Masson's Trichrome (NA) Stain Kit

PRODUCT INFORMATION:

REF

SSP018-NA 100ml
SSP018-NA 250ml
SSP018-NA 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 is a three-colour staining protocol used in histology. The recipes derived from Claude L. Pierre Masson's (1880–1959) original formulation have different specific applications, but all are suitable for distinguishing cells from surrounding connective tissue. Although many techniques are available for differentiation, connective tissues generally fall into the category of 'trichrome stains. The term 'trichrome stain' broadly refers to a method involving three dyes, one of which is a nuclear stain that selectively demonstrates muscle, collagen fibers, fibrin, and erythrocytes. This product is not intended for diagnostic or therapeutic use. The results should be interpreted by qualified personnel in conjunction with other clinical and laboratory findings.

PRINCIPLE OF THE PROCEDURE

As the name suggests, three dyes are used selectively to stain muscle, collagen fibers, fibrin, and erythrocytes. The general rule in trichrome staining is that the smallest dye molecules stain less porous tissues; when a larger dye molecule can penetrate, it does so at the expense of the smaller one. Others propose that the tissue is first stained with the acid dye, Biebrich Scarlet, which binds to acidophilic tissue components. Then, when treated with phospho acids, the less permeable components retain the red stain, while the red is washed out of the collagen. This process also causes a link between the collagen and 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 - 1 (Reagent B)	IPS029A	RT	1gm	2.5gm	5gm
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 reagent performance and stability, replace the dispenser cap after each use and store the vials immediately at room temperature, away from sunlight, in an upright position. During transport, short-term exposure to temperatures between 2-8 °C does not affect product performance.

Laboratory Use Only

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. 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 the expiration date
6. Use protective clothing, a face mask 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
- Whatman Filter Paper

PREPARATION OF WORKING SOLUTION

Refer to the pack size (listed on the box and the empty labelled bottle) that was received before preparing any working solutions.

Measure 95% Alcohol (check the pack size for accurate volume measurement) and pour it into an empty, labelled bottle provided in the kit. Then, add Weigert's Iron Hematoxylin – 1 (Reagent B) (check the pack size for accurate volume measurement) and mix thoroughly. Filter the solution using Whatman Filter Paper once the Hematoxylin is dissolved.

Preparation of (Weigert's Iron Hematoxylin Solution - 1):

Components	Quantity Required		
Pack Size	**100ml**	**250ml**	**500ml**
Weigert's Iron Hematoxylin – 1 (Reagent B)	1g	2.5g	5g
95% Alcohol (Not provided)	100ml	250ml	500ml

Note: Once the stock reagents (Weigert's Iron Hematoxylin Solution – 1) are prepared, they remain stable until the expiration date of the kit.

Preparation of Weigert's Iron Hematoxylin Working Solution:

Measure equal volumes of Weigert's Iron Hematoxylin Solution – 1(Refer to reagent preparation above) and Weigert's Iron Hematoxylin Solution - 2 (Reagent C) and mix well.

STAINING PROCEDURE

MICROWAVE PROTOCOL:

1. Deparaffinize and hydrate to distilled water.
2. Heat Bouin's Fluid (Reagent A) solution at 56ºC to 60ºC in a microwave and then incubate slides in the heated solution for 10-15minutes.
   NOTE: Do not heat slides in Bouin's Fluid (Reagent A); heat Reagent A, remove from microwave, place slides in coplin jar, seal with lid and incubate outside the microwave.

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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 (Refer to the reagent Preparation) for 10min.
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 2min.
7. Rinse in three changes of distilled water.
8. Place slides in Phosphomolybdic and Phosphotungstic Acid Solution (Reagent E) for 15min.
9. Drain slides and transfer to Aniline Blue solution (Reagent F) for 5min.
10. Rinse in three changes of distilled water.
11. Differentiate in Glacial Acetic Acid Solution – A (Reagent G) for 1-2min.
12. Rinse in two changes of distilled water.
13. Dehydrate, clear and do cover slip with DPX mountant.

HOT AIR OVEN PROTOCOL:

1. Deparaffinize and hydrate to distilled water.
2. Incubate in Bouin's Fluid (Reagent A) solution at 56ºC to 60ºC 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 (Refer to the reagent Preparation) for 10min.
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 2min.
7. Rinse in three changes of distilled water.
8. Place slides in Phosphomolybdic and Phosphotungstic Acid Solution (Reagent E) for 15min.
9. Drain slides and transfer to Aniline Blue solution (Reagent F) for 5min.
10. Rinse in three changes of distilled water.
11. Differentiate in Glacial Acetic Acid Solution – A (Reagent G) for 1-2min.
12. Rinse in two changes of distilled water.
13. Dehydrate, clear and do cover slip with DPX mountant.

QUALITY CONTROL

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

PERFORMANCE CHARACTERISTICS

Masson's Trichrome (NA) Stain Kit stains **nuclei** in **black**, **cytoplasm** in **red, muscle fibers** in **red**, and **collagen fibers** in **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.

Laboratory Use Only

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. 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 the Product Material Safety Data Sheet (MSDS)

BIBLIOGRAPHY

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3. Luna L,Manual of Histologic Staining Methods of the AFIP,3rd Ed, 1968, pp 94-95, McGraw-Hill, NY.
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**
	Storage limitation	**RT**	**Room Temperature**
	Date of manufacture	**REF**	**Catalogue number**
	Manufacturer address		

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