PathnSitu Biotechnologies

LUO

Melanin Bleach stain

PRODUCT INFORMATION:

REF

SSP027 25 reactions

SSP027 50 reactions

PERFORMANCE CHARACTERISTICS:

Staining interpretation:

Melanin: Colorless

Nuclei: Pink

SUMMARY AND EXPLANATION

For *Laboratory* use only

Melanin bleach stain is intended to be used in many histopathological investigations. It helps to remove melanin pigment from tissues containing a pale brown to dark brown or even black pigment localized to intracellular cytoplasm. Melanin can mask or alter the staining patterns of certain histological dyes. Bleaching the melanin allows for more accurate application of staining techniques, which can be critical for diagnosing diseases or understanding tissue pathology.

PRINCIPLE OF THE PROCEDURE

When melanin pigment is present in large amounts, cell detail may be obscured. Also, the ability to be bleached serves as an identifying factor for melanin. Removal of melanin from tissue sections can be attained by treating the tissue sections with potassium permanganate followed by incubation with oxalic acid. If any traces of melanin are present, silver nitrate (AgNO3) reacts with melanin to produce metallic silver (Ag), resulting in a black stain that can be visualized with a light microscope, indicating that the melanin pigment was not bleached completely. Any non-specific blackening of the 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.

REAGENT PREPARATION

Kit contents	Product code	Storage conditions	Pack sizes	
			25 tests	50 tests
1% Potassium permanganate (Reagent A)	IPS058	RT	25ml	50ml
1% Oxalicacid (Reagent B)	IPS059	RT	25ml	50ml
10%Silvernitrate (Reagent C)	IPS061	2-8^{0C}	50ml	100ml
5%Sodiumthio sulphate (Reagent D)	IPS051	RT	25ml	50ml
0.01%SafraninO (Reagent E)	IPS097	RT	25ml	50ml

STORAGE AND HANDLING

Storage recommendations: Store at recommended temperature. 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 room temperature away from sunlight in an upright position.

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SPECIMEN PREPARATION

Recommended positive controls: Skin, Malignant 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
6. Use protective clothing and gloves, while handling reagents
7. 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 solution

REAGENT PREPARATION

Ammoniacal Silver Nitrate working solution: Take given volume of 10% Silver Nitrate (Reagent C) 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 to the final volume.

Reference volume for working solution:

10% Silver Nitrate (Reagent C): 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%, 50%) 3 minutes each.
2. Incubate the slides with 1% Potassium Permanganate (Reagent-A) for 5 minutes.
3. Rinse the slides in distilled water for 2 minutes.
4. Incubate the slides with 1% Oxalic acid (Reagent B) for 2 minutes or until section is colourless.
5. Wash slides in distilled water for 2 minutes.
6. Preheat the Ammoniacal Silver nitrate working solution (Refer to reagent preparation above) for 15 min at 60ºc.
7. Place the slides in preheated Ammoniacal silver nitrate working solution for 45 min in water bath at 60ºc.
8. Rinse the slides thoroughly in distilled water.
9. Incubate the slides with 5% Sodium thiosulphate (Reagent D) for 5minutes.
10. Wash the slides thoroughly in distilled water for 3 minutes.
11. Counterstain with Safranin (Reagent E) for 3-5 Seconds.
12. Air dry and dehydrate quickly through graded alcohols (95% ,100% alcohol).
13. Clear the slides in 3 changes of xylene for 2 minutes each.
14. Cover slip with Compatible mounting medium (DPX mountant).

QUALITY CONTROL

The recommended positive tissue control for melanin bleach is the skin, and Pigmented or Malignant/ melanoma.

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PERFORMANCE CHARACTERISTICS

The **Melanin pigment** will be **colourless** after bleaching and the **other tissue elements** will be stained **pink** in colour.

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. Orchard ge, calonje e. The effect of melanin bleaching on immunohistochemical staining in heavily pigmented melanocytic neoplasms. Am j dermatopathol. 1998;20:357–361.
2. Melanin bleaching with potassium permanganate for melanocytic lesions diagnosis in veterinary medicine [Despigmentação de melanócitos com permanganato de potássio para diagnóstico de lesões melanocíticas na medicina veterinária] T.A. Moreira1 , W.T. Blanca2 , L.F. Gundim3 , I.P. Castro1 , A.A. Medeiros-Ronchi1
3. Effective Melanin Depigmentation of Human and Murine Ocular Tissues: An Improved Method for Paraffin and Frozen Sections Caroline Manicam1 *, Susanne Pitz1 , Christoph Brochhausen2 , Franz H. Grus1 , Norbert Pfeiffer1 , Adrian Gericke1

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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