Movat's Pentachrome Stain Kit

Laboratory Use Only

PRODUCT INFORMATION:

REF

SSP025 25 Reactions
SSP025 50 Reactions

PERFORMANCE CHARACTERISTICS:

Staining Interpretation:
Mucins & Ground Substance: Blue - Green
Elastin Fibers & Cell Nuclei: Purple - Black
Collagen Fibers: Yellow
Muscle: Red
Fibrin: Intense Red

SUMMARY AND EXPLANATION

For *laboratory* use only

Movat's Pentachrome Stain Kit is intended for use in simultaneous histological visualization of five major tissue components in contrasting colours. The term 'Pentachrome stain' is a general name for the application of five dyes, which facilitates the selective demonstration of muscle, cell nuclei, mucins and ground substance, elastin fibers, collagen fibers, and Fibrin. 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

Movat's pentachrome is a multichromatic histochemical technique designed to differentiate and demonstrate various connective tissue elements in a single tissue section. The stain uses a series of specific dyes and chemical reactions to color five distinct tissue components in contrasting colours, providing a comprehensive view of tissue architecture and pathological changes. The principle is based on the sequential staining reactions where each dye has selective affinity for particular tissue element: cell nuclei and elastin fibers are stained purple to black by Verhoeff's hematoxylin, where ferric chloride and iodine acts as mordent and oxidizing agents to form black lake with elastin, collagen is stained yellow by tartrazine, which has a high affinity for mature collagen fibers, ground substance and mucin are stained blue to green with alcian blue, due to it's cationic nature that binds to acidic mucopolysaccharides. Muscle and Fibrin are stained in red and bright red by biebrichscarlet acid fuchsin, differentiating it from the surrounding connective tissue. Erythrocytes may typically be found stained bright red. Thus, Movat's pentachrome stain provides a composite color pattern that allows simultaneous visualization of elastin fibers, collagen, ground substance, mucin, cell nuclei and muscle

REAGENTS PROVIDED

Kit Contents	Product Code	Storage Conditions	Pack Sizes	
			25 tests	50 tests
Alcian Blue Solution (Reagent A)	SS005	RT	25ml	50ml
Hematoxylin Solution – A (Reagent B)	IPS088	RT	15ml	30ml
Ferric Chloride Solution - A (Reagent C)	IPS089	RT	7ml	15ml
Lugol's Iodine Solution (Reagent D)	IPS090	RT	7ml	15ml
Ferric Chloride Solution - B (Reagent E)	IPS091	RT	25ml	50ml
Sodium Thiosulphate Solution -A (Reagent F)	IPS051	RT	25ml	50ml
Biebrich Scarlet Acid Fuchsin Solution (Reagent G)	IPS033	RT	25ml	50ml
Phosphomolybdic and Phosphotungstic Acid Solution (Reagent H)	IPS034	RT	75ml	150ml
Tartrazine Solution – A (Reagent I)	IPS096	RT	25ml	50ml

STORAGE AND HANDLING

Storage Recommendations: Store at Room Temperature. When stored at the appropriate conditions, the reagents are stable until expiry. **Do not use the reagents after the expiration date provided on the vial.**

To ensure proper reagent delivery and stability, replace the dispenser cap after every use and immediately place the vials in appropriate storage conditions away from sunlight in an upright position.

During transport, short-term exposure to 2- 8 °C does not affect product performance.

SPECIMEN PREPARATION

Formalin-fixed, Paraffin-embedded tissue sections of 4-5 µm thickness mounted on positively charged glass 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. The product contains Alcohol and is classified as highly flammable; it must be kept away from ignition sources
4. It can cause serious eye and skin irritation. Refer to Material Safety Datasheet for any updated risk, hazard or safety information.
5. Dispose of waste observing all local, state, provincial or national regulations.
6. Do not use reagents after the expiration date
7. Use protective clothing and gloves while handling reagents
8. Avoid contamination of reagents, as it may lead to incorrect result

MATERIALS REQUIRED. BUT NOT PROVIDED

- Xylenes
- Graded alcohols (100%, 70% ,50%)
- DPX Mountant
- Microscopic slides
- Slide holder
- Cover slips
- Coplin jars
- Eppendorf tubes (2ml)
- Distilled water
- Staining/humid chamber

REAGENT PREPARATION

Verhoeff Hematoxylin Working Solution: Verhoeff hematoxylin working solution should be made up fresh for the best results. Prepare the working solution by adding the following reagents in the similar order:

- Hematoxylin Solution - A (Reagent B): 0.5 ml
- Ferric Chloride Solution – A (Reagent C): 0.25 ml
- Lugol's Iodine solution (Reagent D) : 0.25 ml

The volumes mentioned above are sufficient for use for one slide. Mix the above amounts (or the required proportions thereof) thoroughly. Solution should be jet black. Use immediately, and any leftover volume can be added to the slides during the incubation of this working solution.

STAINING PROCEDURE

STANDARD PROTOCOL:

1. Deparaffinize the slides and hydrate to distilled water.
2. Stain the slides with Alcian Blue Solution (Reagent A) and incubate for 20 minutes.
3. Rinse the slides with under running tap water for 10 mins, followed by two changes of distilled water.
4. Stain the slides with Verhoeff's Hematoxylin Working Solution (Refer to the reagent preparation above) and incubate for 30 mins
5. Rinse the slides under running tap water for 5 mins.

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6. Treat the slides with Ferric Chloride Solution-B (Reagent E) for 2 - 5 seconds for differentiation. **Note:** The ferric chloride is both mordant and differentiator. So, differentiate quickly in ferric chloride solution until tissue background lightens but elastic fibers and nuclei remain black. As the time of differentiation is somewhat dependent on the amount of elastic tissue present, it is better not to rely on the control of timing for the differentiation of all sections, and slides must be individually differentiated to get good results.
7. Rinse the slides under running tap water for 2 mins, followed by two changes of distilled water. Observe the slides under microscope. The elastic fibers and nuclei should remain jet black while surrounding tissue becomes noticeably paler.
8. Incubate the slides with Sodium Thiosulphate Solution -A (Reagent F) for 1 min.
9. Rinse the slides under running tap water for 2 mins, followed by two changes of distilled water.
10. Stain the slides with Biebrich Scarlet Acid Fuchsin Solution (Reagent G) and incubate for 3mins
11. Rinse the slides in two changes of distilled water.
12. Incubate the slides with Phosphomolybdic and Phosphotungstic Acid Solution (Reagent – H) with 3 changes for 5 mins each (Apply the reagent and incubate for 5 minutes, and discard. Repeat this step twice more to complete three 5-minute cycles)
    Note: Phosphomolybdic and Phosphotungstic Acid Solution (Reagent H) is applied in short 5-minute intervals to ensure controlled differentiation, preventing over-bleaching and excess red-dye removal while preserving fibrin and muscle staining; this avoids dull nuclear/elastic staining, a washed-out background, and impaired tartrazine uptake.
13. Rinse slides in 2 changes of distilled water.
14. Counter stain with Tartrazine Solution-A (Reagent – I) and incubate the slides for 3 mins
15. Dip the slides rapidly through 3 changes of 100% alcohol.
    Note: Do not use distilled water or graded alcohols for washing slides, as these will wash out the counterstain.
16. Clear the slides in 3 changes of xylene for 2 minutes each.
17. Cover slip with Compatible mounting medium (E.g. DPX mountant).

QUALITY CONTROL

The recommended positive tissue controls for Movat's Pentachrome Stain Kit are Normal Lung, Skin and Colon

PERFORMANCE CHARACTERISTICS

Movat's Pentachrome Stain Kit, stains **Cell Nuclei** and **Elastin Fibers** in **Purple to Black** color, **Mucins and Ground Substance in Blue to Green** Color**, Muscle in Red** color**, Collagen Fibers in Yellow** color, and **Fibrin** in **Intense Red.**

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. General issues occurring in Movat's pentachrome staining

S.no	Staining Issue	Trouble shooting
5.1	Elastin fibers/cell nuclei are colour less	Decrease the incubation time in the Ferric chloride solution B on step 6
5.2	Muscle and collagen are not differentiated	The final stains of the procedure (Biebrichscarlet Acid Fuchsin Solution and Tartrazine solution – A) are a trichrome – type of staining that is quite sensitive to incubation time and temperature. The "differentiating solution" Phosphomolybdic and Phosphotungstic Acid Solution on step 12 is also sensitive to incubation time and temperature.
5.3	Collagen is colour less or not yellow	a) Do not allow the slides to dip in water or graded alcohols because tartrazine solution is water soluble. b) Decrease the incubation time of "differentiating solution" Phosphomolybdic and Phosphotungstic Acid Solution on step 12.
5.4	Muscle and Background are too yellow	Increase the incubation time of "differentiating solution" Phosphomolybdic and Phosphotungstic Acid Solution on step 12.

6. 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. 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.
14. All the staining associated containers must be clean and dust free prior to use. Residual reagents, detergents, or particulate matter can adversely affect staining performance and result interpretation.
15. Use the product only under the recommended assay conditions specified in this datasheet, as performance outside validated conditions cannot be guaranteed.
16. Mix reagents gently, if required, to ensure homogeneity without introducing bubbles or affecting staining performance.
17. Alcian Blue staining is pH-sensitive; improper storage may lead to reagent degradation and affect mucin staining intensity.
18. Over- or under-exposure to Ferric Chloride Solution-B and Phosphomolybdic and Phosphotungstic Acid Solution results in loss of color balance.
19. PathnSitu makes no warranties beyond those expressly stated in the product description.
20. PathnSitu shall not be liable for property damage, personal injury, time or effort, or economic loss arising from the use of this product.
21. Please refer to the complete datasheet for all instructions, precautions, and additional product limitations.
22. For detailed information and specifications on individual components, please refer to Product Material Safety Data Sheet (MSDS)

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EXPLANATION OF SYMBOLS

LOT	Lot number / Batch number		Expiry
°C	Storage limitation	RT	Room Temperature
	Date of manufacture	REF	Catalogue number
	Manufacturer address		

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