

Pathology Core Protocol



Protocol Section: Staining Procedures-Manual

Policy No:

P-CMHD2-MASS-01

Protocol Subject: Masson Trichrome Stain

Date Reviewed:

6 January 2003

Date Revised:

September 22,
2003

Fixation: Buffered formalin, Bouin's or Zenker's.

Sections: 3 to 5 micron paraffin

Solutions:

Celestine Blue

Celestine Blue	2.5 gm
Iron Alum (Ferric ammonium sulphate)	25 gm
Distilled water	500 ml

Dissolve iron alum and celestine blue in distilled water. Bring to a boil, then cool to room temperature. Filter before using.

Harris' Haematoxylin, acid alcohol, Scott's Tapwater Substitute

Acid Fuchsin - Ponceau solution

1% Ponceau de Xylidene in 1% acetic acid	2 parts
1% Acid Fuchsin in 1% acetic acid	1 part

Phosphotungstic Acid - 1% aqueous

Acetic acid - 1% aqueous

Light Green - 2% Light green SF(yellowish) in 1% acetic acid

Procedure:

1. Take sections to water.
2. Mordant formalin-fixed section in Bouin's solution at 60°C for 1 hour, or mordant as detailed in P-CMHD2-MOR.
3. Wash well in warm tapwater.
4. Stain in celestine blue for 5 minutes.
5. Wash in water and stain in Harris' haematoxylin for 5 minutes.
6. Wash in water, and differentiate in acid alcohol, 6-10 dips.
7. Wash in water, and blue in Scott's tapwater, 1 minute.
8. Wash well in running water.
9. Stain in Ponceau-Acid Fuchsin for 10 minutes.
10. Wash in water.
11. Differentiate with 1% phosphotungstic acid for 5 minutes.
12. Rinse slides in 1% acetic acid.
13. Stain in light green for 2-4 minutes.
14. Rinse slides rapidly in 1% acetic acid.
15. Dehydrate, clear and mount.

Results:

Nuclei - black
Muscle, RBC's, Fibrin - red
Collagen - green

Ref: Bancroft, J.D., Stevens, Alan : Theory and Practice of Histological Techniques

Issued by Lab Manager: _____

Date: _____

Approved by Facility Management: _____

Date: _____