PathnSitu Biotechnologies

LUO

Reticulin Stain

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. This product should be used by qualified and trained professional users only.
2. The product contains hazardous reagents, must use gloves while handling.
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 or laboratory aprons, while handling reagents.
7. 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 10% Silver Nitrate (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 3% Sodium Hydroxide (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 10% Silver Nitrate (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 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

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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

1. Authorized and skilled/trained personnel only may use the product.
2. The clinical interpretation of any test results should be evaluated within the context of the patient's medical history and other diagnostic test results.
3. A qualified trained pathologist must perform the evaluation of the test results.
4. Use appropriate volume/concentration to cover entire tissue sections and optimum conditions to avoid false positive and negative results.
5. Use appropriate/recommended buffer/instruments/all consumables with appropriate incubation timings to obtain optimal results.
6. Always recommend using known positive and negative controls to evaluate the test result.
7. Unexpected reactions may occur in untested tissues due to tissue component variability.
8. False positive results can arise from no stringent washing practices and other contributing factors.
9. Maintain recommended storage conditions.
10. Please refer to the entire datasheet for additional instructions, precautions, and limitations.
11. No warranties whether expressed or implied, extend beyond description.
12. The kit has been validated on commonly tested formalin-fixed, paraffin-embedded (FFPE) tissues. Performance on other tissue types must be established by the user.
13. Do not use reagents that appear cloudy, discolored, or show signs of contamination. Discard if any component shows deterioration.
14. Maintain recommended storage conditions.
15. This product is intended for single-use application only. Once applied to the tissue section, it shouldn't be recovered or reused. Reuse may compromise the integrity, specificity, and reliability of immunohistochemical staining.
16. PathnSitu is not liable for property damage, personal injury, time or effort or economic loss caused by this product.

BIBLIOGRAPHY

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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**
LUO **Laboratory Use Only**	**Storage limitation**
Date of manufacture	REF **Catalogue number**
Manufacturer address	RT **Room Temperature**

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