Mucicarmine Staining Kit

REF 860-011

05279275001

IVD Σ 75

Figure 1. Mucicarmine Staining Kit on normal colon tissue.

INTENDED USE

The Mucicarmine Staining Kit is intended for laboratory use as a qualitative histologic stain to demonstrate acid mucopolysaccharides (mucin) by light microscopy in sections of formalin-fixed, paraffin-embedded (FFPE) tissue stained on a BenchMark Special Stains instrument.

This product should be interpreted by a qualified pathologist in conjunction with histological examination, relevant clinical information, and proper controls.

This product is intended for in vitro diagnostic (IVD) use.

SUMMARY AND EXPLANATION

Mucicarmine staining is a modification of Southgate's method. First described in 1896 by Mayer, mucicarmine staining is one of the oldest histochemical staining techniques for acid mucins; the most standardized version used in routine clinical practice is Southgate's method.[1,2] Mucicarmine staining is a technique used to identify acid mucopolysaccharides, also called acid mucins, which are O-linked glycoproteins.[3,4] Mucicarmine stains carboxylated and sulfonated acid mucins; mucicarmine does not stain neutral mucins.[2]

Cryptococcus, a genus of pathogenic fungi, has an acid mucin rich capsule that is its dominant virulence factor.[5] Detection of the acid mucin rich capsule of Cryptococcus using mucicarmine staining can be used to identify Cryptococcus.[6]

The Mucicarmine Staining Kit is used to demonstrate acid mucopolysaccharides (mucins) to aid the pathologist in the diagnosis of infection by Cryptococcus.

PRINCIPLE OF THE PROCEDURE

The staining reaction is based on the reaction of an aluminum-carmine chelate complex attached to acid groups of mucin.[7] Mucicarmine contains an aluminum-carmine chelate complex that stains mucin pink to red. Iron Hematoxylin A and Iron Hematoxylin B stain the nuclei grey to black. Tartrazine Counterstain is applied to provide a contrasting yellow background.

This kit is optimized for use on BenchMark Special Stains instruments. The reagents are applied to tissue on microscope slides and mixed over the entire specimen.

MATERIAL PROVIDED

The reagent vials are supplied in barcode labeled carriers to insert into the reagent tray of the instrument. Each kit contains sufficient reagent for 75 tests.

One 27 mL vial of Mucicarmine Stain reagent.

One 22 mL vial of Iron Hematoxylin A contains approximately 1% hematoxylin in 95% ethanol reagent.

One 27 mL vial of Iron Hematoxylin B contains approximately 1.2% ferric chloride and approximately 1% hydrochloric acid reagent.

One 22 mL vial of Tartrazine Counterstain contains approximately 1% tartrazine and approximately 1% acetic acid reagent.

Four vial inserts with sipping straws.

Reconstitution, Mixing, Dilution, Titration

No reconstitution, mixing, dilution, or titration of kit reagents is required. Further dilution of any of the reagents may result in unsatisfactory staining.

The reagents in this kit have been optimally diluted for use on BenchMark Special Stains instruments.

MATERIALS REQUIRED BUT NOT PROVIDED

Not all products listed in the method sheet may be available in all geographies. Consult your local support representative.

The following reagents and materials may be required for staining but are not provided:

1. Recommended control tissue
2. Microscope slides, positively charged
3. BenchMark Special Stains instrument
4. Benchmark Special Stains Deparaffinization Solution (10X) (Cat. No. 860-036 / 06523102001)
5. Benchmark Special Stains Liquid Coverslip (Cat. No. 860-034 / 06523072001)
6. Benchmark Special Stains Wash II (Cat. No. 860-041 / 08309817001)
7. General purpose laboratory equipment

STORAGE AND STABILITY

Mucicarmine Staining Kit should be stored at 2-8°C. Refrigerated kit components should be brought to room temperature prior to use.

When properly stored, unopened and opened reagents are stable to the date indicated on the label.

Do not use reagent beyond the expiration date indicated on the kit.

There are no obvious signs to indicate instability of these reagents; therefore, controls should be run simultaneously with unknown specimens. Contact your local support representative if positive control material shows a decrease in staining as it could indicate reagent instability.

SPECIMEN PREPARATION

Routinely processed, FFPE tissues are required for use with this product and BenchMark Special Stains instruments. The recommended tissue fixative is 10% neutral buffered formalin.[8]

Perform specimen collection and storage according to CLSI document M29-T2.[9]

Cut sections to the appropriate thickness approximately 4 µm, and place the sections on positively charged glass slides

1. Dry the slides.[8]
2. Print appropriate barcode label(s).
3. Apply barcode labels to the frosted end of the slides prior to loading the slides onto the instrument (see the instrument User Guide for correct application of labels).

Refer to the Instructions for Use section for the recommended protocol for the BenchMark Special Stains instrument.

WARNINGS AND PRECAUTIONS

1. For in vitro diagnostic (IVD) use.
2. For professional use only.
3. **CAUTION**: In the United States, Federal law restricts this device to sale by or on the order of a physician. (Rx Only)
4. Do not use beyond the specified number of tests.
5. Positively charged slides may be susceptible to environmental stresses resulting in inappropriate staining. Ask your Roche representative for more information on how to use these types of slides.
6. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions. In the event of exposure, the health directives of the responsible authorities should be followed.[10,11]
7. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
8. Avoid microbial contamination of reagents as it may cause incorrect results.
9. For further information on the use of this device, refer to the BenchMark Special Stains instrument User Guide, and instructions for use of all necessary components located at dialog.roche.com.
10. Consult local and/or state authorities with regard to recommended method of disposal.
11. Product safety labeling primarily follows EU GHS guidance. Safety data sheet available for professional user on request.

12. To report suspected serious incidents related to this device, contact the local Roche representative and the competent authority of the Member State or Country in which the user is established.

This product contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Table 1. Hazard information.

Hazard	Code	Statement
Danger 	H225	Highly flammable liquid and vapour.
	H290	May be corrosive to metals.
	H302	Harmful if swallowed.
	H314	Causes severe skin burns and eye damage.
	H371	May cause damage to organs.
	H411	Toxic to aquatic life with long lasting effects.
	P210	Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
	P260	Do not breathe dust/ fume/ gas/ mist/ vapours/ spray.
	P273	Avoid release to the environment.
	P280	Wear protective gloves/ protective clothing/ eye protection/ face protection/ hearing protection.
	P303+ P361+ P353	IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water.
	P304+ P340 + P310	IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing. Immediately call a POISON CENTER/ doctor.
	P305 + P351+ P338 + P310	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/ doctor.
	P370+ P378	In case of fire: Use dry sand, dry chemical or alcohol-resistant foam to extinguish.
	P391	Collect spillage

This product contains Tartrazine. May produce an allergic reaction.

INSTRUCTIONS FOR USE

Prepare Reagent Vial

Before first use, a vial insert and sipping straw must be placed in the reagent vial.

Remove the shipping cap from the vial and place the insert and straw into the vial. The insert and sipping straw should be left in the vial, once the vial has been opened.

Staining Procedure

1. Load reagents and slides onto the instrument.
2. Place the soft cap into the slot on the reagent holder when the reagent is in use.
3. Perform the staining run according to the recommended protocol in Table 2 and the instructions in the User Guide.
4. When the run is complete, remove the slides from the instrument.
5. Use the soft cap to cover the reagent vial when reagent is not in use.
6. After use, store the reagents according to the recommended storage conditions.

Recommended Protocol

The parameters for the automated procedures can be displayed, printed and edited according to the procedure in the instrument User Guide.

The following procedures allow flexibility to accommodate user preference. This product has been optimized for use with the BenchMark Special Stains instrument but the user must validate results obtained with this product.

Table 2. Recommended staining protocol for Mucicarmine Staining Kit on a BenchMark Special Stains instrument.

Staining Procedure	S Mucicarmine
Protocol Step	**Method**
Deparaffinization	Select to automate paraffin removal.
Baking (optional)	The default is not selected. 75°C for 4 minutes is recommended.
Optimize Hematoxylin Intensity (Iron Hematoxylin A)	The default time is 8 minutes. Select an incubation time from 8 to 16 minutes:* 8 minutes, lighter staining of nuclei 16 minutes, darker staining of nuclei
Optimize Stain Intensity (Mucicarmine)	The default time is 12 minutes. Select an incubation time from 8 to 16 minutes:* 8 minutes, lighter staining of acid mucins 16 minutes, darker staining of acid mucins
Optimize Counterstain Intensity (Tartrazine)	The default time is 4 minutes. Select an incubation time from 4 to 16 minutes:* 4 minutes, lighter counterstain 16 minutes, darker counterstain

*To adjust staining preferences, increment the incubation time one parameter at a time.

Recommended Post-Instrument Processing

1. Rinse slides in three changes of 95% ethanol to remove the leftover solution, followed by three changes of 100% ethanol.
2. Dehydrate slides in three changes of 100% xylene.
3. Coverslip with permanent mounting media.

Compatible with the VENTANA HE 600 system coverslipping protocol. For further information, refer to the VENTANA HE 600 system User Guide.

QUALITY CONTROL PROCEDURE

An example of a positive control material would be FFPE human tissue with epithelial mucin such as colon. Control tissue should be fresh autopsy, biopsy, or surgical specimen prepared or fixed as soon as possible in a manner identical to test sections. Such tissues should monitor all steps of the analysis, from tissue preparation through staining.

Use of a tissue section fixed or processed differently from the test specimen provides control for all reagents and method steps except fixation and tissue processing. The cellular components of other tissue elements may serve as the negative control.

Optimal laboratory practice is to include a positive control section on the same slide as the test tissue. This helps identify any failures applying reagents to the slide. Control tissue may contain both positive and negative staining elements and serve as both the positive and negative control.

The control tissue must be tested with each run.

Known positive tissue controls should only be utilized for monitoring the correct performance of processed tissues and test reagents, not as an aid in formulating a specific diagnosis of patient samples.

If the positive tissue components fail to demonstrate positive staining, results with the test specimens should be considered invalid. If the negative components demonstrate positive staining, results with patient specimens should also be considered invalid.

Unexplained discrepancies in control results should be referred to the local support representative immediately. If quality control results do not meet specifications, patient

results are invalid. The cause must be identified and corrected, and the patient samples repeated.

STAINING INTERPRETATION / EXPECTED RESULTS

Mucicarmine Staining Kit is tested to demonstrate acid mucopolysaccharides (mucins).

- Acid mucopolysaccharides (mucins): pink to red
- Cryptococcus: pink to red
- Nuclei: gray to black
- Background: yellow

SPECIFIC LIMITATIONS

Only positively charged microscope slides have been used and validated for this assay.

PERFORMANCE CHARACTERISTICS

ANALYTICAL PERFORMANCE

Staining tests for sensitivity, specificity, and precision were conducted and the results are listed below.

Sensitivity and Specificity

Analytical sensitivity and specificity for normal and diseased tissue cases was evaluated. All evaluated tissue cases (64/64) passed for acceptable staining as shown in Table 3 and Table 4.

Table 3. Sensitivity/Specificity of Mucicarmine Staining Kit was determined by testing the following FFPE normal tissues.

Tissue	# Cases Passed / # Tested
Colon	14 / 14
Lung	14 / 14

Table 4. Sensitivity/Specificity of Mucicarmine Staining Kit was determined by testing the following FFPE diseased tissues.

Tissue	# Cases Passed / # Tested
Adenocarcinoma (Colon)	15 / 15
Adenocarcinoma (Lung)	9 / 9
Squamous cell carcinoma (Lung)	9 / 9
Cryptococcus (Lung)	3 / 3

Precision

Precision of Mucicarmine Staining Kit was determined across multiple runs, days, instruments, and reagent lots using multiple cut slides from 2 normal colon, 2 normal lung, and 2 colon adenocarcinoma tissue cases. All acceptance criteria were fully met. Precision studies were performed for the Mucicarmine Staining Kit according to Table 5.

Table 5. Precision slide studies for Mucicarmine Staining Kit.

Parameters Tested	# of conditions	# Slides Passed / # Tested
Run to Run	3 runs, same day	54 / 54
Day to Day	5 days	90 / 90
Instrument to Instrument	3 instruments	54 / 54
Intra Run	same day, same instrument	54 / 54
Lot to lot	3 lots	54 / 54

The results demonstrated no significant difference in staining intensity among the slides.

TROUBLESHOOTING

1. Section thickness may affect quality and intensity of staining. If staining is inappropriate, contact your local support representative for assistance.
2. Necrotic or autolyzed tissue may exhibit nonspecific staining.
3. If the positive control is negative, tissue may have been improperly collected, fixed, or deparaffinized. Follow the proper procedure for collection, storage, and fixation.
4. If the positive control is negative, check that the slide has the proper barcode label. If the slide is labeled properly, check the other positive controls from the same run to determine if the controls were properly stained.
5. If excessive background staining occurs: incomplete paraffin removal could cause staining artifacts or no staining. If all paraffin is not removed from the slide, repeat the staining run using the extended deparaffinization option, if available.
6. If tissue sections wash off the slide, confirm the slides are positively charged.
7. Extended stay of the slides on-board the instrument after run completion may affect quality and intensity of the staining. If the staining is inappropriate, remove slides promptly at the end of the run and proceed to post-instrument processing.
8. For corrective action, refer to the Instructions for Use section, the instrument User Guide or contact your local support representative.

CLINICAL PERFORMANCE

The sensitivity and specificity characteristics relevant to the intended purpose of this device are reported in the analytical performance section.

REFERENCES

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NOTE: A point (period/stop) is always used in this document as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols

Ventana uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see dialog.roche.com for definition of symbols used):

GTIN — Global Trade Item Number

UDI — Unique Device Identification

Indicates the entity importing the medical device into the European Union

INTELLECTUAL PROPERTY

Additions, deletions or changes are indicated by a change bar in the margin.

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