
INTENDED USE

Gill Hematoxylin solutions are nuclear stains intended for use in Histology and Cytology. Hematoxylin Solutions, Gill Nos. 1, 2 and 3 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.

Generally, hematoxylins 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 stains nuclear chromatin. The desired intensity is a function of time. Regressive stains (e.g., Harris hematoxylin) color all nuclear and cytoplasmic structures intensely. To arrive at correct chromatic response, excess dye must be removed by treatment with dilute acid (differentiation).

Gill No. 1 formulation is used as a progressive cytology stain, Gill formulations No. 2 and No. 3 may be used as progressive or regressive stains depending on length of staining time. These hematoxylin solutions are manufactured as half-oxidized hematoxylin; mordanted with aluminum and stabilized with glycols. The positively charged aluminum-hematein complex combines with negatively charged phosphate groups of nuclear DNA forming the blue-purple color characteristic of hematoxylin stains.

REAGENTS

HEMATOXYLIN SOLUTION, GILL NO. 1, Catalog No. GHS-1

Certified hematoxylin, 2 g/l, sodium iodate, 0.2 g/l, aluminum sulfate, 17.6 g/l, and stabilizers.

HEMATOXYLIN SOLUTION, GILL NO. 2, Catalog No. GHS-2

Certified hematoxylin, 4 g/l, sodium iodate, 0.4 g/l, aluminum sulfate, 35.2 g/l, and stabilizers.

HEMATOXYLIN SOLUTION, GILL NO. 3, Catalog No. GHS-3

Certified hematoxylin, 6 g/l, sodium iodate, 0.6 g/l, aluminum sulfate, 52.8 g/l, and stabilizers.

STORAGE AND STABILITY:

Store reagents at room temperature (18-26°C) protected from light. Reagents are stable until the expiration date shown on the label.

DETERIORATION:

Discard if solutions turn brown (over-oxidized from air) or purple (loss of acidity).

PREPARATION:

Hematoxylin Solutions, Gill Nos. 1, 2 and 3, are provided ready for use.

Scott's Tap Water Substitute is prepared by diluting 1 volume Scott's Tap Water Substitute Concentrate with 9 volumes 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 for any updated risk, hazard or safety information.

US Risks and Safety Statements

Gill Hematoxylin Solutions are HARMFUL. Harmful if swallowed. Irritating to eyes, respiratory system and skin. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing. Target organs: Liver and kidneys.

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. Target organs: Eyes and kidneys.

Alcoholic Eosin Solutions, Papanicolaou Stain OG 6, Papanicolaou Stain solutions (Modified EA, EA50 and EA65) are 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.

Gill Modified EA Solution is FLAMMABLE and an IRRITANT. Harmful by inhalation, in contact with skin and if swallowed. Toxic: danger of very serious 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. Avoid contact with skin. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible). Target organs: Nerves and eyes.

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).

Xylene Substitute is FLAMMABLE and an IRRITANT. Irritating to eyes, respiratory system and skin. 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. Target organs: Nerves and kidneys.

EU Risks and Safety Statements

Gill Hematoxylin Solutions are HARMFUL. Harmful if swallowed. Irritating to eyes. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing.

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, Papanicolaou Stain OG 6, Papanicolaou Stain solutions (Modified EA, EA50 and EA65) 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.

Gill Modified EA Solution is HIGHLY FLAMMABLE and TOXIC. Highly flammable. Harmful by inhalation, in contact with skin and if swallowed. Toxic: danger of very serious irreversible effects through inhalation, in contact with skin and if swallowed. Keep away from sources of ignition - no smoking. 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).

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

Xylene Substitute is HIGHLY FLAMMABLE and an IRRITANT. Highly flammable. Irritating to eyes, respiratory system and skin. 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.

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.^{2,3}

SPECIAL MATERIALS REQUIRED BUT NOT PROVIDED:

ACCUMATE™ DIFFERENTIATING SOLUTION, Catalog Nos. A 3179, A 3304 and A 3429

COUNTERSTAINS

(choice depends on specimen and individual preference):

EOSIN Y SOLUTION, ALCOHOLIC, Catalog No. HT110-1

EOSIN Y SOLUTION, AQUEOUS, Catalog No. HT110-2

EOSIN Y SOLUTION, ALCOHOLIC WITH PHLOXINE, Catalog No. HT110-3

PAPANICOLAOU STAIN OG 6, Catalog No. HT40-1

PAPANICOLAOU STAIN, Modified EA, Catalog No. HT40-2

PAPANICOLAOU STAIN EA 50, Catalog No. HT40-3

PAPANICOLAOU STAIN EA 65, Catalog No. HT40-4

GILL MODIFIED EA SOLUTION, Catalog No. 391-5

REAGENT ALCOHOL, Catalog No. R 8382

OR

ETHANOL, 100%

NOTE: A dilution (95%) of Reagent Alcohol or Ethanol is also required.

SCOTT'S TAP WATER SUBSTITUTE CONCENTRATE, Catalog No. S 5134

XYLENE, Catalog No. 24,764-2

XYLENE SUBSTITUTE, Catalog No. A 5597

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.⁴
2. 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.
3. Purple or red-brown nuclei are indicative of inadequate "blueing".
4. 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.
5. Positive control slides should be included in each run.
6. 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:

(Staining Exfoliative Cytology Preparations Using Hematoxylin Solution, Gill No. 1 or Gill No. 2)

1. Fix cytologic smears in 95% ethanol15 minutes
2. Rinse in gently running tap water.....30 seconds
3. Stain in Hematoxylin Solution, Gill No. 1 or Gill No. 2.....1.5-3 minutes
4. Rinse in tap water.
5. Scott's Tap Water Substitute.....15-60 seconds
6. Rinse in tap water.
7. Reagent Alcohol, 95%.....10 dips
8. Counterstain in Papanicolaou Stain OG 6, Catalog No. HT40-11.5 minutes
9. Reagent Alcohol, 95%.....10 dips
10. Papanicolaou Stain EA 50, Catalog No. HT40-3, **Or**
Papanicolaou Stain EA 65, Catalog No. HT40-4, **Or**
Papanicolaou Stain Modified EA, Catalog No. HT40-2, **Or**
Gill Modified EA, Catalog No. 391-5.....2.5-3 minutes
11. Reagent Alcohol, 95%, two changes10 dips each
12. Reagent Alcohol, 100%, two changes.....1 minute each
13. Xylene, two changes.....2 minutes each
14. Coverslip and examine microscopically.

PROCEDURE 2:

(Histology and/or Cytology Staining Using Hematoxylin Solution Gill No. 2 or Gill No. 3)

1. Deparaffinize to water or fix and dehydrate frozen sections.
2. Stain in Hematoxylin Solution, Gill No. 2 or Gill No. 3.....1.5-3 minutes
3. Tap water wash.
4. ACCUMATE™ Differentiating Solution20-60 seconds
5. Tap water wash.
6. Blue in Scott's Tap Water Substitute5-60 seconds
7. Tap water wash.
8. Counterstain:
For Histology
Eosin Y Solution, Alcoholic, Catalog No. HT110-1, **Or**
Acidified Eosin Y Solution, Aqueous, Catalog No. HT110-2, **Or**
Eosin Y Solution, Alcoholic with Phloxine, Catalog No. HT110-3.....30-60 seconds
For Cytology
Papanicolaou Stain OG 6, Catalog No. HT40-3, **And**
Papanicolaou Stain EA 50, Catalog No. HT40-3, **Or**
Papanicolaou Stain EA 65, Catalog No. HT40-4, **Or**
Papanicolaou Stain, Modified EA, Catalog No. HT40-2, **Or**
Gill Modified EA Solution, Catalog No. 391-51-3 minutes
9. Dehydrate, clear and mount.

PERFORMANCE CHARACTERISTICS

EXPECTED RESULTS:

Chromatin appears blue to blue-black and nucleoli should be conspicuous. Cytoplasmic staining with Gill No. 1 and No. 2 Hematoxylin Solutions should be pale or absent; thus, acid differentiation may not be necessary. Gill No. 3 Hematoxylin Solution should be considered a regressive stain.

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

REFERENCES

1. Natural Dyes. IN HJ 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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