Ver.250301

Massons Trichrome Stain Kit

G1340 (7×50mL)

FOR RESEARCH USE ONLY, DO NOT USE IT IN CLINICAL DIAGNOSIS

Principle

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	Storage Conditions	Pack Sizes (50 mL)
Bouin's Fixative (Reagent A)	RT	50 mL
Weigert's Iron Hematoxylin Solution 1 (Reagent B)	RT	25 mL
Weigert's Iron Hematoxylin Solution 2 (Reagent C)	RT	25 mL
Biebrich Scarlet Acid Fuchsin solution (Reagent D	RT	50 mL
Phosphomolybdic and Phosphotungstic Acid Solution (Reagent E)	RT	50 mL
Aniline Blue Solution (Reagent F)	RT	50 mL
1% Glacial Acetic Acid Solution (Reagent G)	RT	50 mL

Storage and Handling

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 56ºC to 60^0C 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.

STANDARD PROTOCOL:

1. Deparaffinize and hydrate to distilled water.
2. Incubate in Bouin's fixative (Reagent A) solution at 56º C to 60^0 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.
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.

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.

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.