Masson's Trichrome Stain Kit

Laboratory Use Only

PRODUCT INFORMATION:

REF

SSP009 100ml
SSP009 250ml
SSP009 500ml

PERFORMANCE CHARACTERISTICS:

Staining Interpretation:
Nuclei : Black
Cytoplasm : Red
Muscle Fibers : Red
Collagen Fibers : Blue

SUMMARY AND EXPLANATION

For *laboratory* use only

Masson's Trichrome Stain Kit is a three-colour staining protocol used in histology.. The term 'trichrome stain' is a general name for the application of three dyes, which facilitates the selective demonstration of muscle, collagen fibres, fibrin and erythrocytes. This method is used to differentiate between smooth muscle and collagen fibers in tissue sections using light microscopy. It is often a routine stain for liver and kidney biopsies, as well as for analyzing muscle and skin tissues. The results should be verified with appropriate controls and interpreted by qualified laboratory personnel.

PRINCIPLE OF THE PROCEDURE

As the name implies, three dyes are employed to selectively stain muscle, collagen fibres, fibrin, and erythrocytes. The general rule in trichrome staining is that the smallest dye molecule colours the less porous tissues; whenever a dye of large molecular size can penetrate, it will always do so at the expense of the smaller molecule. Others suggest that the tissue is stained first with the acid dye, Biebrich Scarlet, which binds with the acidophilic tissue components. Then, when treated with the phospho acids, the less permeable components retain the red, while the red is pulled out of the collagen. At the same time, it causes a link with the collagen to bind with the aniline blue.

REAGENTS PROVIDED

Kit Contents	Product Code	Storage Conditions	Pack Sizes		
			100ml	250ml	500ml
Bouin's Fluid (Reagent A)	IPS036	RT	100ml	250ml	500ml
Weigert's Iron Hematoxylin Solution - 1 (Reagent B)	IPS029	RT	50ml	125ml	250ml
Weigert's Iron Hematoxylin Solution - 2 (Reagent C)	IPS030	RT	50ml	125ml	250ml
Biebrich Scarlet Acid Fuchsin Solution (Reagent D)	IPS033	RT	100ml	250ml	500ml
Phosphomolybdic and Phosphotungstic Acid Solution (Reagent E)	IPS034	RT	100ml	250ml	500ml
Aniline Blue Solution (Reagent F)	IPS035	RT	100ml	250ml	500ml
Glacial Acetic Acid Solution - A (Reagent G)	IPS040	RT	100ml	250ml	500ml

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 regent 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

Sample preparation and fixation: Formalin-fixed, Paraffin-embedded tissue sections of 4-5 µm thickness.

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 results

MATERIALS REQUIRED.BUT NOT PROVIDED

- Xylenes
- Graded alcohols (50%, 70%, 95%, Absolute)
- DPX Mountant
- Microscopic slides
- Slide holder
- Cover slips
- Coplin jars

PREPARATION OF WORKING SOLUTION

Weigert's Iron Hematoxylin Working Solution: Measure equal volume of Reagent B (Weigert's Iron Hematoxylin Solution - 1) and Reagent C (Weigert's Iron Hematoxylin Solution - 2) and mix. Prepare the working solution just before staining and discard once it is used.

STAINING PROCEDURE

MICROWAVE PROTOCOL:

1. Deparaffinize and hydrate to distilled water.
2. Heat the Bouin's Fluid (Reagent A) at 56º C to 60º C in microwave and then incubate slides in heated solution for 10-15 minutes.

   NOTE: Do not heat slides in Bouin's Fluid (Reagent A); heat Bouin's Fluid (Reagent A), remove from microwave, place slides in Coplin jar, seal with lid and incubate outside the microwave.
3. Wash in running tap water until the yellow color disappears and rinse in two changes of distilled water.
4. Stain nuclei with Weigert's Iron Hematoxylin working solution for 10 minutes.
5. Wash in running tap water and rinse in two changes of distilled water.
6. Stain in Biebrich Scarlet Acid Fuchsin Solution (Reagent D) for 2 minutes.
7. Rinse in three changes of distilled water.
8. Place slides in Phosphomolybdic and Phosphotungstic Acid Solution (Reagent E) for 15 minutes.
9. Drain slides and transfer to Aniline Blue Solution (Reagent F) for 5 minutes.
10. Rinse in three changes of distilled water.
11. Differentiate in Glacial Acetic Acid Solution - A (Reagent G) for 1-2 minutes.
12. Rinse in two changes of distilled water.
13. Dehydrate, clear and do cover slip with DPX mountant.

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STANDARD PROTOCOL:

1. Deparaffinize and hydrate to distilled water.
2. Incubate in Bouin's Fluid (Reagent A) solution at 56° C to 60º C for 60 minutes in hot air oven.
3. Wash in running tap water until the yellow color disappears and rinse in two changes of distilled water.
4. Stain nuclei with Weigert's Iron Hematoxylin working solution for 10 minutes.
5. Wash in running tap water and rinse in two changes of distilled water.
6. Stain in Biebrich Scarlet Acid Fuchsin Solution (Reagent D) for 2 minutes.
7. Rinse in three changes of distilled water.
8. Place slides in Phosphomolybdic and Phosphotungstic Acid Solution (Reagent E) for 15 minutes.
9. Drain slides and transfer to Aniline Blue Solution (Reagent F) for 5 minutes.
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11. Differentiate in Glacial Acetic Acid Solution - A (Reagent G) for 1-2 minutes.
12. Rinse in two changes of distilled water.
13. Dehydrate, clear and cover with DPX mountant.

QUALITY CONTROL

The recommended positive tissue controls for Masson's Trichrome Stain Kit are Lung, Uterus, Small Intestine, and Stomach**.**

PERFORMANCE CHARACTERISTICS

Masson's Trichrome Kit stains **nuclei black, cytoplasm red, muscle fibers red, and collagen fibers blue.**

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. Excessive or incomplete counterstaining may compromise the interpretation of the results
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.

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12. Do not use reagents that appear cloudy, discoloured, or show signs of contamination. Discard any components showing signs of deterioration.
13. Bouin's fluid contains picric acid, which is explosive when dry, and formaldehyde, which releases toxic and carcinogenic vapors. It must only be used under controlled laboratory conditions with appropriate safety measures.
14. 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.
15. PathnSitu makes no warranties beyond those expressly stated in the product description.
16. PathnSitu shall not be liable for property damage, personal injury, time or effort, or economic loss arising from the use of this product.
17. Please refer to the complete datasheet for all instructions, precautions, and additional product limitations.
18. For detailed information and specifications on individual components, please refer to Product Material Safety Data Sheet (MSDS)

BIBLIOGRAPHY

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4. Saxena R, Special Stains in Interpretation of Liver Biopsies, pp 94, Connection 2010.
5. Petersen, Hans. Organe der Reizbearbeitung. Histologie und mikro-skopische Anatomie. Schluss-Abschnitt 6. J.F. Bergmann, Munchen, 1935.

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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