PathnSitu Biotechnologies

Laboratory Use Only

Jones Methenamine Silver (JMS) Stain Kit

PRODUCT INFORMATION:
SSP022 25 Reactions
SSP022 50 Reactions

PERFORMANCE CHARACTERISTICS
Staining Interpretation:
Basement membrane: Black
Cell Nuclei : Blue
Cytoplasm : Pink-Orange

SUMMARY AND EXPLANATION

For *Laboratory* use only
The reagents in this kit are intended for *Laboratory use* only. The Jones Methenamine Silver (JMS) Stain Kit is used as a qualitative histologic stain to demonstrate basement membranes, specifically glomerular basement membranes, in formalin-fixed, paraffin-embedded (FFPE) renal tissue by light microscopy. 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 Jones methenamine silver method relies on the production of aldehyde groups from the carbohydrate components of the reticular fibers and basement membranes after their exposure to a periodic acid solution. The released aldehydes then reduce the silver of the methenamine silver complex to visible metallic silver. The gold chloride solution functions to tone the tissue section, and sodium thiosulfate functions to remove excess unreacted silver and gold chloride.

Kit Contents	Product Code	Storage Conditions	Pack Sizes	
			25 Reactions	50 Reactions
Periodic Acid Solution – B (Reagent A)	IPS093	2-8°C	25ml	50ml
Methenamine Solution - B (Reagent B)	IPS079	2-8°C	100ml	200ml
Silver Nitrate Solution – A (Reagent C)	IPS048	2-8°C	50ml	100ml
Borax Solution (Reagent D)	IPS049	RT	75ml	150ml
Gold Chloride Solution (Reagent E)	IPS050	2-8°C	25ml	50ml
Sodium Thiosulphate Solution -B (Reagent F)	IPS094	RT	25ml	50ml

STORAGE AND HANDLING

Storage Recommendations: Store at recommended temperatures. 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:
- Normal Kidney

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

- Harris Hematoxylin
- Ammonia water
- 1% Acid alcohol
- Eosin Y
- Xylenes
- Graded alcohols (50%, 70%, 95%, absolute)
- DPX Mountant
- Microscopic slides (positively charged)
- Slide holder
- Jars
- Hot air oven
- Cover slips
- Distilled water

REAGENT PREPARATION

Working Methenamine Silver solution:
Freshly prepare the working silver methenamine solution by mixing the reagents in the order below:
- Distilled water ---------- 36ml
- Methenamine Solution - B (Reagent B) ---------- 4ml
- Silver Nitrate Solution – A (Reagent C) ---------- 2ml
- Borax Solution (Reagent D) ---------- 3ml

Note: 1. Do not expose the solution to light.
- The solution cannot be reused. Discard after use.
- The working methenamine silver solution stability is essential (e.g., if it precipitates or shows silvering of the container, it indicates that the solution is deteriorated) according to the protocol. Hence, make sure the solution is colourless.

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STAINING PROCEDURE

1. Deparaffinize and hydrate the slides in distilled water.
2. Treat the slides with Periodic Acid Solution – B (Reagent A) for 15 minutes for oxidation.
3. Wash in distilled water
4. Place the slides in the Working methenamine silver solution (Refer to the reagent preparation above) at 70°C for about 20-30 mins in a water bath.
   (Note: Check the slides under the microscope when the slides appear medium brown every 10 minutes. Rinse again in hot water and return to the hot staining solution. As the staining time approaches the endpoint, check the slides as above, every 1-2 mins. The entire procedure must be performed quickly to prevent an uneven staining of the tissues.)
5. Rinse well in distilled water
6. Incubate the slides in Gold Chloride Solution (Reagent E) for 2-3 mins for toning
7. Rinse the slides well in distilled water
8. Incubate the sections with Sodium Thiosulphate Solution -B (Reagent F) for 2 mins
9. Wash in running tap water for 3-5 mins
10. Rinse the slides in distilled water
11. Stain the slides in Harris hematoxylin for 2-3 mins,
12. Rinse the slides in distilled water
13. Differentiate in 1% acid alcohol until sections turn red.
14. Place the slides in ammonia water, 0.3% for 2 mins for bluing.
15. Wash thoroughly in distilled water
16. Counterstain in Eosin Y, 1% solution for 2-3 mins.
17. Quickly dehydrate in graded alcohols.
18. Clear in xylene, three or four changes for 2 mins each
19. Mount with the compatible medium.

QUALITY CONTROL

The recommended positive tissue control for Jones Methenamine Silver (JMS) stain is a normal kidney.

PERFORMANCE CHARACTERISTICS

The Jones Methenamine Silver (JMS)stain highlights **the basement membrane of Glomeruli in black** colour, the **Cell nuclei in blue** colour and the **Cytoplasm in Pink Orange** colour

TROUBLESHOOTING

1. Follow the specific protocol recommendations according to the datasheet provided
2. Tissue staining depends on the tissue's handling and processing before staining.
3. 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
4. Do not allow the section to dry out during the entire staining process
5. Excessive or incomplete counterstaining may compromise the interpretation of the results
6. If unusual results occur, contact PathnSitu Technical Support at +91-40-2701 5544 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 this can cause the chemical to decompose.
14. If working methenamine silver solution preparation is not correct (e.g., precipitates remain, contamination, glassware/ containers not cleaned), the silver mirror effects, background silver deposition, or non-specific darkening can be observed on the stained tissues.
15. 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.
16. PathnSitu makes no warranties beyond those expressly stated in the product description.
17. PathnSitu shall not be liable for property damage, personal injury, time or effort, or economic loss arising from the use of this product.
18. Please refer to the complete datasheet for all instructions, precautions, and additional product limitations.
19. For detailed information and specifications on individual components, please refer to the Product Material Safety Data Sheet (MSDS)

BIBLIOGRAPHY

- Advances in understanding the morphology of glomerular disease DR TURNER* From the Department of Pathology, Guy's Hospital Medical School, Londoni Bridge, Lotndoti SEI 9RT
- Histochemical staining reactions of the normally Functioning and abnormal kidney. WACHSTEIN, Department of pathology St. catherine's Hospital, Brooklyn, New York
- Role of special stains as a useful complementary tool in the diagnosis of renal diseases: a case series study. Veenaa Venkatesh, Vinuta Malaichamy

EXPLANATION OF SYMBOLS

Lot number / Batch number

Expiry

Storage limitation

RT- Room temperature

Date of manufacture

Catalogue number

Manufacturer address

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