

## Trichrome Stain, Masson, Fast Green - Technical Memo

### SOLUTION:

Fast Green Stain 2.5%, Aqueous	250 ml Part 10852A	500 ml Part 10852B
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### Additionally Needed:

Trichrome, Liver Control Slides	Part 4690	or	Trichrome, Multi-Tissue Control Slides	Part 4693
Xylene, ACS	Part 1445			
Alcohol, Ethyl Denatured, 100%	Part 10841			
Alcohol, Ethyl Denatured, 95%	Part 10842			
Bouin Fluid	Part 1020			
Hematoxylin Stain Set, Weigert Iron	Part 1409			
Biebrich Scarlet-Acid Fuchsin Stain, Aqueous	Part 10161			
Phosphotungstic Acid 5%, Aqueous	Part 13345			
Acetic Acid 0.5%, Aqueous	Part 100121			
Coplin Jar, Plastic	Part 5184 (for microwave modification)			

**For storage requirements and expiration date refer to individual product labels.**

### APPLICATION:

Newcomer Supply Trichrome Stain, Masson, Fast Green procedure, with included microwave modification, is used to differentially demonstrate connective tissue elements, collagen and muscle fibers.

### METHOD:

**Fixation:** Formalin 10%, Phosphate Buffered (Part 1090)

**Technique:** Paraffin sections cut at 4 microns

a. See Procedure Note #1.

**Solutions:** All solutions are manufactured by Newcomer Supply, Inc.

All Newcomer Supply stain procedures are designed to be used with Coplin jars filled to 40 ml following the staining procedure.

### PRESTAINING PREPARATION:

- If necessary, heat dry tissue sections/slides in oven.
- Preheat Bouin Fluid (1020) to 56-60°C in oven or water bath. (**Skip if using overnight method or microwave procedure.**)

### STAINING PROCEDURE:

- Deparaffinize sections thoroughly in three changes of xylene, 3 minutes each. Hydrate through two changes each of 100% and 95% ethyl alcohols, 10 dips each. Wash well with distilled water.
    - See Procedure Notes #2 and #3.
  - Mordant in preheated Bouin Fluid (Step #2) for one hour at 56-60°C or overnight at room temperature. Cool at room temperature for 5-10 minutes.
    - Skip Step #4 if tissue was originally Bouin fixed.
- Microwave Modification:** See Procedure Note #4.
- Place slides in a plastic Coplin jar containing Bouin Fluid and microwave for 5 minutes at 60°C. Allow slides to sit an additional 10 minutes in solution.
- Wash well in running tap water; rinse in distilled water.
  - Prepare fresh Weigert Iron Hematoxylin (1409); combine and mix well.
    - Solution A: Ferric Chloride, Acidified 20 ml
    - Solution B: Hematoxylin 1%, Alcoholic 20 ml
  - Stain slides in fresh Weigert Iron Hematoxylin for 10 minutes.
  - Wash in running tap water for 10 minutes; rinse in distilled water.
    - See Procedure Note #5.
  - Place slides in Biebrich Scarlet-Acid Fuchsin Stain, Aqueous (10161) for 2 minutes.
  - Rinse in distilled water.
  - Place slides in Phosphotungstic Acid 5%, Aqueous (13345) for 5 minutes.

- Transfer slides directly into Fast Green Stain 2.5%, Aqueous for 5-6 minutes, depending on stain intensity preference.
- Rinse in distilled water.
- Place slides in Acetic Acid 0.5%, Aqueous (100121) for 2 quick dips.
- Dehydrate in two changes each of 95% and 100% ethyl alcohol. Clear in three changes of xylene, 10 dips each; coverslip with compatible mounting medium.

### RESULTS:

Collagen and mucin	Green
Muscle fibers, cytoplasm and keratin	Red
Nuclei	Blue/black

### PROCEDURE NOTES:

- Using ammonium hydroxide to soak/face tissue blocks will alter the pH of tissue sections and diminish trichrome staining.
- Drain slides after each step to prevent solution carry over.
- Do not allow sections to dry out at any point during procedure.
- The suggested microwave procedure has been tested at Newcomer Supply. This procedure is a guideline and techniques should be developed for your laboratory.
- If Weigert Iron Hematoxylin is not completely washed from tissue sections, nuclear and cytoplasmic staining will be compromised.
- If using a xylene substitute, closely follow the manufacturer's recommendations for deparaffinization and clearing steps.

### REFERENCES:

- Brown, Richard. *Histologic Preparations: Common Problems and Their Solutions*. Northfield, Ill.: College of American Pathologists, 2009. 95-101.
- Carson, Freida L., and Christa Hladik. *Histotechnology: A Self-Instructional Text*. 3rd ed. Chicago, Ill.: American Society of Clinical Pathologists, 2009. 162-165.
- Sheehan, Dezna C., and Barbara B. Hrapchak. *Theory and Practice of Histotechnology*. 2nd ed. St. Louis: Mosby, 1980. 191-192.
- Vacca, Linda L. *Laboratory Manual of Histochemistry*. New York: Raven Press, 1985. 308-310.
- Modifications developed by Newcomer Supply Laboratory.