

INTENDED USE

Sigma-Aldrich Trichrome Stains (Masson) are intended for use in the study of connective tissue, muscle and collagen fibers. Trichrome Stain reagents are for "In Vitro Diagnostic Use".

"Trichrome" stains are used primarily for distinguishing collagen from muscle tissue.¹ In general, they consist of nuclear, collagenous and cytoplasmic dyes in mordants such as phosphotungstic or phosphomolybdic acid. Historically, the first trichrome system was attributed to Mallory.^{2,3} Further modifications were introduced by Masson and Gomori.^{3,4} The procedure described here is based on the work of Masson as modified by Lillie⁵ using aniline blue as a collagen stain instead of a green dye. Tissue sections are treated with Bouin's solution to intensify the final coloration. Nuclei are stained with Weigert's iron hematoxylin, and cytoplasm¹ and muscle are then stained with Beibrich scarlet-acid fuchsin. After treatment with phosphotungstic and phosphomolybdic acid, collagen is demonstrated by staining with aniline blue. Rinsing in acetic acid after staining renders the shades of color more delicate and transparent.⁴ Included is a trichrome stain technique for rapid staining in microwave ovens.^{6,9}

REAGENTS

BIEBRICH SCARLET-ACID FUCHSIN SOLUTION, Catalog No. HT15-1
Biebrich scarlet, 0.9%, acid fuchsin 0.1%, in acetic acid, 1.0%.

PHOSPHOTUNGSTIC ACID SOLUTION, Catalog No. HT15-2
Phosphotungstic acid, 10%.

PHOSPHOMOLYBDIC ACID SOLUTION, Catalog No. HT15-3
Phosphomolybdic Acid, 10%

ANILINE BLUE SOLUTION, Catalog No. HT15-4
Aniline blue, 2.4% and acetic acid, 2%.

STORAGE AND STABILITY:

Store reagents at room temperature (18–26°C). Reagent labels bear expiration dates. Formation of a precipitate in Phosphomolybdic Acid Solution, Catalog No. HT15-3, does not affect performance.

Trichrome Stain solutions, Bouin's solution, Weigert's Iron Hematoxylin, Trichrome TISSUE-TROL, Acetic Acid solution and Scott's Tap Water Substitute Concentrate and Working solution should be stored at room temperature (18–26°C).

Biebrich Scarlet-Acid Fuchsin solution, Bouin's solution, Weigert's Iron Hematoxylin (Parts A and B), Trichrome TISSUE-TROL, and Scott's Tap Water Substitute Concentrate and Working solution are stable until expiration date.

Weigert's Iron Hematoxylin working solution should be prepared fresh for each use.

PREPARATION:

Prepare Working Phosphotungstic/Phosphomolybdic Acid Solution by mixing 1 volume of Phosphotungstic Acid Solution, Catalog No. HT15-2, and 1 volume Phosphomolybdic Acid Solution, Catalog No. HT15-3, with 2 volumes of deionized water. Discard after one use.

Biebrich Scarlet-Acid Fuchsin, Bouin's solution, Gill's 3 hematoxylin are ready to use.

Weigert's Iron Hematoxylin solution is prepared by mixing equal parts of Solution A and Solution B.

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

Prepare 1% Acetic Acid by diluting 8.8 ml 1N Acetic Acid with 41.2 ml 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.

Trichrome TISSUE-TROL control slides are paraffin embedded human tissue containing collagen and muscle tissue and should be considered potentially infectious.

US Risks and Safety Statements

Biebrich Scarlet-Acid Fuchsin Solution, Phosphotungstic Acid Solutions and Phosphomolybdic Acid Solutions are CORROSIVE. Causes Burns. 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).

Aniline Blue Solution is HARMFUL. Harmful in contact with skin. 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: Teeth 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.

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).

Bouin's Solution is HARMFUL. Explosive when dry. Harmful by inhalation, in contact with skin and if swallowed. Limited evidence of a carcinogenic effect. May cause sensitization by skin contact. Irritating to eyes, respiratory system and skin. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible). 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. Use only in well ventilated areas. Keep locked up and out of the reach of children.

Weigert's Iron Hematoxylin Solution, Part A 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. Target organs: Nerves and liver.

Weigert's Iron Hematoxylin Solution, Part B is TOXIC. Toxic by inhalation. Causes burns. 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).

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.

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

1N Acetic Acid Solutions are CORROSIVE. Causes severe burns. 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). Target organs: Kidneys and teeth.

EU Risks and Safety Statements (Caution: Substances not yet fully tested)

Biebrich Scarlet-Acid Fuchsin Solution, Phosphotungstic Acid Solutions and Phosphomolybdic Acid Solutions are CORROSIVE. Causes Burns. 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).

Aniline Blue Solution is HARMFUL. Harmful in contact with skin. 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.

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

Bouin's Solution is HARMFUL. Explosive when dry. Harmful by inhalation, in contact with skin and if swallowed. Limited evidence of a carcinogenic effect. May cause sensitization by skin contact. Irritating to eyes, respiratory system and skin. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. This material and its container must be disposed of in a safe way. Wear suitable protective clothing and gloves.

Weigert's Iron Hematoxylin Solution, Part A 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.

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.

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

1N Acetic Acid Solutions. Avoid contact with skin and eyes. Do not breathe vapor.

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.

Any well fixed paraffin section cut at 5–6 microns may be used. Incorporate appropriate control slides.

SPECIAL MATERIALS REQUIRED BUT NOT PROVIDED:

Positive control slides, such as Sigma Trichrome TISSUE-TROL, Catalog No. T 8800, should be included in each run

1N Acetic Acid

Bouin's Solution, Catalog No. HT10-1-32 or HT10-1-128, saturated aqueous picric acid, 71%, formaldehyde, 24% and acetic acid, 5%

FOR STANDARD PROCEDURE ONLY:

Weigert's Iron Hematoxylin Set, Catalog No. HT10-79

Weigert's Iron Hematoxylin Solution, Part A, Catalog No. HT10-7, 1% certified hematoxylin in ethanol

Weigert's Iron Hematoxylin Solution, Part B, Catalog No. HT10-9, ferric chloride 1.2% (w/v) and Hydrochloric acid, 1% (v/v)

FOR MICROWAVE PROCEDURE ONLY:

Hematoxylin Solution, Gill No. 3, Catalog No. GHS-3

Scott's Tap Water Substitute Concentrate, Catalog No. S 5134

Coplin Jar (plastic) with vented lids

ACCUMATE™ H2100 Microwave Oven, Catalog Nos. A 9084 (110 v) or A 9209 (220 v)

NOTES:

If the Sigma-Aldrich H2100 Microwave Oven is used, please see the Owner's Manual for instructions.

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:

STANDARD PROCEDURE

1. Deparaffinize slides to deionized water.
2. Mordant in preheated Bouin's Solution, Catalog No. HT10-1, at 56°C for **15 minutes** or at **room temperature overnight**.
3. Cool slides in tap water (**18-26°C**) contained in a Coplin jar.
4. Wash in running tap water to remove yellow color from sections.
5. Stain in Working Weigert's Iron Hematoxylin Solution for **5 minutes**.
6. Wash in running tap water for **5 minutes**.
7. Rinse in deionized water.
8. Stain in Biebrich Scarlet-Acid Fuchsin, Catalog No. HT15-1, for **5 minutes**.
9. Rinse in deionized water.
10. Place slides in Working Phosphotungstic/Phosphomolybdic Acid Solution for **5 minutes**.
11. Place slides in Aniline Blue Solution, Catalog No. HT15-4, for **5 minutes**.
12. Place slides in Acetic Acid, 1%, for **2 minutes**. Discard solution.
13. Rinse slides, dehydrate through alcohol, clear in xylene and mount.

ACCUMATE™ H2100 MICROWAVE PROCEDURE:

1. Deparaffinize slides and hydrate to deionized water.
2. Place slides in **40 ml** of Bouin's Solution contained in a plastic Coplin jar. Loosely cover Coplin jars with lid before placing in microwave oven or use Coplin jars with holes drilled into the lids.
3. Microwave on **600 watts** for **25 seconds**. Gently mix solution with beral pipet or applicator stick, incubate slides in heated Bouin's Solution for **5 minutes** in a fume hood or well ventilated area.
4. Rinse slides in running tap water until yellow color disappears.
5. Place slides in **40 ml** Hematoxylin Solution, Gill No. 3, contained in plastic Coplin jar.
6. Microwave on **800 watts** for **5 seconds**.
7. Rinse well in running tap water for **30 seconds** to **1 minute**.
8. Blue in Working Scott's Tap Water Substitute at **room temperature**.
9. Rinse well in running tap water.
10. Place slides in **40 ml** Biebrich Scarlet-Acid Fuchsin Solution contained in plastic Coplin jar.
11. Microwave on **600 watts** for **20 seconds**. Mix gently with beral pipet or applicator stick. Let incubate for **2 minutes**.
12. Rinse quickly in several changes of deionized water.
13. Place slides in **40 ml** Phosphotungstic-Phosphomolybdic Acid Solution contained in plastic Coplin jar.
14. Microwave on **600 watts** for **20 seconds**. Immediately remove slides and rinse in several changes of deionized water.
15. Place slides in **40 ml** Aniline Blue Solution contained in a plastic Coplin jar.
16. Microwave on **600 watts** for **15 seconds**. Mix gently with a beral pipet or applicator stick. Let incubate for **1 minute**.
17. Rinse well in deionized water.
18. Place slides in 1% Acetic Acid for **30 seconds** to **1 minute** at **room temperature**.
19. Rinse slides, dehydrate through alcohol, clear and coverslip.

PERFORMANCE CHARACTERISTICS

Nuclei	— Black, if Weigert's Iron Hematoxylin is used. Blue, if Hematoxylin Solution Gill No. 3 is used.
Cytoplasm	— Red
Muscle fibers	— Red
Collagen	— Blue

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

REFERENCES

1. HJ Conn's Biological Stains, Edited by RD Lillie, 9th ed, Williams and Wilkins, Baltimore (MD), 1977
2. Theory and Practice of , Edited by DC Sheehan and BB Hrapchak, 2nd ed, Mosby, St. Louis, (MO), 1980
3. Lillie RD: Further experiments with the Masson trichrome modification of Mallory's connective tissue stain. Stain Technol 15:17, 1940
4. Gomori G: A rapid one-step trichrome stain. Am J Clin Pathol 10:661, 1950
5. Staining Procedures, Edited by G Clark, 3rd ed. Williams and Wilkins, Baltimore (MD) 1973
6. Maynard JH: A trichrome stain in glycol methacrylate that works. Lab Med 17:471, 1986
7. Leong AS-Y, Milios J: Rapid immunoperoxidase staining of lymphocyte antigens using microwave irradiation. J Pathol 148:183, 1986
8. Brinn NT: Rapid metallic histologic staining using the microwave oven. J Histotechnol 6:125, 1983
9. Valle S: Special stains in the microwave oven. J Histotechnol 9:237, 1986

Sigma-Aldrich, Inc. warrants that its products conform to the information contained in this and other Sigma-Aldrich publications. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see reverse side of the invoice or packing slip for additional terms and conditions of sale.

Procedure No. HT15
Previous Revision: 2003-03
Revised: 2003-09



AR-MED Ltd., Runnymede Malthouse
Egham TW20 9BD United Kingdom

SIGMA-ALDRICH, INC.
3050 Spruce Street, St. Louis, MO 63103 USA 314-771-5765
Technical Service: 800-325-0250 or call collect 314-771-3122
or e-mail at clintech@sial.com
To Order: 800-325-3010 or call collect 314-771-5750
www.sigma-aldrich.com

SIGMA-ALDRICH CHEMIE GmbH
P.O. 1120, 89552 Steinheim, Germany 49-7329-970