



MMPC–Massons Trichrome

Version: 1
 Modified from: Massons Trichrome UC Davis Clinical Pathology lab
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Summary: Massons trichrome staining is used for detection of collagen fibers in tissues such as skin, heart, etc. on formalin-fixed, paraffin-embedded sections, and may be used for frozen sections as well. The collagen fibers will be stained blue and the nuclei will be stained black and the background is stained red.

Reagents and Materials:

Reagent/Material	Vendor	Stock Number
Weigerts Hematoxylin		
Bouins solution		
Biebrich Scarlet-Acid Fuchsin solution		
Phosphomolybdic-Phosphotungstic Acid solution		
Aniline Blue solution		
Acetic acid, 1%		
coverslip	Corning	2935-245

Protocol:

WARNING:

Formalin is, toxic, flammable and considered a carcinogen

All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions established by CDC when handling and disposing of infectious agents.

1. Hydrate to water.
2. Place in Bouins for one hour in 55 degree oven or overnight at room temperature if needed.
3. Wash well in running water until yellow is gone.
4. Stain in Weigerts Hematoxylin for 10-20 minutes.
5. Wash well in running water for 10 minutes. Rinse distilled water.
6. Place slides in Biebrich Scarlet-Acid Fuchsin for 5 minutes.
7. Rinse in distilled water.
8. Place slides in the Phosphomolybdic-Phosphotungstic Acid Solution in a plastic coplin jar and put in the Bio Rad for 25 seconds using the total time setting. Let stand 30 seconds.
9. Place slides in Aniline Blue solution for 10 minutes.
10. Rinse in distilled water.
11. Place in 1% Acetic acid for 3 minutes if desired.
12. Dehydrate and coverslip.

Reagent Preparation:

Reagent 1: Biebrich Scarlet-Acid Fuchsin solution

Reagents and Materials:

Biebrich scarlet, aqueous, 1%	90 ml
Acid fuchsin, aqueous, 1%	10 ml
Glacial acetic acid	1.0 ml

Reagent 2: Phosphomolybdic- Phosphotungstic Acid solution

Reagents and Materials:

Phosphomolybdic acid	1.25 gm
Phosphotungstic acid	1.25 gm
Distilled water	50 ml

Procedure: Mix just before use

Reagent 3: Aniline Blue solution

Aniline blue	2.5 gm
Glacial Acetic acid	2.0 ml
Dist. water	100 ml

Procedure: Mix just before use

Reagents and Materials