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Part 9124
Revised June 2015

Gram, Brown-Hopps Stain Kit - Technical Memo

KIT INCLUDES:	**Part 9124A**
Solution A: Crystal Violet Stain 1%, Aqueous, Brown-Hopps	250 ml
Solution B: Iodine, Gram, Aqueous	250 ml
Solution C: Basic Fuchsin Stain 0.25%, Aqueous	250 ml
Solution D: Gallego Solution	250 ml
Solution E: Picric Acid-Acetone 0.05%	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
Acetone, ACS	Part 10014
Acetone-Xylene 1:1	Part 10015

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

APPLICATION:

Newcomer Supply Gram, Brown-Hopps Stain Kit procedure, a modification of the original Gram Stain technique, uses Gallego Solution to differentiate staining of gram-positive and gram-negative bacteria in tissue sections.

METHOD:

Fixation: Formalin 10%, Phosphate Buffered (Part 1090)
Technique: Paraffin sections cut at 5 microns
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 #1 and #2.*
2. Stain slides in Solution A: Crystal Violet Stain 1%, Aqueous, Brown-Hopps for 2 minutes.
3. Rinse well in distilled water to remove excess stain.
4. Mordant in Solution B: Iodine, Gram, Aqueous for 5 minutes. Sections should turn black.
5. Rinse well in running tap water to remove excess iodine.
6. Blot one slide at a time and individually decolorize in Acetone (10014) until the blue color stops running, 1-2 dips. Sections should be very light gray in color.
7. Quickly rinse in running tap water to remove excess Acetone.
8. Place in Solution C: Basic Fuchsin Stain 0.25%, Aqueous for 5 minutes.
9. Rinse well in running tap water.
10. Differentiate sections in Solution D: Gallego Solution for 5 minutes.
11. Rinse thoroughly in running tap water. Blot excess water off slide, but not to dryness.
    a. *Proceed with Steps #12 to #15 one slide at a time.*
12. Dip quickly in Acetone (10014), 1-2 dips.
13. Dip directly in Solution E: Picric Acid-Acetone 0.05%, 3-10 dips.
14. Dip quickly in Acetone-Xylene 1:1 (10015), 5 dips.
15. Clear in three changes of xylene, 10 dips each; coverslip with compatible mounting medium.

RESULTS:

Gram-positive bacteria	Blue/violet
Gram-negative bacteria	Red
Nuclei	Red
Background tissue	Yellow

PROCEDURE NOTES:

1. Drain staining rack/slides after each step to prevent solution carry over.
2. Do not allow sections to dry out at any point during staining procedure.
3. 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. 188-190.
2. Chladny, M. Jane. "Batch Staining for Demonstration of Gram Positive and Gram Negative Bacteria in Tissue Sections." *The Journal of Histotechnology* 15.1 (1992): 49-50.
3. Luna, Lee G. *Histopathologic Methods and Color Atlas of Special Stains and Tissue Artifacts*. Gaitheresburg, MD: American Histolabs, 1992. 194-195.
4. Modifications developed by Newcomer Supply Laboratory.
