Diagnostic BioSystems

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Trichrome Stain Kit (Modified Masson's)

Catalog Number: KT034

**This data sheet is applicable to all sizes (volume) of product. Actual volume is indicated on vial.

Document #: DS-3026-A
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Intended Use
For In Vitro Diagnostic Use

Summary and Explanation
The Trichrome Stain Kit (Modified Masson's) is intended for use in the histological visualization of collagenous connective tissue fibers in tissue sections. This kit may be used on formalin-fixed, paraffin-embedded or frozen sections.

Collagen: Blue
Muscle Fibers: Red
Nuclei: Black/Blue

Control Tissue
Tissues fixed in 10% formalin are suitable for use prior to paraffin embedding. Consult references (Kiernan, 1981: Sheehan & Hrapchak, 1980) for further details on specimen preparation.

1. Cut sections, usually 3 to 5 µm and pick the sections up on glass slides.
2. Bake the slides for at least 30 minutes at approximately 70°C.
3. Allow to cool.

Recommended Positive Control
1. Lung
2. Uterus
3. Small Intestine
4. Stomach

Reagents Provided

Kit Contents	Volume	Storage
Bouin's Fluid	125 mL	15-30°C
Weigert's Iron, Hematoxylin (A)	125 mL	15-30°C
Weigert's Iron, Hematoxylin (B)	125 mL	15-30°C
Biebrich Scarlet / Acid Fuchsin Sol.	125 mL	15-30°C
Phosphomolybdic/Phosphotungstic Acid Solution	125 mL	15-30°C
Aniline Blue Solution	125 mL	15-30°C
Acetic Acid Solution (1%)	125 mL	15-30°C

Storage and Handling
Do not use product after the expiration date printed on vial. If reagents are stored under conditions other than those specified here, they must be verified by the user. Diluted reagents should be used promptly.

Staining Procedure
1. Deparaffinize sections if necessary and hydrate to distilled water.
2. Preheat Bouin's Fluid in a water bath to 56° - 64° centigrade in a fume hood or very well ventilated area.
3. Place slides in preheated Bouin's Fluid for 60 minutes followed by a 10 minute cooling period.
4. Rinse slide in tap water until section is completely clear.
5. Rinse once in distilled water.
6. Mix equal parts of Weigert's (A) and Weigert's (B) and stain slide with working Weigert's Iron Hematoxylin for 5 minutes.
7. Rinse slide in running tap water for 2 minutes.
8. Apply Biebrich Scarlet / Acid Fuchsin Solution to slide for 15 minutes.
9. Rinse slide in distilled water.
10. Differentiate in Phosphomolybdic/Phosphotungstic Acid Solution for 10-15 minutes or until collagen is not red.
11. Without rinsing, apply Aniline Blue Solution to slide for 5-10 minutes.
12. Rinse slide in distilled water.
13. Apply Acetic Acid Solution (1%) to slide for 3-5 minutes.
14. Dehydrate very quickly in 2 changes of 95% Alcohol, followed by 2 changes of Absolute Alcohol.
15. Clear in Xylene or Xylene Substitute, and mount in synthetic resin.

Limitations of the Procedure
1. Histological staining is a multiple step diagnostic process that requires specialized training in the selection of the appropriate reagents, tissue selections, fixation, processing, preparation of the slide, and interpretation of the staining results.
2. Tissue staining is dependent on the handling and processing of the tissue prior to staining.
3. Improper fixation, freezing, thawing, washing, drying, heating, sectioning, or contamination with other tissues or fluids may produce artifacts or false negative results.
4. The clinical interpretation of any positive staining, or its absence, must be evaluated within the context of clinical history, morphology and other histopathological criteria. It is the responsibility of a qualified pathologist to be familiar with the special stain and methods used to produce the slide.
5. Staining must be performed in a certified licensed laboratory under the supervision of a pathologist who is responsible for reviewing the stained slides and assuring the adequacy of positive and negative controls.

Precautions
1. Consult local and/or state authorities with regard to recommended method of disposal.
2. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions.
3. Avoid microbial contamination of reagents. Contamination could produce erroneous results.
4. This reagent may cause irritation. Avoid contact with eyes and mucous membranes.
5. If reagent contacts these areas, rinse with copious amounts of water.
6. Do not ingest or inhale any reagents.

Troubleshooting
If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem is suspected, contact Diagnostic BioSystems Technical Support at (925) 484-3350, extension 2 or techsupport@dbiosys.com.

References

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I. Sheehan, DC, Hrapchak, BB. Theory and Practice of Histotechnology; 1980, page 190.
II. A.F.I.P. Laboratory Methods in Histotechnology; 1992, pages 132-133.

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