Reticulin Stain

Laboratory Use Only CE

PRODUCT INFORMATION:

SSP013 100ml Ready to use
SSP013 250ml Ready to use
SSP013 500ml Ready to use

PERFORMANCE CHARACTERISTICS:

Staining Interpretation:
Reticulum : Black
Nuclei : Red/Pink

SUMMARY AND EXPLANATION

For *laboratory* use only

The Reticulin stain 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
1% Potassium Permanganate (Reagent A)	IPS058	2 – 8 °C	100ml	250ml	500ml
1% Oxalic Acid (Reagent B)	IPS059	2 – 8 °C	100ml	200ml	500ml
2.5% Iron Alum (Reagent C)	IPS060	2 – 8 °C	100ml	200ml	500ml
10% Silver Nitrate (Reagent D)	IPS061	2 – 8 °C	50ml	125ml	250ml
3% Sodium Hydroxide Solution (Reagent E)	IPS062	2 – 8 °C	50ml	125ml	250ml
0.2% Gold Chloride Solution (Reagent F)	IPS050	2 – 8 °C	100ml	250ml	500ml
5% Sodium thiosulphate Solution (Reagent G)	IPS051	2 – 8 °C	100ml	250ml	500ml
Nuclear Fast Red (Reagent H)	SS006	RT	100ml	250ml	500ml

STORAGE AND HANDLING

Storage Recommendations: Store at 2- 8° C. Nuclear Fast Red Solution should store at room temperature. 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.

SPECIMEN PREPARATION

Recommended positive controls: Liver, Kidney, Bone marrow, and Spleen tissues

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 microbial 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: Take given volume of 10% Silver Nitrate (Reagent D) in a clean conical flask. While shaking or swirling the flask continuously, add concentrated ammonium hydroxide (not provided), drop by drop, until the precipitate formed is completely dissolved. Do not add excess ammonium hydroxide.

Add given volume of 3% Sodium Hydroxide (Reagent E) to the flask. Solution will turn black and precipitate will form. Continuously swirl the flask and add concentrated ammonium hydroxide (not provided), drop by drop, until the precipitate just dissolves. At this stage the solution should not be completely clear.

NOTE: If no cloudiness remains, add 10% Silver Nitrate (Reagent D) drop by drop, until one drop causes the solution to become permanently cloudy. Only a faint cloudiness is desirable.

Dilute the resulting solution to given volume with distilled or deionized water. Filter into a chemically clean container. **Once prepared, the working solution can stable for 3 days if stored in plastic container at 2°-8° C.**

Table: Reference volume for working solution.

10 % Silver Nitrate Reagent D	3% Sodium Hydroxide Reagent E	DI water	Total volume
1 ml*	1 ml*	8 ml	10 ml
3 ml*	3 ml*	24 ml	30 ml
5 ml*	5 ml*	40 ml	50 ml

* Do not add excess ammonium hydroxide. Maximum drops required are 5, 15 and 30 respectively for total volume of 10, 30, and 50 ml.

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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 1% Potassium Permanganate (Reagent A) for 5 minutes.
3. Rinse slides in distilled water for 2 minutes.
4. Bleach/Reduction in 1% Oxalic acid (Reagent B) for 2 minutes or until section is colorless.
5. Wash slides in distilled water for 2 minutes.
6. Mordant / Sensitize sections in 2.5% Iron Alum (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 for 2 minutes.
9. Rinse slides briefly with distilled water. Do not wash 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 0.2% Gold Chloride (Reagent F) for 10 minutes.
13. Rinse slides in distilled water.
14. Fix slides in 5% Sodium thiosulphate (Reagent G) for 1 minute.
15. Wash slides in distilled water for 2 minutes.
16. Counterstain with Nuclear Fast Red (Reagent H) for 4 minutes. Generally, all sections except those from 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 3 minutes each and mount with appropriate mounting medium.

PERFORMANCE CHARACTERISTICS

Reticulin Stain for Reticulin fibers stains black color and nuclei stains red or pink 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. If unusual results occur, contact PathnSitu Technical Support at +91-40-2701 5544 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.

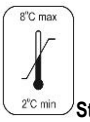
BIBLIOGRAPHY

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- Lot number / Batch number

- Expiry



Storage limitation

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RT- Room Temperature

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