
INTENDED USE

Alcoholic and aqueous Eosin Y solutions are intended for use as a general purpose counterstains. Eosin Y Solutions are for “In Vitro Diagnostic Use”.

Eosin Y is the most commonly used cytoplasmic stain.¹ As early as 1885 it was advocated by List as a counterstain for methyl green. It is also employed in conjunction with basic blue dyes, and when used in combination with hematoxylin it is referred to as “H & E” stains.

Eosin Y is an acid stain which interacts with cellular proteins rich in basic amino acids. A dye protein complex is formed that is characterized by vivid pink cytoplasmic staining.

REAGENTS

EOSIN Y SOLUTION, ALCOHOLIC, Catalog No. HT110-1
Certified eosin Y, 0.5% (w/v), in acidified ethanol, 90% (v/v).

EOSIN Y SOLUTION, AQUEOUS, Catalog No. HT110-2
Certified eosin Y, 0.5% (w/v).

EOSIN Y SOLUTION, ALCOHOLIC, WITH PHLOXINE, Catalog No. HT110-3
Certified eosin Y, 0.1% (w/v), and certified phloxine B, 0.1% (w/v) in acidified ethanol.

HEMATOXYLIN SOLUTION, Catalog No. HHS
Certified hematoxylin, 7.0 g/l, sodium iodate, aluminum ammonium sulfate • 12 H₂O, and stabilizers.

HEMATOXYLIN SOLUTION, GILL NO. 2, Catalog No. GHS-2
Certified hematoxylin, 4 g/l, sodium iodate, 0.4 g/l, aluminum ammonium sulfate • 12 H₂O, 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 ammonium sulfate • 12 H₂O, 52.8 g/l, and stabilizers.

SCOTT'S TAP WATER SUBSTITUTE CONCENTRATE, Catalog No. S 5134
Magnesium sulfate, 200 g/l, sodium bicarbonate, 20 g/l, and preservative.

STORAGE AND STABILITY:

All reagents are stored at room temperature (18–26°C). Reagent labels bear expiration date.

PREPARATION:

Eosin Y Alcoholic Solutions; Eosin Y Solution, Alcoholic, with Phloxine; Harris Hematoxylin; and Gill's 1, 2 and 3 Hematoxylin solutions should be filtered before use.

Acidified Eosin Y Solution, Aqueous is prepared by slowly adding up to 0.5 ml of glacial acetic acid per 100 ml of stain.

Scott's Tap Water Substitute Working solution is prepared by diluting 1 part of Scott's Tap Water Substitute Concentrate with 9 parts of 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

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). Target organs: Eyes and nerves.

Aqueous Eosin Solution. Caution: Substance not yet fully tested.
Avoid contact and inhalation of Harris Hematoxylin Solution. Target organs: heart and nerves.

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.

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).

EU Risks and Safety Statements

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.

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

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).

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

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 the necessary details.^{2,3}

SPECIAL MATERIALS REQUIRED BUT NOT PROVIDED:

Ethanol, 95% and 100% or Reagent Alcohol
Xylene
Synthetic Mounting Media
ACCUMATE™ Differentiation Solution, Catalog No. A 3179

NOTES:

- 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
- Positive control slides should be included in each run.
- 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 such as Scott's Tap Water Substitute.
- Purple or red-brown nuclei are indicative of inadequate “blueing”.
- 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.
- Add new stock to depleted working solutions of hematoxylin or eosin is not recommended.
- Avoid excessive water carry-over into alcoholic eosin solutions.
- 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.

PROCEDURES:

EOSIN Y SOLUTIONS, ALCOHOLIC

- Deparaffinize to water or fix and hydrate frozen sections.
- Stain in hematoxylin.
- Rinse slides in running tap water.
- Differentiate if regressive hematoxylin is used. Rinse in running tap water.
- Blue in Scott's Tap Water Substitute.
- Rinse in running tap water.
- Rinse slides in 95% ethanol or reagent alcohol for 30 seconds.
- Counterstain in Alcoholic Eosin Y Solution (30 seconds – 3 minutes).
- Dehydrate, clear and mount.

EOSIN Y SOLUTION, AQUEOUS

- Deparaffinize to water or fix and hydrate frozen sections.
- Stain in hematoxylin.
- Rinse slides in running tap water.
- Differentiate if regressive hematoxylin is used. Rinse in running tap water.
- Blue in Scott's Tap Water Substitute.
- Rinse in running tap water.
- Aqueous Eosin Y Solution may be acidified by adding up to 0.5 ml of glacial acetic acid per 100 ml of stain.
- Counterstain in Acidified Aqueous Eosin Y Solution (30 seconds – 3 minutes).
- Dehydrate, clear and mount.

PERFORMANCE CHARACTERISTICS

EXPECTED RESULTS

Cytoplasm should be pink to red. Nuclei should be blue to blue-black, depending on the hematoxylin used.



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 & Wilkins, Baltimore, MD, 1977, p 342
2. Theory and Practice of Histotechnology, 2nd ed., DC Sheehan and BB Hrapchak, Editors, CV Mosby, 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, NY, 1968
4. Theory and Practice of Histological Techniques, Edited by Bancroft JD and Gamble, M, Churchill Livingstone, New York, NY, 2002, p 129

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