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## INTENDED USE

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Sigma-Aldrich ACCUSTAIN® Mucicarmine Staining reagents are intended for staining tissue mucins of epithelial origin. Mucicarmine staining reagents are for "In Vitro Diagnostic Use".

Tissue acid-mucopolysaccharides may be demonstrated by a variety of techniques. Mayer's mucicarmine procedure, as modified by Southgate,<sup>1</sup> achieves excellent contrast between red staining mucin and other cellular components. Aluminum, contained in the formulation, is believed to form a chelate complex with mucin, to which carmine attaches by dye-lake formation.<sup>2</sup> The stain displays a specificity towards mucins of epithelial origin, whereas mucins of fibroblastic origin stain poorly.<sup>3</sup> Its major use may be in identification of primary tumor sites and distinguishing mucin-negative undifferentiated squamous cell lesions from mucin-positive adenocarcinomas.<sup>2</sup> The procedure described by Sigma-Aldrich is similar to that of Southgate. A stable solution of tartrazine has been substituted for metanil yellow. Included is a mucicarmine stain technique for rapid staining in microwave oven.<sup>4,6</sup>

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## REAGENTS

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**MUCICARMINE STOCK SOLUTION**, Catalog Nos. HT30-1-8 or HT30-1-16  
Carmine, certified, 10 g/l, aluminum hydroxide, 10 g/l, and aluminum chloride, 5 g/l, in ethanol, 50% v/v.

**TARTRAZINE SOLUTION**, Catalog Nos. HT30-2-4 or HT30-2-8  
Tartrazine, 0.25% [w/v] and Acetic acid, 0.25% [v/v].

### STORAGE AND STABILITY

Store Mucicarmine Stock Solution in refrigerator (2–8°C). Reagent is stable until expiration date shown on label.

Working Mucicarmine Solution is stable for 2–3 days when stored in refrigerator (2–8°C).

### PREPARATION:

Working Mucicarmine Solution is prepared by diluting Mucicarmine Stock Solution 1:4 with tap water (e.g., 10 ml Mucicarmine Stock Solution plus 30 ml tap water).

Tartrazine solution and Gill's 3 hematoxylin are ready to use. Solutions are stable until the stated expiration date.

Scott's Tap Water Substitute is prepared by mixing 1 part of Scott's Tap Water Substitute Concentrate with 9 parts deionized water. Solution is stable until the stated expiration date.

### 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.

Mucicarmine 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

Mucicarmine Stock Solution is **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. Target organs: Eyes and nerves.

Scott's Tap Water Substitute. Caution: Substances 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).

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.

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.

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

Mucicarmine Stock Solution is **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.

Scott's Tap Water Substitute. Caution: Substances 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.

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.

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## PROCEDURE

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### 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 sections cut 4–6 microns.

### SPECIAL MATERIALS REQUIRED, BUT NOT PROVIDED:

Positive control slides, such as Sigma Mucin TISSUE-TROL, Catalog No. M 9543, should be included in each run

Tartrazine Solution, Catalog Nos. HT30-2-4 or HT30-2-8

Reagent Alcohol

Xylene

### STANDARD PROCEDURE ONLY:

Weigert's Iron Hematoxylin Set  
Staining Jars, Catalog No. S 5134

### MICROWAVE PROCEDURE ONLY:

Hematoxylin Solution, Gill No. 3

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

Coplin Jar with vented lids, Catalog No. C 8338

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

### NOTES:

Do not substitute deionized or distilled water for tap water when preparing the mucicarmine working solution.

Masking of mucin may occur if the nuclear stain or counterstains are too intense.

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 procedure.
2. Deparaffinize slides to deionized water.
3. Stain in Working Weigert's Iron Hematoxylin Solution for **5 minutes**.
4. Wash in running tap water for **5 minutes**.
5. Stain in Working Mucicarmine Solution for **30 minutes** or longer at **room temperature**. Check control slide under microscope.

NOTE: The time may be decreased if staining is performed at 37°C or 56°C.

6. Rinse in deionized water.

7. Stain in Tartrazine Solution for **1–5 seconds**.

8. 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 Hematoxylin Solution, Gill No. 3, contained in a plastic Coplin jar. Loosely cover with lid before placing in oven, or use Coplin jar lids with holes drilled into them.
3. Microwave on **800 WATTS** for **10 seconds**.
4. Rinse in running tap water for **1–2 minutes**.
5. Place slides in **40 ml** of Mucicarmine Working Solution.
6. Microwave on **800 WATTS** for **20 seconds**. Mix gently with beral pipet or applicator stick. Let incubate for **5 minutes**.
7. Rinse well in deionized water.
8. Counterstain in Tartrazine Solution for **30 seconds** at **room temperature**.
9. Rinse quickly in deionized water.
10. Dehydrate quickly through alcohols and clear in xylene. Mount slides.

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## PERFORMANCE CHARACTERISTICS

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|------------------------|---|------------------|
| Nuclei                 | — | Blue             |
| Mucin                  | — | Deep Rose to Red |
| Capsule of Cryptococci | — | Deep Rose to Red |
| Other Tissue Elements  | — | Yellow           |

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

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## REFERENCES

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1. Handbook of Histopathological Techniques, 2nd ed., CFA Culling, Editor, Butterworth Inc., London, 1963
2. Theory and Practice of Histotechnology, 2nd ed. DC Sheehan, BB Hrapchak, Editors, Mosby, St. Louis, MO, 1980
3. Selected Histochemical and Histopathologic Methods, SW Thompson, RD Hunt, Editors, CC Thomas, Springfield, IL, 1966, p 453
4. Leong AS-Y, Milios J: Rapid immunoperoxidase staining of lymphocyte antigens using microwave irradiation. J Pathol 148:183, 1986
5. Brinn NT: Rapid metallic histologic staining using the microwave oven. J Histotechnol 6:125, 1983
6. Valle S: Special stains in the microwave oven. J Histotechnol 9:237, 1986

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