PathnSitu Biotechnologies

LUO

Masson Fontana Stain

PRODUCT INFORMATION

REF

SSP026	25 Reactions
SSP026	50 Reactions

PERFORMANCE CHARACTERISTICS:

Staining Interpretation:

Melanin: Black

Nuclei: pink

SUMMARY AND EXPLANATION

For Laboratory use only

The Fontana-Masson stain is intended for use in the histological visualization of melanin pigment in FFPE sections. Melanin is a nonlipid, no hematogenous pigment present in the hair, skin, retina and iris. This stain is particularly useful for identifying melanocytes and melanotic tumors. The method involves using silver-based reagents that bind to melanin, making it visible under a microscope.

PRINCIPLE OF THE PROCEDURE

Any amount of melanin present in the target tissue, the silver nitrate ($AgNO_3$) reacts with melanin to produce metallic silver (Ag), resulting in a black stain that can be visualized with a light microscope. The gold chloride tones the same left-over melanin pigment. Any non-specific blackening of slide due to remaining unreduced silver can be omitted by sodium thiosulphate. The safranin is used as a counter stain to stain other tissue elements.

REAGENTS PROVIDED

Kit Contents	Product Code	Storage Conditions	Pack Sizes	
			25 tests	50 tests
10% Silver nitrates (Reagent A)	IPS061	2-8ºc	50ml	100ml
0.2% Gold chloride (Reagent B)	IPS050	2-8ºc	25ml	50ml
5% Sodium thio-Sulphate (Reagent C)	IPS051	RT	25ml	50ml
0.01% Safranin (Reagent D)	IPS097	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:

Skin, malignant/pigmented melanoma, melanocytic melanoma

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 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
7. Use protective clothing and gloves, while handling reagents
8. 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
- Cover slips
- Coplin jars
- Concentrated Ammonium hydroxide

REAGENT PREPARATION

Ammoniacal Silver Nitrate working solution: Take given volume of 10% Silver Nitrate (Reagent A) 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 solution. Dilute the resulting solution to given volume with distilled or deionized water to make it the final volume.

Reference volume for working solution:

- 10% Silver nitrate (Reagent A) : 2ml
- Distilled Water : 48ml

STAINING PROCEDURE:

1. Deparaffinize in three changes of xylene and hydrate to distilled water via decreasing concentrations of alcohols (100%, 70%, and 50%) for 3 minutes each.
2. Preheat the Ammoniacal silver nitrate working solution (Refer to reagent Preparation above) for 15 min at 60^{o}c.
3. Place the slides in preheated Ammoniacal silver nitrate solution for 4min In water bath at 60 ^{o}c
4. Rinse the slides in distilled water.
5. Incubate the sections with 0.2% Gold chloride (Reagent B) for 5 minutes.
6. Rinse slides thoroughly in distilled water.
7. Incubate slides with 5% sodium thiosulphate (Reagent C) for 5 minutes.
8. Rinse the slides in distilled water for 2 minutes.
9. Counterstain with 0.01% Safranin (Reagent D) for 5-10 Seconds.
10. Rinse slides in distilled water for 30 seconds.
11. Dehydrate quickly through 95% alcohol and 2 changes of 100% alcohol.
12. Clear in 3 changes of xylene for 2 minutes each.
13. Cover slip with compatible mounting medium.

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QUALITY CONTROL

The recommended positive tissue control for Fontana Masson
Is the skin, Malignant/pigmented melanoma/Melanocytic Melanoma

PERFORMANCE CHARACTERISTICS

Fontana Masson stain highlights **Melanin** in **black** color, and **other tissue elements** will be stained **pink** in color.

TROUBLESHOOTING

Follow the specific protocol recommendations according to data sheet provided
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
Do not allow the section to dry out during the entire staining process
Excessive or incomplete counterstaining may compromise the interpretation of the results 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. The product comes with no warranties beyond the provided description
5. Use appropriate volume/concentration to cover entire tissue sections and optimum conditions to avoid false positive and negative results.
6. Use appropriate/recommended buffer/instruments/all consumables with appropriate incubation timings to obtain optimal results.
7. Always recommend using known positive and negative controls to evaluate the test result.
8. Unexpected reactions may occur in untested tissues due to tissue component variability.
9. False positive results can arise from no stringent washing practices and other contributing factors.
10. In instances where localization differs from the specifications outlined in the datasheet, clinical coordination or prompt technical support is advised.
11. Maintain recommended storage conditions.
12. Refer entire data sheet to know any further limitations about the product.
13. No warranties whether expressed or implied, extend beyond the description.
14. PathnSitu is not liable for property damage, personal injury, time or effort or economic loss caused by this product.

BIBLIOGRAPHY

1. A Novel Histochemical Method for a Simultaneous Staining of Melanin and Collagen Fibers Víctor S. Carriel, Jose Aneiros-Fernandez, Salvador Arias-Santiago, Ingrid J. Garzón, Miguel Alaminos, and Antonio Campos
2. Relationship between skin response to ultraviolet exposure and skin color type S. Del Bino, J. Sok, E. Bessac and F. Bernerd*
3. Melanoma of the Urinary Bladder: A Review of the Literature Anthony Kodzo-Grey Venyo

EXPLANATION OF SYMBOLS

Lot number / Batch number


Expiry


Storage limitation

RT-Room temperature


Date of manufacture

Catalogue number


Manufacturer address

LUO- Laboratory use only

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