PathnSitu Biotechnologies

Grocott's Methenamine Silver (GMS) Stain Kit

Laboratory Use Only

PRODUCT INFORMATION: **PERFORMANCE CHARACTERISTICS:**

REF

SSP011 25 Reactions- **Staining Interpretation:**
SSP011 50 Reactions - **Fungi** : Brown/Black
Back ground : Pale green

SUMMARY AND EXPLANATION

For *laboratory* use only

Grocott's Methenamine Silver (GMS) Stain Kit remains a helpful ancillary study for use on cyto and histopathology specimens in order to help detect infrequent, often miniscule, fungal organisms. GMS reliably stains viable and nonviable fungal organisms and is therefore often the preferred method for identifying pathogenic fungi. This product is not intended for diagnostic or therapeutic use The results are to be interpreted by qualified personnel in conjunction with other clinical and laboratory findings.

PRINCIPLE OF THE PROCEDURE

The mechanism of action of Modified Grocott's Methenamine Silver Stain is based upon the capacity of aldehyde groups to reduce cationic silver (Ag+) to metallic silver. Chromic acid is used to generate aldehyde groups by the oxidation of 1-2 glycol groups within polysaccharide rich tissue components.

When cationic silver is added to the section in the form of a Methenamine-Silver ion complex, the aldehyde groups reduce the silver ions to metallic silver. Sections are subsequently toned with Gold Chloride Solution to produce metallic gold which is more stable than metallic silver and produces superior contrast.

The light green is used as a counterstain where in the fungi appears black and sharply delineated.

REAGENTS PROVIDED

Kit Components	Product Code	Storage Conditions	Pack Sizes	
			25 Reactions	50 Reactions
Chromic Acid (25X) (Reagent A)	IPS045	RT	50ml	100ml
Sodium Metabisulphite Solution (Reagent B)	IPS046	RT	50ml	100ml
Methenamine Solution - B (Reagent C)	IPS079	2-8º C	50ml	100ml
Silver Nitrate Solution - A (Reagent D)	IPS048	2-8º C	25ml	50ml
Borax Solution (Reagent E)	IPS049	RT	50ml	100ml
Gold Chloride Solution (Reagent F)	IPS050	2-8º C	50ml	100ml
Sodium Thiosulphate Solution -A (Reagent G)	IPS051	RT	50ml	100ml
Light Green Solution - B (Reagent H)	IPS052	RT	50ml	100ml

STORAGE AND HANDLING

Storage Recommendations: Store at recommended storage conditions of respective reagents. When stored at the appropriate conditions, the reagents are stable until expiry.

Do not use the reagents after expiration date provided on the vial.

To ensure proper regent performance delivery and stability, replace the dispenser cap after every use and immediately place the vials at recommended temperatures away from sunlight in an upright position.

During transport, short-term exposure to 2-8ºC does not affect product performance.

SPECIMEN PREPARATION

Sample preparation and fixation: Formalin-fixed, paraffin-embedded tissue sections of 3- 5 µm thickness on microscopic slides

PRECAUTIONS

1. Normal precautions exercised in handling laboratory reagents should be followed.
2. This product should be used by qualified and trained professional users only
3. It can cause serious eye and skin irritation. Refer to Material Safety Datasheet for any updated risk, hazard or safety information.
4. Dispose of waste observing all local, state, provincial or national regulations.
5. Do not use reagents after expiration date.
6. Use protective clothing and gloves, while handling reagents.
7. Avoid contamination of reagents as it may lead to incorrect results.

MATERIALS REQUIRED, BUT NOT PROVIDED

- Xylenes
- Graded Alcohols (50%, 70%, 95%, absolute)
- DPX Mountant
- Microscopic Slides (positively charged)
- Slide Holder
- Glass Coplin Jar
- Jars
- Cover Slips
- Disposable Droppers
- Distilled water

WORKING SOLUTION PREPARATION

1. Chromic Acid Working Solution

Components	Quantity Required	
	50ml	100ml
Chromic Acid (25X) (Reagent A)	2ml	4ml
Distilled water	48ml	96ml

2. GMS Working Solution: (For 40ml, preferably use glass coplin jar)

Methenamine Solution - B ----------2ml
(Reagent C)

Silver Nitrate Solution - A ----------1ml
(Reagent D)

Distilled Water ----------35ml

(Warm the above mixture to 60ºC. Add Borax Solution just before use and mix well)

Borax Solution ---------- 2ml
(Reagent E)

STAINING PROCEDURE

Protocol (I): **(Conventional Method)**

1. Deparaffinize sections and hydrate to distilled water.
2. Oxidize in Chromic Acid working solution (Refer to working solution preparation above) at 60°C for 10-15 minutes using water bath.
3. Wash in running tap water for a few seconds.
4. Incubate the slides in Sodium Metabisulphite Solution (Reagent B) for 1 minute.
5. Rinse in two changes of distilled water.
6. Place the slides in GMS working solution (Refer to working solution preparation above) at 60ºC in water bath for 30mins (Preferably use glass coplin jar).

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Note: The sections turn to golden brown. Use paraffin coated/plastic forceps to remove slide from solution. Dip slide in distilled water and check for adequate silver impregnation under microscope. Fungi should be dark brown at this stage. If not, place the slide back into the working solution for few more minutes until golden brown color develops.

7. Rinse in 4 changes of distilled water.
8. Tone the sections in Gold Chloride Solution (Reagent F) for 2 minutes.
9. Rinse in two changes of distilled water
10. Incubate slide in Sodium Thiosulphate Solution -A (Reagent G) for 2 minutes.
11. Rinse in two changes of distilled water
12. Counter stain with Light Green Solution - B (Reagent H) for 2-3 minutes.
13. Quickly rinse in distilled water.
14. Quickly dehydrate through graded alcohols (2 dips each)
15. Clear the slide in Xylene and mount using DPX mountant.

Protocol (II) (Microwave Method):

1. Deparaffinize sections and hydrate to distilled water.
2. Oxidize sections in Chromic Acid working solution (Refer to working solution preparation above) at High power microwave for 20-30 seconds. Allow you to stand for 5mins.
3. Wash under running tap water and 2 changes of distilled water.
4. Incubate the slides in Sodium Metabisulphite Solution (Reagent B) for 1 minute.
5. Rinse in two changes of distilled water.
6. Place the slides in GMS working solution (Refer to working solution preparation above) at High Power Microwave for 20 seconds.

Note: The sections turn to Golden brown. Use paraffin coated/plastic forceps to remove slide from solution. Dip slide in distilled water and check for adequate silver impregnation under microscope. Fungi should be dark brown at this stage. If not, place the slide back into the working solution for more minutes.

7. Rinse in 4 changes of distilled water.
8. Tone the sections in Gold Chloride Solution (Reagent F) for 2 minutes.
9. Rinse in two changes of distilled water.
10. Incubate slide in Sodium Thiosulphate Solution -A (Reagent G) for 2 minutes.
11. Rinse in two changes of distilled water.
12. Counter stain with Light Green Solution - B (Reagent H) for 2-3 minutes.
13. Quickly rinse in distilled water.
14. Quickly dehydrate through graded alcohols (2 dips each).
15. Clear the slide in Xylene and mount using DPX mountant.

QUALITY CONTROL

The recommended positive tissue control for Grocott's Methenamine Silver (GMS) Stain Kit is fungal infected tissue.

PERFORMANCE CHARACTERISTICS

Grocott's Methenamine Silver (GMS) Stain Kit positive substances for fungi stains **sharply delineated hyphae** in **brown or black** color and **background** stains **pale green** color.

TROUBLESHOOTING

1. Follow the specific protocol recommendations according to data sheet provided
2. Tissue staining is dependent on the handling and processing of the tissue prior to staining. Improper fixation, tissue processing, freezing, thawing, washing, drying, heating, sectioning or contamination with other tissues or fluids may produce artifacts, reagent trapping or inaccurate results.
3. Do not allow the section to dry out during the entire staining process.
4. Gently mix all the reagents prior to use.
5. Excessive or incomplete counterstaining may compromise the interpretation of the results.
6. If unusual results occur, contact PathnSitu Technical Support at +91-40-27015544 or E-mail: techsupport@pathnsitu.com.

LIMITATIONS AND WARRANTY

1. This product is intended for use only by authorised, trained, and qualified personnel.
2. A qualified and trained pathologist/personnel must interpret the results of the test.
3. Interpretation of test results must be made in conjunction with relevant background information and additional laboratory findings.
4. Always use the recommended volume and concentration of reagents to ensure complete coverage of the tissue section and to minimise the risk of false-positive or false-negative results.
5. Use appropriate buffers, instruments, consumables, and incubation conditions as recommended to achieve optimal staining performance.
6. It is strongly recommended to include known positive and negative controls when performing the test to ensure the validity of results.
7. The product has been validated on formalin-fixed, paraffin-embedded (FFPE) tissues. The end user must establish performance on other tissue types.
8. Unexpected results may occur in untested tissues due to inherent variability in tissue components.
9. False-positive reactions may occur due to insufficient washing, inappropriate protocol conditions, or other contributing factors.
10. In instances where the staining pattern or localisation differs from the specifications outlined in this datasheet, please get in touch with technical support for guidance.
11. Maintain the product under the recommended storage conditions to preserve reagent stability and performance.
12. Do not use reagents that appear cloudy, discoloured, or show signs of contamination. Discard any components showing signs of deterioration.
13. Silver Nitrate is light sensitive. Avoid exposing silver nitrate to bright light, including direct sunlight, as it can cause the chemical to break down.
14. This product is intended for single-use application only. Once applied to a tissue section, reagents should not be recovered or reused, as this may compromise test integrity and specificity.
15. PathnSitu makes no warranties beyond those expressly stated in the product description.
16. PathnSitu shall not be liable for property damage, personal injury, time or effort, or economic loss arising from the use of this product.
17. Please refer to the complete datasheet for all instructions, precautions, and additional product limitations.
18. For detailed information and specifications on individual components, please refer to Product Material Safety Data Sheet (MSDS)

BIBLIOGRAPHY

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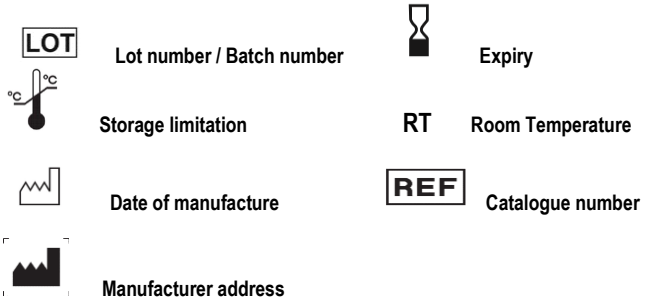
3. Officer B. HIML251 Lecture notes: Silver Stains: Grocott's Methenamine Silver, Jone's Methenamine Silver, and Masson Fontana, March 18, 2009
4. Rapid Methenamine Silver Stain; Arch Pathol Lab Med; 1978, 102: 351- 352.
5. Luna L.G. Histopathological Methods and color atlas of special stains and tissue artefacts, *American Histo Labs Inc.*, Publications Division 1992

EXPLANATION OF SYMBOLS



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