

# Trichrome, Gomori One-Step, Aniline Blue Stain Kit - Technical Memo

## KIT INCLUDES:

Solution A: Bouin Fluid  
Solution B: Ferric Chloride, Acidified  
Solution C: Hematoxylin 1%, Alcoholic  
Solution D: Trichrome Stain, Gomori One-Step, Aniline Blue  
Solution E: Acetic Acid 0.5%, Aqueous

	Part 9176B	Part 9176A
	250 ml	500 ml
	125 ml	250 ml
	125 ml	250 ml
	250 ml	500 ml
	250 ml	500 ml

**COMPLIMENTARY POSITIVE CONTROL SLIDES:** Enclosed with this kit are two complimentary unstained positive control slides to be used 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](http://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, Gomori One-Step, Aniline Blue Stain Kit procedure, with included microwave modification, uses a one-step solution combining a plasma stain and a connective tissue stain to differentially demonstrate collagen and muscle fibers.

## METHOD:

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

**Technique:** Paraffin sections cut at 5 microns

a. See Procedure Note #1.

**Solutions:** All solutions 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.

## STAINING PROCEDURE:

- Preheat Solution A: Bouin Fluid to 56-60°C in oven or water bath. **(Skip if using overnight method or microwave 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 Solution A: Bouin Fluid for one hour at 56-60°C or overnight at room temperature. Cool at room temperature for 5-10 minutes.
    - Skip Step #3 if tissue was originally Bouin fixed.
- Microwave Modification:** See Procedure Note #4.
- 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.
- Wash well in running tap water; rinse in distilled water.
  - Prepare fresh Weigert Iron Hematoxylin; combine and mix well.
    - Solution B: Ferric Chloride, Acidified 20 ml
    - Solution C: Hematoxylin 1%, Alcoholic 20 ml
  - Stain in fresh Weigert Iron Hematoxylin for 10 minutes.
  - Wash in running tap water for 10 minutes; rinse in distilled water.
    - See Procedure Note #5.
  - Stain with Solution D: Trichrome Stain, Gomori One-Step, Aniline Blue for 20 minutes.
  - Directly differentiate in Solution E: Acetic Acid 0.5%, Aqueous for 2 minutes.
  - Rinse quickly in distilled water.

- 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:

- Using ammonium hydroxide to soak or face tissue blocks will alter the pH of tissue sections and greatly diminish trichrome staining.
- Drain staining rack/slides after each step to prevent solution carry over.
- Do not allow sections to dry out at any point during staining procedure.
- The suggested microwave procedure has been tested at Newcomer Supply using an "EB Sciences", 850 watt microwave oven with temperature probe and agitation tubes. This procedure is reproducible in our laboratory. It is nonetheless a guideline and techniques should be developed for your laboratory which meet the requirements of your situation. Microwave devices should be placed in a fume hood or vented into a fume hood, according to manufacturer's instructions, to prevent exposure to chemical vapors.
- If Weigert Iron Hematoxylin is not completely washed from tissue sections, nuclear and cytoplasmic staining may 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. 165-166.
- 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.