
INTENDED USE

Harris Hematoxylin Solutions are nuclear stains intended for use in Histology and Cytology. Harris Hematoxylin Solutions are for "In Vitro Diagnostic Use".

Hematoxylin, a common nuclear stain, is isolated from an extract of logwood (Haematoxylon campechianum).¹ The first successful biologic application of hematoxylin was described by Bohmer¹ in 1865. 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.

Hematoxylin Solutions are regressive stains for use in routine histology and cytology. The positively charged aluminum-hematein complex combines with negatively charged phosphatase of nuclear DNA forming the blue purple color characteristic of hematoxylin stains.

Harris Hematoxylin Solution, may also be used in conjunction with Papanicolaou staining procedures for cytology use. See Sigma-Aldrich Procedure No. HT40.

REAGENT

HARRIS HEMATOXYLIN SOLUTION, Catalog No. HHS

Certified hematoxylin, 7.0 g/l, sodium iodate, aluminum ammonium sulfate 12 H₂O, preservative and stabilizers.

STORAGE AND STABILITY:

Store reagent at room temperature (18-26°C) protected from light. Reagent is stable until expiration date shown on the label.

DETERIORATION:

Discard if staining time becomes excessive or if solution color changes from plum to blue or brown.

PREPARATION:

Filter Harris Hematoxylin Solution before each 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 for any updated risk, hazard or safety information.

US Risks and Safety Statements

Avoid contact and inhalation of Harris Hematoxylin Solution. Target organs: Heart and nerves.

Reagent Alcohol is **FLAMMABLE** and an **IRRITANT**. Irritating to eyes, respiratory system and skin. Keep container tightly closed. Keep away from sources of ignition - no smoking. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing. Target organs: Nerves and liver.

Scott's Tap Water Substitute. Caution: Substance not yet fully tested.

ACCUMATE™ Differentiation Solution is **CORROSIVE** and **FLAMMABLE**. Causes burns. Wear suitable protective clothing. Keep away from sources of ignition - no smoking. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

Alcoholic Eosin Solutions are **FLAMMABLE** and **HARMFUL**. Harmful by inhalation, in contact with skin, and if swallowed. Harmful: possible risk of irreversible effects through inhalation, in contact with skin, and if swallowed. Irritating to eyes, respiratory system and skin. Keep away from sources of ignition - no smoking. Wear suitable protective clothing and gloves. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

Aqueous Eosin Solution. Caution: Substance not yet fully tested.

Xylene is **FLAMMABLE** and **HARMFUL**. Possible risk of impaired fertility. May cause harm to the unborn child. Harmful by inhalation and in contact with skin. Irritating to respiratory system and skin. Risk of serious damage to eyes. Keep away from sources of ignition - no smoking. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves and eye/face protection. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

Hydrochloric Acid Solution is **TOXIC**. Toxic by inhalation. Causes burns. Irritating to respiratory system. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

EU Risks and Safety Statements

Harris Hematoxylin Solution. Do not breathe vapor. Avoid contact with skin and eyes.

Reagent Alcohol is **HIGHLY FLAMMABLE** and an **IRRITANT**. Highly Flammable. Irritating to eyes, respiratory system and skin. Keep container tightly closed. Keep away from sources of ignition - no smoking. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing.

Scott's Tap Water Substitute. Caution: Substance not yet fully tested.

ACCUMATE™ Differentiation Solution is **TOXIC**. Flammable. Causes burns. Keep away from sources of ignition - no smoking. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

Alcoholic Eosin Solutions are **HIGHLY FLAMMABLE** and **HARMFUL**. Highly flammable. Harmful by inhalation, in contact with skin, and if swallowed. Harmful: possible risk of irreversible effects through inhalation, in contact with skin, and if swallowed. Keep away from sources of ignition - no smoking. Wear suitable protective clothing and gloves. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

Aqueous Eosin Solution. Caution: Substance not yet fully tested.

Xylene is **HARMFUL**. Flammable. Harmful by inhalation and in contact with skin. Irritating to skin. Avoid contact with eyes.

Hydrochloric Acid Solution is **CORROSIVE**. Causes burns. Irritating to respiratory system. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves, and eye/face protection. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

PROCEDURE

SPECIMEN COLLECTION:

It is recommended that specimen collection be carried out in accordance with NCCLS document M29-A2. 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.^{2,3}

SPECIAL MATERIALS REQUIRED BUT NOT PROVIDED:

ACCUMATE™ Differentiating Solution, Catalog Nos. A 3179 or A 3429

Eosin Y Solution Counterstains:

Alcoholic, Catalog No. HT110-1

OR

Aqueous, Catalog No. HT110-2

OR

Alcoholic with Phloxine, Catalog No. HT110-3

Reagent Alcohol OR Ethanol, 100%

Scott's Tap Water Substitute Concentrate

Xylene

Hydrochloric Acid, Concentrated

Microscope, Microscope slides, coverslips, and staining dishes

NOTES:

1. Staining times may be varied for individual color preference.
2. Other dilute alkaline solutions may be used in place of Scott's Tap Water Substitute.
3. A 0.25% acid alcohol solution may be used in place of **ACCUMATE™** Differentiating Solution. Prepare by adding 0.25 ml concentrated Hydrochloric Acid to 100 ml of 70% alcohol.
4. 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.⁴
5. 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.
6. Purple or red-brown nuclei are indicative of inadequate "blueing".
7. 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.
8. Positive control slides should be included in each run.
9. 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. Prepare a 95% alcohol solution by adding 5 ml deionized water to 95 ml Reagent Alcohol, No. R 8382, or Ethanol (100%).
2. Prepare Scott's Tap Water Substitute solution as indicated on bottle label.
3. Deparaffinize to water or fix and dehydrate frozen sections.
4. Stain in Harris Hematoxylin Solution2.0 to 2.5 min.
5. Rinse slide in running tap water.
6. **ACCUMATE™** Differentiating Solution.....1-2 dips.
7. Rinse slide in running tap water.
8. Blue in Scott's Tap Water Substitute.....5-60 secs.
9. Reagent Alcohol, 95%30 sec.
10. Eosin Y Solution Counterstain:
Alcoholic, No. HT110-1
OR
Alcoholic with Phloxine, No. HT110-3.....30-60 secs.
11. Dehydrate, clear and mount.

PERFORMANCE CHARACTERISTICS

EXPECTED RESULTS:

Nuclear chromatin should be blue. Nucleoli should be conspicuous and crisply outlined. Cytoplasm will display various shades of pink to pink-orange depending upon the counterstain used and RBC's will be red.

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

REFERENCES

1. Natural Dyes, IN J Conn's Biological Stains, 9th ed., RD Lillie, Editor, Williams and Wilkens Co., Baltimore, MD, 1977, pp 468, 472
2. Theory and Practice of Histotechnology, 2nd ed., DC Sheehan, BB Hrapchak, Editors, CV Mosby Co., St. Louis, MO, 1980
3. Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology, 3rd ed., LG Luna, Editor, McGraw Hill, New York, 1968
4. Theory and Practice of Histological Techniques, Edited by Bancroft JD and Gamble, M, Churchill Livingstone, New York, 2002, p129

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