PathnSitu Biotechnologies

Grocott's Methenamine Silver (GMS Stain)

Laboratory Use Only CE

PRODUCT INFORMATION:
SSP011 25 Reactions- Ready to use
SSP011 50 Reactions -Ready to use

PERFORMANCE CHARACTERISTICS:
Staining Interpretation:
Fungi : Brown/Black
Back ground : Pale green

SUMMARY AND EXPLANATION

For *laboratory* use only

Grocott's Methenamine Silver Stain (GMS Stain) remains a helpful ancillary study for use on cyto and histopathology specimens in order to help detect infrequent, often miniscule, fungal organisms. The stain is also useful to highlight the distinctive morphologic features of commonly encountered fungi, including Mucor, Pneumocystis jiroveci, Cryptococcus, Candida Histoplasma, Blastomyces, and Aspergillus. GMS reliably stains viable and nonviable fungal organisms and is therefore often the preferred method for identifying pathogenic fungi.

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 (Reagent A)	IPS045	RT	50ml	100ml
Sodium Metabisulphite (Reagent B)	IPS046	RT	50ml	100ml
Methenamine (Reagent C)	IPS079	2-8º C	50ml	100ml
Silver Nitrate (Reagent D)	IPS048	2-8º C	25ml	50ml
Borax (Reagent E)	IPS049	RT	50ml	100ml
Gold Chloride (Reagent F)	IPS050	2-8º C	50ml	100ml
Sodium Thiosulphate (Reagent G)	IPS051	RT	50ml	100ml
Light Green (Reagent H)	IPS052	RT	50ml	100ml

STORAGE AND HANDLING

Storage Recommendations: Store at appropriate 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 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.

SPECIMEN PREPARATION

Recommended positive controls: Fungal infected tissue control.

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

Reagent Preparation

1. Chromic Acid Working Solution

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

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

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

Silver Nitrate Solution----------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)

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 microbial 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
- Hot Air Oven
- Jars
- Cover Slips
- Disposable Droppers

STAINING PROCEDURE

Protocol (I): (Conventional Method)

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

   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 in to the working solution

US Office:1257, Quarry Ln, Suite150, Pleasanton, CA-94566, USA Ph: +1 925-218-6939,
India Office:PS Heights, Plot# A-28/1/11B/B, Road#, 15, Industrial Development Area, Nacharam, Secunderabad-76
Phone: 040-27015544/33,Fax:040-2701 5544 , **E-Mail:** customerservice@pathnsitu.com **Web:** www.pathnsitu.com

for few more minutes until golden brown color developed.

7. Rinse in 4 changes of distilled water.
8. Tone the sections in gold chloride solution (Reagent F) (using a dropper) for 2 minutes.
9. Rinse in two changes of distilled water
10. Incubate slide in Sodium Thiosulphate solution (Reagent G) (using a dropper) for 2 minutes.
11. Rinse in two changes of distilled water
12. Counter stain with Light green solution (Reagent H) (using a dropper) for 2-3 minutes.
13. Quick 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 reagent Preparation) at High power microwave for 20-30 seconds. Allow 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) (using a dropper) for 1 minute.
5. Rinse in two changes of distilled water.
6. Place the slides in GMS working solution (Refer Reagent Preparation) at High Power Microwave for 20 seconds.

Note: The sections turns 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 in to the working solution for more minutes.

7. Rinse in 4 changes of distilled water.
8. Tone the sections in gold chloride solution (Reagent F) (using a dropper) for 2 minutes.
9. Rinse in two changes of distilled water.
10. Incubate slide in Sodium Thiosulphate solution (Reagent G) (using a dropper) for 2 minutes.
11. Rinse in two changes of distilled water.
12. Counter stain with light green solution (Reagent H) (using a dropper) for 2-3 minutes.
13. Quick 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 GMS stain is fungal infected tissue.

PERFORMANCE CHARACTERISTICS

GMS positive substances for fungi stains sharply delineated hyphae in brown or black color and back ground 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. Excessive or incomplete counterstaining may compromise the interpretation of the results.
5. If unusual results occur, contact PathnSitu Technical Support at +91-40-27015544 or E-mail: techsupport@pathnsitu.com.

LIMITATIONS AND WARRANTY

Authorized and skilled personnel only may use the product. The clinical interpretation of any test results should be evaluated within the context of the patient's medical history and other diagnostic test results. A qualified pathologist must perform the evaluation of the test results. There are no warranties, expressed or implied, which extend beyond the description. PathnSitu is not liable for property damage, personal injury, time or effort on economic loss caused by this product.

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BIBLIOGRAPHY

1. Aberrant staining with Grocott's methenamine silver: utility beyond fungal organisms: Angela M. WrightMDDina R.ModyMDRose C.AntonMDMary R.SchwartzMD
2. New Grocott Stain without Using Chromic Acid Kazuya Shiogama,[1] Kayo Kitazawa,[2] Yasuyoshi Mizutani,[1] Takanori Onouchi,[1] Ken-ichi Inada,[1] and Yutaka Tsutsumi[1]
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

LOT- **Lot number / Batch number**

- Expiry

Storage limitation

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RT- Room Temperature

US Office:1257, Quarry Ln, Suite150, Pleasanton, CA-94566, USA Ph: +1 925-218-6939,
India Office:PS Heights, Plot# A-28/1/11B/B, Road#, 15, Industrial Development Area, Nacharam, Secunderabad-76
Phone: 040-27015544/33,Fax:040-2701 5544 , **E-Mail:** customerservice@pathnsitu.com **Web:** www.pathnsitu.com