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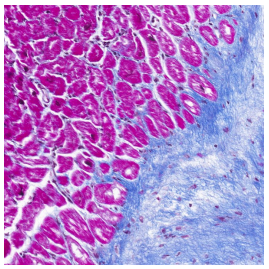
Masson's Trichrome staining for histology



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We use this protocol and it's working

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Abstract

Masson's trichrome is a histological staining method commonly used to distinguish collagen from muscle, cytoplasm and other tissue components.

The method presented here remains close to the original used by Masson in 1929.
While there are many variants of the original method, there is commonality in that they all utilise three components - a nuclear stain (usually an iron haematoxylin), a fibre stain (to specifically dye the collagen) and a plasma stain (which acts as a counterstain). They also consistently use a polyacid to differentiate the plasma staining.

In this method it is noted that, if using routine formalin-fixed paraffin-embedded tissues, including the optional mordanting step will increase staining intensity and enhance contrast between tissue components. The same effect can also be achieved by using Bouin's fixative.

Nuclear stain: This method uses Weigert's haematoxylin as the nuclear stain by staining the nuclei dark purple-black. Some variants use Verhoeff's haematoxylin to additionally highlight elastin by staining it black.

Plasma stain: This method uses the original red dyes for the plasma stain, specifically a mixture of Acid fuchsin and Ponceau de xylidene. The latter has a number of alternate names, including Xylidine Ponceau, Xylidine Ponceau 2R, Ponceau 2R, Ponceau G, Ponceau Red, Acid Red 26. All have the same C.I. 16150.
Common variants use a mixture of Acid fuchsin and Biebrich scarlet, which provides a similar result, with a slightly darker red.

Heteropolyacid: Like the original, this method uses a 1% phosphomolybdic acid (PMA) solution for the heteropolyacid. Most variants use the same, but some recommend combining with phosphotungstic acid (PTA) in a 2.5% PMA + 2.5% PTA solution.

Fibre stain: The fibre stain used here is Methyl blue which stains collagen a vibrant, royal blue. This is similar to the orginal Masson's method, which was thought to use Aniline blue water soluble (a mixture of Water blue and Methyl blue). Some other variants use green fibre stains, such as Light green or Fast Green FCF. The Fast Green FCF resists fading better than Light green, which fades over time, causing sections to lose their contrast. One uncommon variant uses Metanil yellow to stain the collagen yellow.

Protocol materials

- Hydrochloric Acid **Banksia Scientific Company (AJAX FineChem)** **Catalog #**AJA256
- Ethanol **POCD Scientific** **Catalog #**ETHABS70WV5
- Ethanol **Hurst Scientific** **Catalog #**EIMS95-5L
- Xylene **POCD Scientific** **Catalog #**XYL5P
- DPX Mountant for histology **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**06522
- Ethanol **POCD Scientific** **Catalog #**ETHABS20M
- Haematoxylin **Fronine Laboratory Supplies** **Catalog #**JL555S
- Iron (III) chloride hexahydrate **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**F2877
- Acid Fuchsin **Fronine Laboratory Supplies** **Catalog #**JL301
- Ponceau de Xylidene **E. Gurr Ltd**
- Glacial acetic acid **Ajax Finechem (ThermoFisher Scientific)** **Catalog #**AJA1-500ML
- Phosphomolybdic acid **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**221856
- Methyl Blue **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**M5528
- Picric acid **May & Baker LTD**

Deparrafinisation and rehydration

32m

1 **Dewax and rehydrate paraffin sections** 32m

Mordanting (optional)

1h 15m

2 Incubate in saturated picric acid solution at 57 °C 1h

Picric acid **May & Baker LTD** Saturated picric acid solution:

[M] 1.3 Mass Percent in distilled water

Safety information

Picric acid is an unstable explosive when dry, where it becomes highly sensitive to shock, heat and friction. Ensure it remains hydrated at all times.
Picric acid reacts with metals to form unstable picrate salts which are more explosive. Do not use metal containers, lids or racks.
Picric acid is toxic if swallowed, inhaled or absorbed through the skin.
Consult the MSDS and follow all recommended safety precautions.

3 Cool to Room temperature 10m

4 Wash until no longer yellow 5m

Nuclear staining

13m

5 Stain in Weigert's Haematoxylin (combine solution A & B immediately before use) 10m

Solution A: 1 g Haematoxylin in 100 ml of 95% Ethanol
Solution B: 1.6 g Ferric chloride hexahydrate (Iron III chloride; $FeCl_3.6H_2O$) + 95 ml distilled water + 1 ml concentrated HCl

Haematoxylin **Fronine Laboratory Supplies Catalog #**JL555S CI: 75290

Ethanol **Hurst Scientific Catalog #**EIMS95-5L

Iron (III) chloride hexahydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**F2877

Hydrochloric Acid **Banksia Scientific Company (AJAX FineChem) Catalog #**AJA256

6 Wash with running tap water to remove excess stain 2m

7 Differentiate with acid alcohol (1-2 dips) to clear the slides

[1% Hydrochloric Acid (HCl) in 70% Ethanol]

Hydrochloric Acid **Banksia Scientific Company (AJAX FineChem) Catalog #**AJA256

Ethanol **POCD Scientific Catalog #**ETHABS70WV5

8 Wash in tap water 1m

9 Examine slides under microscope to assess nuclear staining. Nuclei should be dark purple-black and cytoplasm or collagenous tissue components should be grey. If nuclei are under-stained, return to step 5. If sections are over-stained, return to step 7.

Plasma stain 11m

10 Stain in Acid Fuchsin + Ponceau de xylidine solution (make fresh, lasts 2-3 days) 5m

Solution: 1 g Acid Fuchsin + 1 g Ponceau de xylidine + 99 ml distilled water + 1 ml Glacial acetic acid

Acid Fuchsin **Fronine Laboratory Supplies Catalog #**JL301 CI: 42685

Ponceau de Xylidene **E. Gurr Ltd** CI: 16150

Glacial acetic acid **Ajax Finechem (ThermoFisher Scientific) Catalog #**AJA1-500ML

11 Wash in distilled water

Heteropolyacid (differentiation of plasma stain) 11m

12 Differentiate and mordant in 1% PMA solution 2 – 6 mins (check progress of differentiation under the microscope every 2 mins, until collagen is colourless) 6m

PMA Solution: 1 g Phosphomolybdic acid + 100 ml distilled water

Phosphomolybdic acid **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**221856

13 Drain – do not rinse

Fibre stain

2m 30s

14 Stain in Methyl blue solution

2m

Solution: 2 g Methyl blue + 2.5 ml Glacial acetic acid + 100 ml distilled water (solution can be re-used, lasts many years)

Methyl Blue **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**M5528 CI: 42780

Glacial acetic acid **Ajax Finechem (ThermoFisher Scientific) Catalog #**AJA1-500ML

15 Wash in distilled water

16 Differentiate with 1% Glacial acetic acid

30s

Glacial acetic acid **Ajax Finechem (ThermoFisher Scientific) Catalog #**AJA1-500ML

Dehydration, clearing and mounting

11m 30s

17 95% ethanol

30s

Ethanol **Hurst Scientific Catalog #**EIMS95-5L

18 100% ethanol

30s

Ethanol **POCD Scientific Catalog #**ETHABS20M

Note: ensure whole slide and slide rack is fully immersed in ethanol to remove all traces of water, preventing water contamination of the xylene

19 100% ethanol 30s

Ethanol **POCD Scientific Catalog #**ETHABS20M

Note: ensure whole slide and slide rack is fully immersed in ethanol to remove all traces of water, preventing water contamination of the xylene

20 100% Xylene 5m

Xylene **POCD Scientific Catalog #**XYL5P

21 100% Xylene 5m

Xylene **POCD Scientific Catalog #**XYL5P

22 Coverslip with a resinous mounting medium, such as DPX

DPX Mountant for histology **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**06522

Results

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Expected result

Nuclei: Purple-black
Cytoplasm: Red
Keratin: Red
Erythrocytes: Red
Muscle: Red
Collagen: Blue