIRED BUT NOT PROVIDED:

Eosin Y Solution Counterstains:
Alcoholic, Catalog No. HT1101
(HT110116-500ML / HT110132-1L / HT110180-2.5L / HT1101128-4L)
Aqueous, Catalog No. HT1102
(HT110216-500ML / HT110232-1L / HT110280-2.5L / HT1102128-4L)
or Alcoholic with Phloxine Catalog No. HT1103
(HT110316-500ML / HT110332-1L / HT110380-2.5L / HT1103128-4L)
Reagent Alcohol, Catalog No. R8382-1GA OR Ethanol, 100%
Scott's Tap Water Substitute Concentrate, Catalog No. S5134
Xylene or Xylene Substitute
Microscope, microscope slides, coverslips, and staining dishes

NOTES:

1. The times given in the insert are approximate. Personal preferences will vary and the times can be adjusted to suit personal preferences. Stain solutions which are heavily used will lose their staining powers and the staining times should be lengthened or new solutions should be used.[6]
2. Dilute alkaline solutions may be used in place of warm running tap water. This will shorten the time needed for the staining procedure. If using a dilute alkali solution, be sure to wash slides an additional 2–3 minutes in running tap water before proceeding to Eosin staining.
3. Some tap water supplies are acidic and unsuitable for use in the "blueing" portion of this procedure. If tap water is acidic, use a dilute alkaline solution.
4. Purple or red-brown nuclei are indicative of inadequate "blueing".
5. If eosin staining is excessive, nuclear staining may be masked. Proper eosin staining will demonstrate a 3-tone effect. To increase differentiation of eosin, extend time in alcohols or use a first alcohol with a higher water content. The times in the alcohols may be adjusted to obtain the proper degree of Eosin staining.
6. Filter working stain solution daily. Rotate alcohols and xylene/xylene substitute daily.
7. Adding new stock to depleted working solutions of Mayer's Hematoxylin or Eosin is not recommended.
8. Avoid excessive water carry-over into Mayer's Hematoxylin.
9. Positive control slides should be included in each run.
10. The data obtained from this procedure serves only as an aid to diagnosis and should be reviewed in conjunction with other clinical diagnostic tests or information.

PROCEDURE 1:
HEMATOXYLIN AND EOSIN STAINING

1. Prepare a 95% alcohol solution by adding 5 ml deionized water to 95 ml Reagent Alcohol or Ethanol (100%).
2. Deparaffinize to water or fix and hydrate frozen sections.
3. Stain in Mayer's Hematoxylin Solution.....15 minutes
4. Rinse in warm running tap water.....15 minutes
5. Place in distilled water.....30 seconds
6. If Alcoholic Eosin is to be used:
   Place in Reagent Alcohol, 95%.....30 seconds
7. Place in Eosin Y Solution Counterstain:
   Alcoholic,
   Aqueous
   or Alcoholic with Phloxine.....30–60 seconds
8. Dehydrate and clear through 2 changes each of
   95% Reagent Alcohol, absolute Reagent Alcohol, and xylene.....2 minutes each
9. Mount with resinous mounting medium.

PROCEDURE 2:
NUCLEAR COUNTERSTAIN FOR SPECIAL STAINS

1. Complete individual staining procedure.
2. Rinse in deionized water.
3. Stain in Mayer's Hematoxylin Solution 1–5 minutes.
4. Rinse in running tap water or dilute alkaline solution until nuclei are blue.
5. Rinse in deionized water.
6. If any portion of the stain is alcohol soluble, mount in aqueous mounting media. If stain is alcohol insoluble, dehydrate in alcohol, clear in xylene or xylene substitute and mount in resinous mounting media.

PERFORMANCE CHARACTERISTICS

EXPECTED RESULTS

Nuclear chromatin should be blue. Nucleoli should be visible. Cytoplasm will display various shades of pink to pink-orange (depending upon the counterstain used) and red blood cells will be red.

If observed results vary from expected results, please contact Sigma-Aldrich Technical Service for assistance.

REFERENCES

1. Conn's Biological Stains, 9th ed., RD Lillie, Editor, Williams and Wilkens Co., Baltimore (MD), pp 468, 472, 1977
2. Mayer P, (1903) Notiz über Hämateïn und Hämalaun. Zeitschrift für wissenschaftliche Mikroskopie und für mikroskopische Technick, 20, 409
3. Theory and Practice of Histological Techniques, 2nd ed., Bancroft JD and Stevens A, Editors, Churchill Livingstone, New York (NY), page 111, 1982
4. Theory and Practice of Histotechnology, 2nd ed., Sheehan DC, Hrapchak BB, Editors, CV Mosby Co, St Louis (MO) 1980
5. Laboratory Methods in Histotechnology of the Armed Forces Institute of Pathology, 4th ed., Prophet EB, Mills B, Arrington JB and Sobin LH, Editors, American Registry of Pathology, Washington DC 1992
6. Theory and Practice of Histological Techniques, Edited by Bancroft JD and Gamble, M, Churchill Livingstone, New York, 2002, p129

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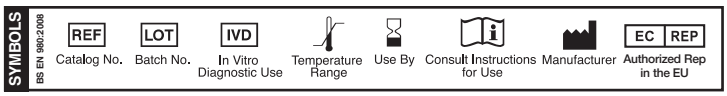

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