Fronine

First for Pathology

A THERMO FISHER SCIENTIFIC COMPANY

Masson's Trichrome Staining

For In-Vitro Diagnostics

For Professional Use Only

Masson's Trichrome is a three colour staining histological protocol used to differentiate tissue structures such as collagen from muscle in contrasting colours.

Mode of Action

The mechanism of the stain is not fully understood. One theory is that the Biebrich Scarlet acid dye is used to stain the acidophilic cytoplasm and muscle fibres. Although collagen fibres are also acidophilic, they colour differently from the muscle and cell cytoplasm. It is believed that this is the result of the action of the phosphomolybdic/phosphotungstic acids. The phosphomolybdic/phosphotungstic acid is taken up by the connective tissue and replaced by the aniline blue.

Warning and Precautions

Phosphomolybdic/Phosphotungstic acid is an irritant. Use suitable personal protective equipment when handling this product.

Please consult the SDS and packaging labels before use.

Ingredients

Biebrich Scarlet Acid

Chemical	CAS	Conc
Biebrich Scarlet	85-83-6	<1%
Fuchsin Acid	3244-88-0	<1%
Water	7732-18-5	Balance

Aniline Blue

Chemical	CAS	Conc
Aniline Blue	28983-56-4	<3%
Acetic Acid	64-19-7	<1%
Water	7732-18-5	Balance

Phosphomolybdic/Phosphotungstic Acid

Chemical	CAS	Conc
dodeca-molybdo-phosphoric acid	12026-57-2	<3%
dodeca-tungsto-phosphoric acid	12067-99-1	<1%
Water	7732-18-5	Balance

Specifications

Beibrich Scarlet Acid

Appearance	Clear Red Liquid
Absorption (λmax)	520-560nm

Aniline Blue

Appearance	Clear Blue Liquid
Absorption (λmax)	Approx 600nm

Phosphomolybdo/Phosphotungstic Acid

Appearance	Clear Yellow Liquid
pH @ 20°C	0.8 to 1.6

Stability

All Masson Trichrome solutions are stable for 12 months when stored at room temperature in a sealed bottle away from heat and light.

Sample Preparation

Bouin fixed tissue is preferred but 10% Neutral Buffered Formalin may be used. Cut paraffin sections at 5µm.

Technical Procedure[1]

1. Deparaffinise sections, and hydrate to distilled/deionised water
2. Rinse well in distilled/deionised water
3. Mordant formalin-fixed sections in Bouin solution for 1 hour @ 56°C
4. Remove slides from oven, allow to cool and wash in water until yellow colour disappears
5. Rinse in distilled/deionised water
6. Stain sections in Weigert Iron Haematoxylin for 10 minutes
7. Wash in running water for 10 minutes
8. Rinse in distilled/deionised water
9. Stain sections with Biebrich Scarlet Acid fuchsin solution (Masson1A) for 2 minutes
10. Rinse in distilled/deionised water
11. Place slides in Phosphomolybdic/phosphotungstic acid solution for 10-15 minutes. Discard solution
12. Stain sections in Aniline blue solution for 5 minutes
13. Rinse slides in distilled/deionised water
14. Place slides in 1% acetic acid solution for 3 to 5 minutes. Discard solution
15. Dehydrate with 95% and absolute alcohol, 2 changes each.
16. Clear with 2 or 3 changes of xylene and mount with synthetic resin.

Results and Interpretation

Only experienced and suitably qualified persons should carry out interpretation of stained slides.

Nuclei	Black
Cytoplasm, keratin, muscle fibres	Red
Collagen and mucin	Blue

[1] *Carson, Hladik, Histotechnology pp 163-4*

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Notes

- Collagen may be counterstained with light green (available separately) instead of aniline blue if desired. The following changes to the procedure are made:

11. Place sections in a 5% solution of phosphomolybdic/phosphotungstic acid;

12. Stain 5 minutes in 2% Light Green.

- Light Green is a better counterstain when collagen is predominant.
- Decreasing red staining indicates that the staining solution has aged or has been overused. Discard
- Faded blue staining of the connective tissue indicates overdifferentiation in the acetic acid solution
- Sections fixed in 10% Neutral Buffered Formalin will stain poorly and unevenly if not mordanted in Bouin's solution
- Iron Haematoxylin solution is used in preference to an Alum haematoxylin as it is more resistant to decolourisation by the acidic dye solutions
- Never leave the cap open
- Always prepare fresh solutions just before use and discard used solutions. Do not return used stains to its original container as this may adversely affect the overall performance of the unused product.

References

1. *Carson, Freida L, Hladik, Christa; Histotechnology – A Self Instuctional Text, American Society for Clinical Pathology Press, 3rd Edition, 2009*
2. *Sheehan, Dezna, Hrapchak, Barbara, Theory and Practice of Histotechnology, Battelle Press, 2nd Edition 1980*
3. *Cook, H.C; Manual of Histological Demonstration Techniques, Butterworth & Co., 1974*
4. *Sabnis, R.W, Handbook of Biological Dyes and Stains, John Wiley & Sons, 2010*

Ordering Information

Code	Product Name	Size
FNNMASSONS1	Masson Trichrome Stain Kit (contains Masson1A, 1B and 1C)	100mL
FNNMASSON1A	Biebrich Scarlet Acid Fuchsin	100mL
FNNMASSON1B	Aniline Blue 2.5%	100mL
FNNMASSON1C	Phosphomolybdic / Phosphotungstic acid	100mL
FNNMASSON1D	Light Green 2%	100mL
FNNFG033	Bouins Fixative	500mL
FNNFG075	Weigerts Haematoxylin A	500mL
FNNFG076	Weigerts Haematoxylin B	500mL

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