

Trichrome, Masson, Aniline Blue Stain Kit - Technical Memo

KIT INCLUDES:

	Part 9179A	Part 9179B
Solution A: Bouin Fluid	250 ml	500 ml
Solution B: Ferric Chloride, Acidified	125 ml	250 ml
Solution C: Hematoxylin 1%, Alcoholic	125 ml	250 ml
Solution D: Biebrich Scarlet-Acid Fuchsin Stain, Aqueous	250 ml	500 ml
Solution E: Phosphomolybdic-Phosphotungstic Acid, Aqueous	250 ml	500 ml
Solution F: Aniline Blue Stain, Aqueous	250 ml	500 ml
Solution G: Acetic Acid 0.5%, Aqueous	250 ml	500 ml

COMPLIMENTARY POSITIVE CONTROL SLIDES: Enclosed are two complimentary unstained positive control slides for the initial verification of staining techniques and reagents. Verification must be documented by running one Newcomer Supply complimentary positive control slide along with your current positive control slide for the first run. Retain the second complimentary control slide for further troubleshooting, if needed.

Individual stain solutions and additional control slides may be available for purchase under separate part numbers at www.newcomersupply.com.

Additionally Needed:

Xylene, ACS	Part 1445
Alcohol, Ethyl Denatured, 100%	Part 10841
Alcohol, Ethyl Denatured, 95%	Part 10842
Coplin Jar, Plastic	Part 5184 (for microwave modification)

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

APPLICATION:

Newcomer Supply Trichrome, Masson, Aniline Blue Stain Kit 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 Kits are designed to be used with Coplin jars filled to 40 ml following the staining procedure provided below. Some solutions in the kit may contain extra volumes.

PRESTAINING PREPARATION:

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

STAINING PROCEDURE:

3. 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.
 - a. See Procedure Notes #2 and #3.
4. Mordant in preheated Solution A: Bouin Fluid (Step #2) for one hour at 56-60°C or overnight at room temperature. Cool at room temperature for 5-10 minutes.
 - a. Skip Step #4 if tissue was originally Bouin fixed.

Microwave Modification: See Procedure Note #4.

- b. Place slides in a plastic Coplin jar containing Solution A: Bouin Fluid and microwave for 5 minutes at 60°C. Allow slides to sit an additional 10 minutes in solution.
5. Wash well in running tap water; rinse in distilled water.
 6. Prepare fresh Weigert Iron Hematoxylin; combine and mix well.
 - a. Solution B: Ferric Chloride, Acidified 20 ml
 - b. Solution C: Hematoxylin 1%, Alcoholic 20 ml
 7. Stain in fresh Weigert Iron Hematoxylin for 10 minutes.
 8. Wash in running tap water for 10 minutes; rinse in distilled water.
 - a. See Procedure Note #5.
 9. Place in Solution D: Biebrich Scarlet-Acid Fuchsin Stain, Aqueous for 2 minutes.

10. Rinse in distilled water.
11. Place in Solution E: Phosphomolybdic-Phosphotungstic Acid, Aqueous for 10-15 minutes.
12. Move directly to Solution F: Aniline Blue Stain, Aqueous; 5 minutes.
13. Rinse in distilled water.
14. Place in Solution G: Acetic Acid 0.5%, Aqueous for 3-5 minutes.
15. 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	Blue
Muscle fibers, cytoplasm and keratin	Red
Nuclei	Blue/black

PROCEDURE NOTES:

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

REFERENCES:

1. Brown, Richard. *Histologic Preparations: Common Problems and Their Solutions*. Northfield, Ill.: College of American Pathologists, 2009. 95-101.
2. Carson, Freida L., and Christa Hladik. *Histotechnology: A Self-Instructional Text*. 3rd ed. Chicago, Ill.: American Society of Clinical Pathologists, 2009. 162-165.
3. Sheehan, Dezna C., and Barbara B. Hrapchak. *Theory and Practice of Histotechnology*. 2nd ed. St. Louis: Mosby, 1980. 190.
4. Vacca, Linda L. *Laboratory Manual of Histochemistry*. New York: Raven Press, 1985. 308-310.
5. Modifications developed by Newcomer Supply Laboratory.