

Masson's Trichrome Stain Kit

Catalog No. 25088

DESCRIPTION

This method is used to differentiate between smooth muscle and of collagen fibers in tissue sections. The Trichrome Stain can assess the degree of fibrosis in the liver, which gives important information about stage and progression of a disease. Liver diseases such as hepatitis B and C viral infections, fatty liver disease, alcoholic liver disease and chronic biliary diseases show the formation of fibrous tissue including bridging fibrous septa leading to the end-stage process called cirrhosis.¹ The stain is used to make treatment decisions; utilized to assess the effect of therapy, medications in clinical trials and needed for liver biopsy specimen and tissues such as skin and heart.

KIT CONTENTS

| Description | Product Code | 100ml Kit | 500ml Kit |
|--|-------------------|-----------|-------------|
| Bouin's Fixative | 25088A | 100 ml | 500 ml |
| Weigert's Iron Hematoxylin (A and B) | 25088B1 & 25088B2 | 75ml each | 250 ml each |
| Biebrich Scarlet - Acid Fuchsin Solution | 25088C | 100 ml | 500 ml |
| Phosphotungstic/Phosphomolybdic Acid | 25088D | 100 ml | 500 ml |
| Aniline Blue | 25088E | 100 ml | 500 ml |
| 1% Acetic Acid | 25088F | 100 ml | 500 ml |

PROCEDURE (FORMALIN FIXED PARAFFIN EMBEDDED TISSUE) - *recommended method for best results*

Important (Please READ Safety Notes under Storage and Handling before proceeding)

Place slides in oven at 60°C for approximately 15 minutes to dry slides

1. Quickly transfer slides to Xylene (2 changes for 5 minutes each)
2. Transfer slides to 100% Ethanol (2 changes 3 minutes each)
3. Transfer slides to 95% Ethanol (1 change at 3 minutes)
4. Rinse in tap water
5. Preheat Bouin's Fixative to 60°C and mordant slides in solution for 1 hour (Conventional Method) or Microwave Bouin's solution for 1 minute and allow slides to mordant in solution for 15 minutes.
6. Gently wash in running tap water to remove the picric acid for 5 minutes.
7. Stain in Weigert's Iron Hematoxylin Working Solution for 10 minutes.

To prepare Weigert's Iron Hematoxylin Working Solution:

Mix Weigert's Hematoxylin A and Weigert's Hematoxylin B at a 1:1 ratio

8. Wash in running tap water for 5 minutes, rinse in distilled water.
9. Stain in Biebrich Scarlet - Acid Fuchsin Solution for 5 minutes.
10. Rinse in distilled water.
11. Transfer to Phosphotungstic/phosphomolybdic acid for 10 minutes, discard solution.
12. Drain slide and transfer to Aniline Blue for 5 minutes.
13. Rinse in distilled water (3 changes).
14. Transfer to 1% Acetic acid for 1 minute, discard solution, rinse in distilled water.
15. Dehydrate in 95% ethanol, then 100% ethanol for 1-2 minutes each.
16. Clear in xylene for 1-2 minutes.
17. Mount with a xylene based mounting medium, Poly-Mount (Cat. # 08381)

PROCEDURE: (FROZEN TISSUE)

1. Fix sections in 10% NBF at room temperature for 1 hour.
2. Mordant in pre-warmed Bouin's at 35C for 1 hour or Mordant overnight at room temperature.
3. Follow procedure for Formalin Fixed Paraffin Embedded Tissue, **beginning at step 6.**

RESULTS

| | |
|--------------------------|-------------------|
| Collagen Fibers.....Blue | Cytoplasm.....Red |
| Muscle.....Red | Nuclei.....Black |

STORAGE AND HANDLING

Use a vented heating source (i.e. oven, waterbath) or place in a fume hood when heating or handling hazardous reagents, such as Bouin's Fixative. Loosen the cap on container of reagent prior to heating. Close cap securely when transferring from heating source to avoid exposure to harmful vapors. Cap of container can be removed after it is placed in the ventilated hood.

Store at room temperature. Use ventilation hood and gloves when handling materials.

REFERENCES

1. Sheehan D, Hrapchak B, Theory and Practice of Histotechnology, 2nd Ed, 1980, pp189-190, Battelle Press, Ohio.