

Special Stain

Masson Trichrome Stain

Staining to differentiate between collagen and smooth muscle

Material and Techniques

Solution should be prepared prior day to staining.

Bouin Solution. (may be obtain by Poly Scientific)

- Picric acid, saturated aqueous solution75 mL
- Formaldehyde, 37% to 40%.....25 mL
- Glacial Acetic Acid.....5mL

Weiger Iron Hematoxylin (best to be prepared in house)

Solution A

- Hematoxylin10 g
- Alcohol, 95%.....1 L

Solution B

- Ferric Chloride, 29% aqueous solution.....20 mL
- Distilled Water475 mL
- Glacial Acetic Acid5 mL

Working Solution

- Mix equal parts of solution A and B. (1:1)

Biebrich Scarlet-Acid Fuchsin Solution

- Biebrich scarlet, 1% aqueous solution.....360 mL
- Acid Fuchsin, 1% aqueous solution40mL
- Glacial Acetic Acid.....5mL

Phosphomolybdic / Phosphotungstic Acid solution

- Phosphomolybdic acid25 g
- Phosphotungstic acid.....25g
- Distilled water.....1 L

Aniline Blue Solution

- Aniline blue.....25 g
- Glacial Acetic Acid.....20 mL
- Distilled water.....1 L

Acetic Acid Solution 1%

- Glacial acetic Acid.....1 mL
- Distilled Water.....99 mL

Procedure for Masson Trichrome Stain

1. Deparaffinize sections and hydrate to distilled water.
2. Rinse well in distilled water.
3. Mordant the section in Bouins solution for at least 1 hour at 56° C.
4. Remove slides from oven and allow cooling at room temperature for 10 t 15 minutes.
5. Wash in running tap water until the yellow color disappears. .Rinse in distilled water
6. **Stain** sections in Weigert Hematoxylin for 10 minutes and wash in tap water for 10 minutes.
7. Rinse in distilled water.
8. **Stain** sections in filtered Biebrich scarlet-acid fuchsin solution for 2 to 4 minutes. The solution may be saved for future use.
9. Rinse in distilled water until water is dye free.
10. Continue by placing slides in phosphomolybdic / phosphotungstic acid solution for 15 to 20 minutes. Discard solution after each use.
11. Transfer sections directly into filtered Aniline Blue solution for 10 to 20 minutes. Save solution.
12. Rinse sections in distilled water until clear water is seen.
13. Place sections in 1% Acetic Acid solution for 3 to 5 minutes. Look at slides and note differentiation to red, black and blue.
14. Dehydrate with 2X95% alcohol, 2X100% alcohol, two minutes each. Continue by 2Xxylene, five minutes each followed by mounting with synthetic resin.

Results

NucleiBlack
Cytoplasm, Keratin, muscle fibers.....Red
Collagen and Mucus.....Blue