PathnSitu Biotechnologies

Laboratory Use Only

Vonkossa Stain Kit

PRODUCT INFORMATION:

REF

SSP023	**100 ml (Ready to use)**
SSP023	**250 ml (Ready to use)**
SSP023	**500 ml (Ready to use)**

PERFORMANCE CHARACTERISTICS:

Staining Interpretation:
Calcium Salts: Black
Cell Nuclei : Red
Cytoplasm : Light Pink

SUMMARY AND EXPLANATION

For *Laboratory use* only

Vonkossa Stain Kit is intended for use in the histological visualization of calcium deposits in Formalin fixed paraffin embedded sections. 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

Von Kossa stain is widely used in histology to detect the presence of mineral deposits, such as abnormal calcium deposits, in the body. The principle of this staining is based on the transformation of calcium salts into silver salts: Von Kossa's technique is clearly a two-step reaction: first, silver cations react with the components of calcium deposits, resulting in a transient yellow colouration. This colouration is transient and gradually changes from grey to black in the second step, where the organic material reduces the bound silver to black metallic silver with the aid of light. Counterstaining with nuclear fast red allows for the staining of cell nuclei in red and their cytoplasm in pink.

Kit Contents	Product Code	Storage Conditions	Pack Sizes		
			100ml	250ml	500ml
Silver Nitrate Solution - A (Reagent A)	IPS048	2-8°C	100ml	250ml	500ml
Sodium Thiosulphate Solution -A (Reagent B)	IPS051	RT	100ml	250ml	500ml
Nuclear Fast Red Solution (Reagent C)	IPS085	RT	100ml	250ml	500ml

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 temperature away from sunlight in an upright position.

SPECIMEN PREPARATION

RECOMMENDED POSITIVE CONTROLS:

- Tissues with Calcium deposits

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 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 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
- Incandescent Light source
- Distilled water

STAINING PROCEDURE:

1. Deparaffinize the tissue sections and hydrate to distilled water.
2. Incubate slide in Silver Nitrate Solution - A (Reagent A) for 30 minutes or longer while exposing to either light at 100 watts or Sunlight. (For best results, keep light source nearer to the slide) (~ 60cm) during Silver Nitrate impregnation procedure.

Note: 1. Inadequate time, light/exposure may yield weak stain.

- With prolonged time of exposure to light, the black/dark deposits may become so dense that fine features of calcium deposits or adjacent tissue morphology are obscured or lost, background silver deposition and over-darkening may be observed in the stained slides.

Hence, keep monitoring the slides under the light microscope after 30 minutes to stop incubation when the deposits reach optimal intensity.

3. Rinse in 3 changes of distilled water.
4. Incubate slide in Sodium Thiosulphate Solution -A (Reagent B) for 2 minutes.
5. Rinse for 2 minutes in running tap water followed by 2 changes of distilled water.
6. Stain tissue section with Nuclear Fast Red Solution (Reagent C) for 5 minutes.
7. Rinse for 2 minutes in running tap water followed by 2 changes of distilled water.
8. Dehydrate quickly in Graded Alcohols.
9. Clear in 3 changes of xylene and mount the slide with compatible medium.

QUALITY CONTROL

The recommended positive tissue control for Vonkossa stain is the tissue with calcium deposits

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PERFORMANCE CHARACTERISTICS

The Von Kossa stain for Calcium Salts stains Calcium salts in black, the Cell Nuclei in red, and the cytoplasm in light pink.

TROUBLESHOOTING

1. Follow the specific protocol recommendations according to the 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. Excessive or incomplete counterstaining may compromise the interpretation of the results
5. 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 in the reagent bottle to bright light, including direct sunlight, as this can cause the chemical to decompose. Keep the reagent containers closed after every use.
14. Silver Nitrate reagents are easily contaminated; background silver deposition may occur if Containers/glassware are not cleaned correctly.
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

- Von Kossa and his staining technique; Marlon R. Schneider1, Accepted: 3 November 2021 / Published online: 20 November 2021
- Identification and Characterization of Vascular Calcification–Associated Factor, a Novel Gene Upregulated During Vascular Calcification In Vitro and In Vivo ; M. Yvonne Alexander, Fiona L. Wilkinson, John Paul Kirton, Claire Farrington Rock, Georgina D.M. Collett, Maria Jeziorska, J. Vincent Smyth, Anthony M. Heagerty, Ann E. Canfield
- HISTOCHEMICAL M ETHODS FOR CALCIUM, S.M McGEE Russell, Department of zoology, Birkbeck college, University of London, Male street W.C, London, June 6, 1957

EXPLANATION OF SYMBOLS

LOT **Lot number / Batch number**

Expiry

Storage limitation

RT- Room temperature

Date of manufacture

REF **Catalogue number**

Manufacturer address

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