PathnSitu Biotechnologies

**Laboratory Use Only**

# Melanin Bleach Stain Kit

**PRODUCT INFORMATION:**

**REF**

**SSP027 25 reactions**

**SSP027 50 reactions**

**PERFORMANCE CHARACTERISTICS:**

**Staining interpretation:**

**Melanin: Colourless**

**Cell Nuclei: Pink**

## SUMMARY AND EXPLANATION

**For *laboratory* use only**

The reagents of the Melanin Bleach stain kit are intended for *Laboratory use* only. The Melanin Bleach stain Kit is used as a qualitative histologic stain to remove melanin pigment from tissues containing pale brown to dark brown or even black pigment localised to the intracellular cytoplasm in formalin-fixed, paraffin-embedded (FFPE) tissue. This product is not intended for diagnostic or therapeutic use The results are to be interpreted by qualified personnel in conjunction with other clinical and laboratory findings.

## PRINCIPLE OF THE PROCEDURE

When melanin pigment is present in large amounts, cell detail may be obscured. Additionally, the ability to be bleached serves as a distinguishing 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 ($AgNO_3$) 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 counterstain to stain other tissue elements.

## REAGENT PREPARATION

| Kit contents | Product code | Storage conditions | Pack sizes | |
|---|---|---|---|---|
| | | | 25 Reactions | 50 Reactions |
| Potassium Permanganate Solution (Reagent A) | IPS058 | RT | 25ml | 50ml |
| Oxalic Acid Solution (Reagent B) | IPS059 | RT | 25ml | 50ml |
| Silver Nitrate Solution – B (Reagent C) | IPS061 | 2-8ºC | 50ml | 100ml |
| Sodium Thiosulphate Solution -A (Reagent D) | IPS051 | RT | 25ml | 50ml |
| Safranin Solution (Reagent E) | IPS097 | RT | 25ml | 50ml |

## STORAGE AND HANDLING

**Storage recommendations**: Store at recommended storage conditions. 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 reagent performance delivery and stability, replace the dispenser cap after every use and immediately place the vials at recommended temperature away from sunlight in an upright position.

## SPECIMEN PREPARATION

**Recommended positive controls: Normal** Skin, Pigmented 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 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
- Conical flask
- Concentrated ammonium hydroxide solution
- Distilled water
- Water bath

## REAGENT PREPARATION

**Ammoniacal Silver Nitrate working solution:** Take given volume of Silver Nitrate Solution - B (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:**

Silver Nitrate Solution - B (Reagent C): 2ml

Distilled Water : 48ml

Note: 1. Do not expose the working solution to light.

2. The solution cannot be reused. Discard after use.

3. The Ammoniacal Silver Nitrate working solution stability is important (e.g., if it precipitates or shows silvering of the container, it indicates that the solution is deteriorated) according to the protocol. Hence, make sure the solution is colourless.

## 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 Potassium Permanganate Solution (Reagent-A) for 5 minutes.
3. Rinse the slides in distilled water for 2 minutes.
4. Incubate the slides with Oxalic Acid Solution (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 Sodium Thiosulphate Solution -A (Reagent D) for 5minutes.
10. Wash the slides thoroughly in distilled water for 3 minutes.
11. Counterstain with Safranin Solution (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).



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## QUALITY CONTROL

The recommended positive tissue control for melanin bleach is Normal skin and pigmented melanoma.

## PERFORMANCE CHARACTERISTICS

The **Melanin pigment** will be **colourless** after bleaching, and the **Cell Nuclei** will be stained **pink** in colour.

## TROUBLESHOOTING

Follow the specific protocol recommendations as outlined in the provided data sheet. 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. 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.
7. The product has been validated on formalin-fixed, paraffin-embedded (FFPE) tissues. The end user must establish performance on other tissue types.
8. Unexpected results may occur in untested tissues due to inherent variability in tissue 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 this can cause the chemical to decompose.
14. If the Ammoniacal silver working solution preparation is not correct (e.g., precipitates remain, contamination, glassware/containers not cleaned), the silver mirror effects, background silver deposition, or non-specific darkening can be observed.
15. 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.
16. PathnSitu makes no warranties beyond those expressly stated in the product description.
17. PathnSitu shall not be liable for property damage, personal injury, time or effort, or economic loss arising from the use of this product.
18. Please refer to the complete datasheet for all instructions, precautions, and additional product limitations.
19. For detailed information and specifications on individual components, please refer to the Product Material Safety Data Sheet (MSDS)

## BIBLIOGRAPHY


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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 number / Batch number**


**Expiry**

**Storage limitation**

**RT-Room temperature**


**Date of manufacture**

**Catalogue number**


**Manufacturer address**



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