

INTENDED USE

Sigma-Aldrich Trichrome Stains (Gomori) 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, Foot and Gomori.^{3,4} The procedure described here is based on the work of Gomori and is a one-step system combining the cytoplasmic and connective-fiber stain in a phosphotungstic acid/acetic acid solution.⁴ Tissue sections are treated with Bouin's solution to intensify the final coloration. Nuclei are then stained with Weigert's iron hematoxylin, followed by chromotrope 2R (stains cytoplasm and muscle fibers) and fast green FCF or aniline blue (stains collagen fibers). Rinsing in acetic acid after staining makes the shades of color more delicate and transparent.⁴ Included is a Gomori trichrome stain technique for rapid staining in microwave ovens.⁵⁻⁶

REAGENTS

TRICHROME STAIN LG SOLUTION, Catalog No. HT10-3-16

Chromotrope 2R, 0.6% (w/v), fast green FCF, (certified), 0.1% (w/v), phosphotungstic acid, 0.8% (w/v) and acetic acid, 1.0% (v/v). Reagent stains collagen green.

TRICHROME STAIN AB SOLUTION, Catalog No. HT10-5-16

Chromotrope 2R, 0.6% (w/v), aniline blue, (certified), 0.3% (w/v), phosphotungstic acid, 0.8% (w/v) and acetic acid, 1.0% (v/v). Reagent stains collagen blue.

STORAGE AND STABILITY:

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).

Trichrome Stain solutions, Bouin's solution, Weigert's Iron Hematoxylin (Parts A and B), Trichrome TISSUE-TROL, Acetic Acid solution 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:

Trichrome Stain LG Solution, Trichrome Stain AB and Bouin's solution 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 0.5% Acetic Acid by diluting 4.4 ml 1N acetic acid with 45.6 ml 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

Trichrome Stain LG Solution - Caution: Substance not yet fully tested.

Trichrome Stain AB Solution - Caution: Substance not yet fully tested.

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.

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 respiratory system and skin. Risk of serious damage to eyes. 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

Trichrome Stain LG Solution - Caution: Substance not yet fully tested.

Trichrome Stain AB Solution - Caution: Substance not yet fully tested.

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. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

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

1N Acetic Acid Solutions. Avoid contact with skin and eyes. After contact with skin, take off immediately all contaminated clothing, and wash immediately with plenty of water and seek medical advice. 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 micron or less may be used.

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 Solution

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

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. Prepare Working Weigert's Iron Hematoxylin Solution according to label instructions.
2. Deparaffinize slides and hydrate to deionized water.
3. Place slides in preheated Bouin's Solution at 56°C for **15 minutes** or at **room temperature overnight**.
4. Cool slides in tap water (**18–26°C**) contained in a Coplin jar.
5. Wash in running tap water to remove yellow color from sections.
6. Stain in Working Weigert's Iron Hematoxylin Solution for **5 minutes**.
7. Wash in running tap water for **5 minutes**.
8. Stain in Trichrome Stain (LG or AB) Solution for **5 minutes**.
9. Place in 0.5% Acetic Acid for **1 minute**.
10. 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 in plastic Coplin jar. Loosely cover jar with lid or use lids with vented caps.
3. Microwave on **600 watts** for **25 seconds**. Agitate solution with applicator stick or beral pipet then incubate for **5 minutes** in a fume hood or well ventilated area.

4. Rinse in running tap water until yellow color disappears.
5. Place slides in **40 ml** of Hematoxylin Solution, Gill No. 3, contained in a plastic Coplin jar.
6. Microwave on **800 watts** for **5 seconds**.
7. Rinse well in 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** of Trichrome Stain (LG or AB) Solution contained in a plastic Coplin jar.
11. Microwave on **800 watts** for **15 seconds**. Gently agitate solution using an applicator stick or beral pipet. Let incubate for **1 minute**.
12. Rinse quickly in tap water.
13. Place slides in 1% Acetic Acid for **30 seconds** to **1 minute** at **room temperature**.
14. Rinse slides, dehydrate through alcohol, clear in xylene and mount.

PERFORMANCE CHARACTERISTICS

| | |
|-------------------|---------------------------|
| Nuclei | - Blue to Black |
| Collagen | - Green (LG) or Blue (AB) |
| Cytoplasm, Muscle | - Red |



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

REFERENCES

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