

### Instructions for Use

## HTRM-IFU

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## Trichrome Stain Kit (Modified Masson's)

#### Description

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



#### Uses/Limitations

Not to be taken internally. Histological applications.

Do not use if reagents become cloudy.

Do not use past expiration date. Use caution when handling reagents.

Non-Sterile.

#### **Control Tissue**

Lung Liver Colon Stomach

Product	Volume	Storage
Bouin's Fluid	125 ml	18-25°C
Weigert's Iron, Hematoxylin (A)	125 ml	18-25°C
Weigert's Iron, Hematoxylin (B)	125 ml	18-25°C
Biebrich Scarlet / Acid Fuchsin	125ml	18-25°C
Solution		
Phosphomolybdic/Phosphotungstic	125 ml	18-25°C
Acid Solution		
Aniline Blue Solution	125 ml	18-25°C
Acetic Acid Solution (1%)	125 ml	18-25°C

#### **Precautions**

Avoid contact with skin and eyes.

Harmful if swallowed.

Follow all Federal, State, and local regulations regarding disposal.

#### 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



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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. Rinse slide in distilled water.
- 9. Apply Biebrich Scarlet / Acid Fuchsin Solution to slide for 15 minutes.
- 10. Rinse slide in distilled water.
- 11. Differentiate in Phosphomolybdic/Phosphotungstic Acid Solution for 10-15 minutes or until collagen is not red.
- 12. Without rinsing, apply Aniline Blue Solution to slide for 5-10 minutes.
- 13. Rinse slide in distilled water.
- 14. Apply Acetic Acid Solution (1%) to slide for 3-5 minutes.
- 15. Dehydrate very quickly in 2 changes of 95% Alcohol, followed by 2 changes of Absolute Alcohol.
- 16. Clear in Xylene or Xylene Substitute, and mount in synthetic resin.

#### References

- 1. Sheehan, DC., Hrapchak, BB. Theory and Practice of Histotechnology; 1980, page 190.
- 2. A.F.I.P. Laboratory Methods in Histotechnology; 1992, pages 132-133.
- 3. Bekeredjian R., Walton C.B., MacCannell K.A., Ecker J., Kruse F., et al. Conditional HIF-1α Expression Produces a Reversible Cardiomyopathy. PLoS ONE 5(7): e11693. Doi:10.1371/journal.pone.0011693. (2010)