

# 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 formalin-fixed tissue sections.

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


**Uses/Limitations:** For In-Vitro Diagnostic use only.  
Histological applications.  
Do not use past expiration date.  
Use caution when handling these reagents.  
Has not been tested on and may not be suitable for frozen sections.

**Control Tissue:** Lung  
Uterus  
Small Intestine  
Stomach

**Availability/Contents:**

<u>Kit Contents</u>	<u>Volume</u>	<u>Storage</u>
Bouin's Fluid	125 ml	Room Temperature
Weigert's Iron, Hematoxylin (A)	125 ml	Room Temperature
Weigert's Iron, Hematoxylin (B)	125 ml	Room Temperature
Biebrich Scarlet / Acid Fuchsin Sol.	125ml	Room Temperature
Phosphomolybdic/Phosphotungstic Acid Solution	125 ml	Room Temperature
Aniline Blue Solution	125 ml	Room Temperature
Acetic Acid Solution (1%)	125 ml	Room Temperature

**Precautions:** Keep away from open flame.  
Avoid contact with skin and eyes.  
Harmful if swallowed.  
Follow all Federal, State, and local regulations regarding disposal.  
Use in chemical fume hood whenever possible.

Storage: 18° C  25° C

**Store Components at Room  
Temperature.**


### Procedure (Standard):

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 2-10 minutes. Stain is alcoholic and prone to evaporation – monitor and add stain as necessary to ensure stain does not dry on slide. Dried stain may result in excess grey background.
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 5-10 minutes.
10. Rinse slide in distilled water.
11. Differentiate in Phosphomolybdic/Phosphotungstic Acid Solution for 10-15 minutes.
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.

Notes: If final background is a dark or dull red/blue, it may be caused by excess grey background from the Weigert's Iron Hematoxylin. If this is the case, reduce staining time of Working Weigert's Iron Hematoxylin for future slides (step 6). A standard Acid Alcohol solution (not provided) may also be used to remove excess grey background immediately after step 6 as well.

### 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.

Storage: 18° C  25° C

**Store Components at Room Temperature.**