Reticulin Stain Kit

Laboratory Use Only

PRODUCT INFORMATION: **PERFORMANCE CHARACTERISTICS:**

REF

SSP013 100ml
SSP013 250ml
SSP013 500ml

Staining Interpretation:
Reticulum: Black
Nuclei : Red/Pink

SUMMARY AND EXPLANATION

For *laboratory* use only

The Reticulin Stain Kit is extensively used in the histopathology laboratory for staining liver, kidney, spleen specimens but can also be used to identify fibrosis in bone marrow core biopsy specimens. Fibrosis or the excess formation of fibrous tissue is commonly demonstrated in bone marrow biopsy specimens that have myeloproliferative disorders (conditions that cause blood cells to grow abnormally in the paraffin processed bone marrow) such as polycythemia vera, primary or idiopathic myelofibrosis, essential thrombocytosis, or chronic myeloid leukemia (CML). Additionally, fibrosis can be noted on bone marrow specimens that have significant tumor metastasis. Because several neoplastic and non-neoplastic pathologic conditions can be associated with increased reticulin fibrosis, the pathologist must be certain to evaluate both the quantity and thickness of the fibers. Reticulin fibers cannot be visualized in a hematoxylin & eosin (H&E) stained slide. Reticulin fibers are agyrophilic, meaning that these tissue elements will stain black with a silver solution using the aid of a chemical reducer, which brings the silver into a visible form. This silver staining process is known as silver impregnation. The reticulin stain used to demonstrate reticulin fibers for this course is Gordon & Sweets.

PRINCIPLE OF THE PROCEDURE

Reticulin fibres have little natural affinity for silver solutions so, they must be treated with potassium permanganate to produce sensitised sites on the fibres where silver deposition can be initiated. The silver is in a form readily able to precipitate as metallic silver (diamine silver solution). The optimal pH for maximum uptake of silver ions is pH 9.0. A reducing agent, formalin, causes deposition of silver in the form of metal. Any excess silver in the unprecipitated state is removed by treating with sodium thiosulphate. Gold chloride treatment renders the preparation permanent and produces a neutral black colour of high intensity.

REAGENTS PROVIDED

Kit Contents	Product Code	Storage Conditions	Pack Sizes		
			100ml	250ml	500ml
Potassium Permanganate Solution (Reagent A)	IPS058	RT	100ml	250ml	500ml
Oxalic Acid Solution (Reagent B)	IPS059	RT	100ml	200ml	500ml
Iron Alum Solution (Reagent C)	IPS060	RT	100ml	200ml	500ml
Silver Nitrate Solution – B (Reagent D)	IPS061	2 – 8 °C	50ml	125ml	250ml
Sodium Hydroxide Solution (Reagent E)	IPS062	RT	50ml	125ml	250ml
Gold Chloride Solution (Reagent F)	IPS050	2 – 8 °C	100ml	250ml	500ml
Sodium Thiosulphate Solution - A (Reagent G)	IPS051	RT	100ml	250ml	500ml
Nuclear Fast Red Solution (Reagent H)	SS006	RT	100ml	250ml	500ml

STORAGE AND HANDLING

Storage Recommendations: Store at recommended storage conditions. When stored at the appropriate conditions, the reagents are stable until expiry date.
Do not use the reagents after expiration date provided on the vial.
To ensure proper reagent performance delivery and stability, replace the dispenser cap after every use and immediately place the vials at recommended storage conditions and keep away from sunlight and heat.
During transport, short-term exposure to 2-8°C does not affect product performance.

SPECIMEN PREPARATION

Sample preparation and fixation: Formalin-fixed, Paraffin-embedded tissue sections of 4- 5 µm thickness.

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. The product contains hazardous reagents, must use gloves while handling.
4. It can cause serious eye and skin irritation. Refer to Material Safety Datasheet for any updated risk, hazard or safety information.
5. Dispose of waste observing all local, state, provincial or national regulations.
6. Do not use reagents after expiration date.
7. Use protective clothing or laboratory aprons, while handling reagents.
8. Avoid contamination of reagents as it may lead to incorrect results.

MATERIALS REQUIRED. BUT NOT PROVIDED

- Positive control and Negative control slides
- Formalin solution- 10%
- Ammonium Hydroxide, concentrated
- Xylenes
- Alcohol (50%, 70%, 95%, Absolute)
- Mounting Medium
- Microscopic slides (Positively charged)
- Slide holder
- Cover slips
- Coplin jars

PREPARATION OF WORKING SOLUTION

Ammoniacal Silver Nitrate working solution:

1. Take the required volume of Silver Nitrate Solution - B (Reagent D) in a clean conical flask.
2. While continuously shaking or swirling the flask, add concentrated ammonium hydroxide (**not provided**) drop by drop until the precipitate formed is completely dissolved. (**Do not add excess ammonium hydroxide**)
3. Add the required volume of Sodium Hydroxide Solution (Reagent E) to the flask.
4. The solution will turn black and a precipitate will form.
5. Continue swirling the flask and add concentrated ammonium hydroxide (**not provided**) drop by drop until the black precipitate just dissolves.
6. At this stage, the solution should retain slight cloudiness.
7. If no cloudiness is observed, add Silver Nitrate Solution - B (Reagent D) drop by drop until faint cloudiness appears (Note: If the addition of a more drop results in permanent cloudiness, ensure that only faint cloudiness is present).
8. Dilute the resulting solution to the given volume using distilled or deionized water.
9. Filter the solution into a chemically clean container.

Note: Once prepared, the working solution can be stable for 3 days if stored in a plastic container at 2-8 °C.

Table: Reference volume for working solution.

Silver Nitrate Solution – B (Reagent D)	Sodium Hydroxide Solution (Reagent E)	DI water	Total volume
1 ml*	1 ml*	8 ml	10 ml
3 ml*	3 ml*	24 ml	30 ml

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5 ml*	5 ml*	40 ml	50 ml

STAINING PROCEDURE

1. Deparaffinize in three changes of xylene and hydrate to distilled water via decreasing concentrations of alcohols (100%, 70%, and 50%), 3 minutes each.
2. Oxidize sections in Potassium Permanganate Solution (Reagent A) for 5 minutes.
3. Rinse slides in distilled water for 2 minutes.
4. Bleach/Reduction in Oxalic acid Solution (Reagent B) for 2 minutes or until the section is colourless.
5. Wash slides in distilled water for 2 minutes.
6. Mordant / Sensitize sections in Iron Alum Solution (Reagent C) for 15 minutes.
7. Wash slides in several changes of distilled water.
8. Impregnate sections by placing slides in Ammoniacal Silver Nitrate working Solution (Refer to the Preparation of working solution above) for 2 minutes.
9. Rinse slides briefly with distilled water. Do not wash for more than 1 minute.
10. Reduce sections for 2 minutes in 10% Formalin Solution (not provided).
11. Wash slides in distilled water for 3 minutes.
12. Tone sections in Gold Chloride Solution (Reagent F) for 10 minutes.
13. Rinse slides in distilled water.
14. Fix slides in Sodium Thiosulphate Solution - A (Reagent G) for 1 minute.
15. Wash slides in distilled water for 2 minutes.
16. Counterstain with Nuclear Fast Red Solution (Reagent H) for 4 minutes. Generally, all sections except those from the liver are counterstained. Wash well in running tap water.
17. Dehydrate in 2 changes each of 95% ethanol and absolute ethanol.
18. Clear in 3 changes of xylene for 3 minutes each and mount with appropriate mounting medium.

QUALITY CONTROL

The recommended positive tissue controls for the Reticulin Stain Kit are Liver, Kidney, Bone marrow, and Spleen tissues.

PERFORMANCE CHARACTERISTICS

Reticulin Stain Kit stains **Reticulin fibres in black colour and nuclei in red or pink colour**.

TROUBLESHOOTING

1. Follow the specific protocol recommendations according to the 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 +91-40-2701 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.

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7. The product has been validated on buccal smear. The end user must establish performance on other smear types.
8. Unexpected results may occur in untested smear due to inherent variability in smear 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

1. Sheehan DC, Hrapchak BB: Theory and Practice of Histotechnology, 2nd ed, CV Mosby Co., St. Louis, MO, 1980, pp 181–182
2. Carson FL: Histotechnology: A Self-Instructional Text, ASCP Press, Chicago, IL, 1990, pp 150-155.
3. Wallington, EF (1965): The explosive properties of ammoniacal-silver solutions. J Med Lab Technol, 22, 220–223Saxena R, Special Stains in Interpretation of Liver Biopsies, pp 94, Connection 2010.

EXPLANATION OF SYMBOLS

Lot number / Batch number

Expiry

Storage limitation

RT **Room Temperature**

Date of manufacture

Catalogue number

Manufacturer address

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