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Part 4693
Revised January 2020

Trichrome, Multi-Tissue Control Slides – Technical Memo

CONTROL SLIDES:	**Part 4693A**	**Part 4693B**
	10 Slide/Set	98 Slide/Set

PRODUCT SPECIFICATIONS:
Tissue: Positive staining liver, positive staining kidney and positive staining uterus.
Fixation: Formalin 10%, Phosphate Buffered (Part 1090).
Section/Glass: Paraffin sections cut at 4 microns on Superfrost™ Plus slides.
Quality Control Stain: Gomori One-Step Aniline Blue quality control stained slide(s) included.
Reactivity: Guaranteed product specific reactivity for one year from date of receipt. Revalidate after one year to verify continued reactivity.
Storage: 15-30°C in a light deprived and humidity controlled environment.
Intended Use: To verify histological techniques and reagent reactivity.

Before using unstained control slides, review the enclosed stained slide(s) to ensure that this tissue source is acceptable for testing needs.

CONTROL SLIDE VALIDATION:

With Trichrome, Gomori One-Step, Aniline Blue Stain Kit:	**Part 9176B/A**	**Individual Stain Solution**
Solution A: Bouin Fluid	250/500 ml	Part 1020
Solution B: Ferric Chloride, Acidified	125/250 ml	Part 1409
Solution C: Hematoxylin 1%, Alcoholic	125/250 ml	Part 1409
Solution D: Trichrome Stain, Gomori One-Step, Aniline Blue	250/500 ml	Part 1403
Solution E: Acetic Acid 0.5%, Aqueous	250/500 ml	Part 100121

APPLICATION:

Newcomer Supply Trichrome, Multi-Tissue Control Slides, use a combination of tissue sources for the positive histochemical staining of connective tissue and to differentially demonstrate collagen and muscle fibers.

PRESTAINING PREPARATION:

1. Heat dry sections in oven according to your laboratory protocol.
2. Preheat Solution A: Bouin Fluid to 56-60°C in oven or water bath. ***(Skip if using overnight method or microwave procedure.)***

NEWCOMER SUPPLY VALIDATION 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 #1 and #2.*
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 #3.*
   b. *Place slides in a plastic Coplin jar containing Solution A: Bouin Fluid and microwave for 5 minutes at 60°C.*
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 #4.*
9. Stain with Solution D: Trichrome Stain, Gomori One-Step, Aniline Blue for 20 minutes.
10. Differentiate in Solution E: Acetic Acid 0.5%, Aqueous; 2 minutes.
11. Rinse quickly in distilled water.
12. 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:

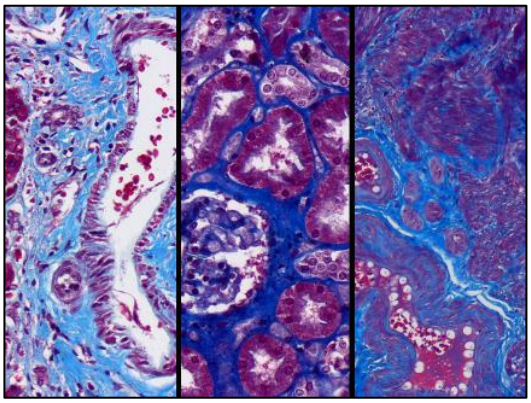
Collagen and mucin	Blue
Muscle fibers, cytoplasm and keratin	Red
Nuclei	Blue/black

PROCEDURE NOTES:

1. Drain slides after each step to prevent solution carry over.
2. Do not allow sections to dry out at any point during procedure.
3. 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.
4. If Weigert Iron Hematoxylin is not completely washed from tissue sections, nuclear and cytoplasmic staining may be compromised.
5. Trichrome, Multi-Tissue Control Slides are validated with Trichrome Stain Kit, Gomori One-Step, Aniline Blue but can be used as positive controls with any preferred Trichrome procedure.
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.



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