

INTENDED USE

Alcoholic and aqueous Eosin Y solutions are intended for use as 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. HT1101
(HT110116-500 ml; HT110132-1L; HT110180-2.5L; HT1101128-4L)
Certified eosin Y, 0.5% (w/v), in acidified ethanol, 90% (v/v).

EOSIN Y SOLUTION, AQUEOUS, Catalog No. HT1102
(HT110216-500 ml; HT110232-1L; HT110280-2.5L; HT1102128-4L)
Certified eosin Y, 0.5% (w/v).

EOSIN Y SOLUTION, ALCOHOLIC, WITH PHLOXINE, Catalog No. HT1103
(HT110316-500 ml; HT110332-1L; HT110380-2.5L; HT1103128-4L)
Certified eosin Y, 0.1% (w/v), and certified phloxine B, 0.1% (w/v) in acidified ethanol.

HEMATOXYLIN SOLUTION, HARRIS MODIFIED, Catalog No. HHS
(HHS16-500 ml; HHS32-1L; HHS80-2.5L; HHS128-4L)
Certified hematoxylin, 7.0 g/l, sodium iodate, aluminum ammonium sulfate • 12 H₂O, and stabilizers.

HEMATOXYLIN SOLUTION, GILL NO. 1, Catalog No. GHS1
(GHS116-500 ml; GHS132-1L; GHS1128-4L)
Certified hematoxylin, 2 g/l, sodium iodate, 0.2 g/l, aluminum ammonium sulfate • 12 H₂O, 17.6 g/l, and stabilizers.

HEMATOXYLIN SOLUTION, GILL NO. 2, Catalog No. GHS2
(GHS216-500 ml; GHS232-1L; GHS280-2.5L; GHS2128-4L)
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. GHS3
(GHS316-500 ml; GHS332-1L; GHS380-2.5L; GHS3128-4L)
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. S5134-6x100 ml
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). Reagents are stable until expiration date shown on the label.

PREPARATION:

Eosin Y Alcoholic Solutions; Eosin Y Solution, Alcoholic, with Phloxine; and Harris Modified Hematoxylin solutions should be filtered before use. Hematoxylin solutions, Gill Nos. 1, 2 and 3 are provided ready to 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 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 Safety Data Sheet and product labeling for any updated risk, hazard or safety information.

PROCEDURE

SPECIMEN COLLECTION:

All samples must be clearly labeled. Suitable instruments must be used for obtaining and preparing samples. Follow manufacturer's instructions for use. 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 or xylene substitute

Synthetic Mounting Media

Differentiation Solution, Catalog Nos. A3179-1L or A3429-4L

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.
- Addition of 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.

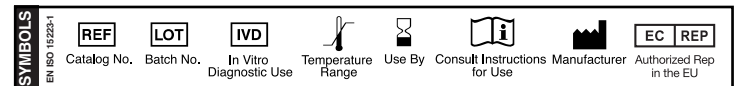
If observed results vary from expected results, please contact Sigma-Aldrich Technical Service for assistance.

REFERENCES

- Natural Dyes, In HJ Conn's Biological Stains, 9th ed., RD Lillie, Editor, Williams & Wilkins, Baltimore, MD, 1977, p 342
- Theory and Practice of Histotechnology, 2nd ed., DC Sheehan and BB Hrapchak, Editors, CV Mosby, St. Louis, MO, 1980
- Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology, 3rd ed., LG Luna, Editor, McGraw Hill, New York, NY, 1968
- 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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