SIGMA-ALDRICH®

HARRIS HEMATOXYLIN SOLUTION

(Procedure No. HHS)

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 Bohmer[1] 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
(HHS16-500ml; HHS32-1L; HHS80-2.5L; HHS128-4L)
Certified hematoxylin, 7.0 g/l, sodium iodate, aluminum ammonium sulfate • 12 H_2O, 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.

Scott's Tap Water Substitute is prepared by mixing 1 part of Scott's Tap Water Substitute Concentrate with 9 parts deionized water.

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.[2,3]

SPECIAL MATERIALS REQUIRED BUT NOT PROVIDED:
Differentiation Solution, Catalog Nos. A3179-1L or A3429-4L

Eosin Y Solution Counterstains

Eosin Y Solution, Alcoholic, Catalog No. HT1101
(HT110116-500ml; HT110132-1L; HT110180-2.5L; HT1101128-4L)
OR
Eosin Y Solution, 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, 10x, Catalog No. S5134-6x100ml
Xylene or Xylene substitute
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 Differentiation 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 or Ethanol (100%).
2. Deparaffinize to water or fix and dehydrate frozen sections.
3. Stain in Harris Hematoxylin Solution.....2.0 to 2.5 min.
4. Rinse slide in running tap water.
5. Differentiation Solution.....1-2 dips.
6. Rinse slide in running tap water.
7. Blue in Scott's Tap Water Substitute.....5-60 secs.
8. Reagent Alcohol, 95%.....30 sec.
9. **Eosin Y Solution Counterstain:**

   Eosin Y Solution, Alcoholic, Catalog No. HT1101
   OR
   Eosin Y Solution, Alcoholic, With Phloxine, Catalog No. HT1103.....30-60 secs.
10. 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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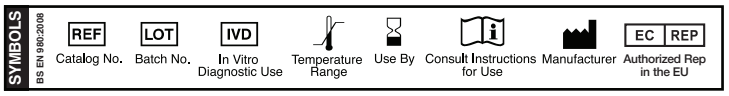

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