Masson's Trichrome Stain

Laboratory Use Only CE

PRODUCT INFORMATION:

SSP009	**100ml Ready to use**
SSP009	**250ml Ready to use**
SSP009	**500ml Ready to use**

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-color staining protocol used in histology. The recipes evolved from Claude L. Pierre Masson's (1880–1959) original formulation have different specific applications, but all are suited for distinguishing cells from surrounding connective tissue. Even though many techniques are available for the differentiation, connective tissues fall into the category of 'trichrome stains'. The term 'trichrome stain' is a general name for implication of three dyes in which one is nuclear stain which foes the selective demonstration of muscle, collagen fibers, fibrin and erythrocytes. This method is used to differentiate between smooth muscle and of collagen fibers in tissue sections. Most recipes produce red keratin and muscle fibers, blue or green collagen and bone, light red or pink cytoplasm, and dark brown to black cell nuclei.

PRINCIPLE OF THE PROCEDURE

As the name implies, three dyes are employed selectively staining muscle, collagen fibers, fibrin, and erythrocytes. The general rule in trichrome staining is that the less porous tissues are colored by the smallest dye molecule; whenever a dye of large molecular size is able to 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 causing 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 Fixative (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
1% Glacial Acetic Acid Solution (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 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 room temperature away from sunlight in an upright position.

SPECIMEN PREPARATION

Recommended positive controls: Formalin-fixed ,paraffin-embedded, Human Lung, Uterus, Small Intestine, Stomach**.** Cut the sections, usually 4-5 µm

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, 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 expiration date
7. Use protective clothing and gloves, while handling reagents
8. Avoid microbial 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 fixative (Reagent A) solution 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 fixative (Reagent A); heat Bouin's fixative (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 1% Glacial Acetic Acid Solution (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 fixative (Reagent A) solution at 56º 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.
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13. Dehydrate, clear and do cover slip with DPX mountant.

PERFORMANCE CHARACTERISTICS

Masson's Trichrome for Nuclei stains Black, Cytoplasm stains Red, Muscles Fibers stains Red and Collagen Fibers stains 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. 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

Authorized and skilled personnel only may use the product. The clinical interpretation of any test results should be evaluated within the context of the patient's medical history and other diagnostic test results. A qualified pathologist must perform the evaluation of the test results. There are no warranties, expressed or implied, which extend beyond the description. PathnSitu is not liable for property damage, personal injury, time or effort on economic loss caused by this product.

BIBLIOGRAPHY

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EXPLANATION OF SYMBOLS

LOT- **Lot number / Batch number**

- Expiry

RT- Room Temperature

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