

Masson's Trichrome Method

Fixation: Bouin's or 10% NBF

Technique: Cut paraffin sections at 6 microns

Control: heart, skin

Solutions:

Bouin's

Picric acid, saturated aqueous solution	750.0 ml
Formalin, 37-40%	250.0 ml
Glacial acetic acid	50.0 ml

Weigert's Iron Hematoxylin

Solution A

5% Alcianin Hematoxylin
95% Ethanol

200ml
800ml

Solution B

Ferric chloride, 29% aqueous	40 ml
Distilled water	950 ml
Hydrochloric acid, conc.	10 ml

Working Solution

Equal parts of solution A and solution B.

Biebrich Scarlet-Acid Fuchsin Solution

Biebrich scarlet, aqueous, 1%	900 ml
Acid fuchsin, 1%	100 ml
Glacial acetic acid	10 ml

Phosphomolybdic-Phosphotungstic Acid

Phosphomolybdic acid	25 g
Phosphotungstic acid	25 g
Distilled water	1000 ml

Aniline Blue

Aniline blue	25 g
Glacial acetic acid	20 ml
Distilled water	1000 ml

2% Light Green

Light green, SF yellowish	2.0 g
Distilled water	98.0 ml
Glacial acetic acid	1.0 ml

1% Glacial Acetic Acid

Glacial acetic acid	10ml
Distilled water	1000 ml

Staining Procedure:

1. Deparaffinize and hydrate to distilled water.
2. Mordant in Bouin's for 1 hour at 56°C, or overnight at room temperature, if formalin fixed.
3. Cool and wash in running water until yellow color disappears.
4. Rinse in distilled water.
5. Weigert's iron hematoxylin for 10 minutes. Wash in running water for 10 minutes.
6. Rinse in distilled water.
7. Biebrich scarlet-acid fuchsin for 2 minutes. Filter and save solution.
8. Rinse in distilled water.
9. Phosphomolybdic-phosphotungstic acid for 15 minutes if using aniline blue as counterstain. If using light green as counterstain use aqueous 5% phosphotungstic acid for 15 minutes. Filter before use and discard solution.
10. Aniline blue for 5 minutes, or light green for one minute. Filter and save solution.
11. Rinse in distilled water.
12. Glacial acetic acid for 5 minutes. Discard solution.
13. Dehydrate in twice in both 95% alcohol, absolute alcohol then clear in two changes of xylene before coverslipping.

Results:

Nuclei	Black
Cytoplasm, keratin, muscle fibers and intercellular fibers	Red
Collagen	Blue

Reference:

Luna, L. 1968, Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology, 3rd edition, pg 94-95. McGraw-Hill Book Co.