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

Part 9177
Revised March 2016

Trichrome, McLetchie, Aniline Blue Stain Kit - Technical Memo

KIT INCLUDES:	**Part 9177A**
Solution A: Biebrich Scarlet-Acid Fuchsin Stain, Aqueous	250 ml
Solution B: Iodine, Weigert & Lugol, Aqueous	250 ml
Solution C: Phosphotungstic Acid 2%, Alcoholic	250 ml
Solution D: Aniline Blue Stain, Aqueous	250 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.

Additionally Needed:

Xylene, ACS	Part 1445
Alcohol, Ethyl Denatured, 100%	Part 10841
Alcohol, Ethyl Denatured, 95%	Part 10842

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

APPLICATION:

Newcomer Supply Trichrome, McLetchie, Aniline Blue Stain Kit procedure is useful for the demonstration of collagen and muscle fibers, has excellent staining results with bone marrow and renal biopsies and provides time effective trichrome results. This modified protocol differs from a standard trichrome procedure by not using a Bouin Fluid mordant or a hematoxylin nuclear stain.

METHOD:

Fixation: Formalin 10%, Phosphate Buffered (Part 1090)
Technique: Paraffin sections cut at 5 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.

STAINING PROCEDURE:

1. 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.*
2. Place slides in Solution A: Biebrich Scarlet-Acid Fuchsin Stain, Aqueous for 5 minutes.
3. Rinse slides in several changes of distilled water.
4. Place slides in Solution B: Iodine, Weigert & Lugol, Aqueous for 2 minutes.
5. Rinse slides in several changes of distilled water.
6. Differentiate slides one at a time in Solution C: Phosphotungstic Acid 2%, Alcoholic, for 15-30 seconds. Gently agitate slides once.
   a. *To deter over-differentiation do not exceed 30 seconds in Solution C: Phosphotungstic Acid 2%, Alcoholic.*
   b. *If sections are over-differentiated, wash slides well in distilled water and repeat Steps #2 through #6.*
7. Rinse slides immediately in several changes of distilled water.
8. Place in Solution D: Aniline Blue Stain, Aqueous for 1-3 minutes.
9. Rinse slides in several changes of distilled water.
10. 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	Blue
Muscle fibers, cytoplasm and keratin	Magenta to red
Nuclei	Dark red

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 nuclear detail with this method is dark red with crisp definition.
5. If using a xylene substitute, closely follow the manufacturer's recommendations for deparaffinization and clearing steps.

REFERENCES:

1. Carson, Freida, *Histotechnology: A Self-Instructional Text*. 2nd ed. Chicago: ASCP Press, 1997. 134-136.
2. McLetchie, Norman G.B. "Trichrome McLetchie Modification". Laboratory Procedure: Lakes Region General Healthcare, Laconia, NH.
3. Modifications developed by Newcomer Supply Laboratory.
