2505 Parview Road • Middleton, WI 53562-2579 • 800-383-7799 • www.newcomersupply.com • info@newcomersupply.com

Part 1402
Revised April 2017

Trichrome Stain, Gomori One-Step, Light Green - Technical Memo

SOLUTION:	**250 ml**		**500 ml**	
Trichrome Stain, Gomori One-Step, Light Green	Part 1402C		Part 1402B	

Additionally Needed:

Trichrome, Kidney Control Slides	Part 4691	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			
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, Gomori One-Step, Light Green 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 procedures are designed to be used with Coplin jars filled to 40 ml following the staining procedure provided below.

STAINING PROCEDURE:

1. Preheat Bouin Fluid (1020) to 56-60°C in oven or water bath. ***(Skip if using overnight method or microwave procedure.)***
2. 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.
3. Mordant in Bouin Fluid for 1 hour at 56-60°C or overnight at room temperature. Cool at room temperature for 5-10 minutes.
   a. Skip Step #3 if tissue was originally Bouin fixed.
   Microwave Modification: *See Procedure Note #4.*
   b. 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.
4. Wash well in running tap water; rinse in distilled water.
5. Prepare fresh Weigert Iron Hematoxylin; combine and mix well.
   a. Solution A: Ferric Chloride, Acidified 20 ml
   b. Solution B: Hematoxylin 1%, Alcoholic 20 ml
6. Stain slides in fresh Weigert Iron Hematoxylin for 10 minutes.
7. Wash in running tap water for 10 minutes; rinse in distilled water.
   a. See Procedure Note #5.
8. Stain in Trichrome Stain, Gomori One-Step, Light Green for 20 minutes.
9. Directly differentiate in Acetic Acid 0.5%, Aqueous (100121) for 2 minutes.
10. Rinse quickly in distilled water.
11. 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:

1. Using ammonium hydroxide to soak or face tissue blocks will alter the pH of tissue sections and greatly diminish trichrome staining.
2. Drain staining rack/slides after each step to prevent solution carry over.
3. Do not allow sections to dry out at any point during staining procedure.
4. 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.
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. 165-166.
3. Sheehan, Dezna C., and Barbara B. Hrapchak. *Theory and Practice of Histotechnology*. 2nd ed. St. Louis: Mosby, 1980. 191-192.
4. Vacca, Linda L. *Laboratory Manual of Histochemistry*. New York: Raven Press, 1985. 308-310.
5. Modifications developed by Newcomer Supply Laboratory.

2505 Parview Road • Middleton, WI 53562-2579 • 800-383-7799 • www.newcomersupply.com • newly@newcomersupply.com

Part 1402
Revised April 2017

Trichrome Stain, Gomori One-Step, Light Green for Frozen Muscle Biopsies Technical Memo

SOLUTION:	**250 ml**		**500 ml**	
Trichrome Stain, Gomori One-Step, Light Green	Part 1402C		Part 1402B	
Additionally Needed:				
Hematoxylin Stain, Harris Modified	Part 1201	or	Hematoxylin Stain, Harris	Part 12013
Acetic Acid 0.5%, Aqueous	Part 100121			
Alcohol, Ethyl Denatured, 95%	Part 10842			
Alcohol, Ethyl Denatured, 100%	Part 10841			
Xylene, ACS	Part 1445			

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

APPLICATION:

Newcomer Supply Trichrome Stain, Gomori One-Step, Light Green for frozen muscle biopsies uses a one-step solution that combines a plasma stain with a connective tissue stain. This procedure provides excellent staining results on fresh non-fixed frozen muscle biopsy sections for the demonstration of muscle fiber morphology and surrounding connective tissue elements.

METHOD:

Technique: Frozen muscle sections cut at 8 microns on adhesive slides or coverglass
   a. *See Procedure Note #1.*

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

STAINING PROCEDURE:

1. Air-dry frozen muscle sections a minimum of 10 minutes prior to staining.
   a. *See Procedure Note #2.*
2. Stain air-dried frozen muscle sections in Hematoxylin Stain, Harris Modified or Hematoxylin Stain, Harris for 5 minutes.
3. Rinse in running tap water for 3 minutes.
   a. *Do not differentiate or use a bluing agent after hematoxylin staining.*
4. Stain in Trichrome Stain, Gomori One-Step, Light Green for 18-20 minutes in a 38°C-40°C oven.
   a. *Allow Trichrome Stain, Gomori One-Step, Light Green to reach room temperature prior to use.*
5. Prepare Acetic Acid 0.25%, Aqueous; combine and mix well.
   a. *Acetic Acid 0.5%, Aqueous* *20 ml*
   b. *Distilled Water* *20 ml*
6. Differentiate sections in Acetic Acid 0.25%, Aqueous; 1-2 quick dips.
7. 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:

Muscle fibers	Green
Interstitial connective tissue	Light green
Mitochondria	Red
Nemaline rods	Red
Myelinated nerve twigs	Red
Nuclei	Blue

PROCEDURE NOTES:

1. For optimal results and minimal tissue section artifact, fresh non-fixed muscle biopsies should be expediently snap frozen using an isopentane (2-Methylbutane) – liquid nitrogen freezing method.
2. Do not fix sections or use a Bouin Fluid mordant prior to staining. Exposure to a fixative or mordant will alter staining results.
3. If using a xylene substitute, closely follow the manufacturer's recommendations for clearing step.

REFERENCES:

1. Carson, Freida L., and Christa Hladik. *Histotechnology: A Self-Instructional Text*. 3rd ed. Chicago, Ill.: American Society of Clinical Pathologists, 2009. 328-329.
2. Dubowitz, Victor, and Caroline A. Sewry. *Muscle Biopsy: A Practical Approach*. 2nd ed. London: Baillière, 1985.30.
3. Mitchell, Jean and Andrew Waclawik. "Muscle Biopsy in Diagnosis of Neuromuscular Disorders: The Technical Aspects, Clinical Utility, and Recent Advances." *The Journal of Histotechnology* 30.4 (2007): 257-269.
4. Sheehan, Dezna C., and Barbara B. Hrapchak. *Theory and Practice of Histotechnology*. 2nd ed. St. Louis: Mosby, 1980. 191-192.
5. Modifications developed by Newcomer Supply Laboratory.