

Gram, Brown-Brenn Modified Stain Kit - Technical Memo

KIT INCLUDES:

Solution A: Crystal Violet-Oxalate Stain, Alcoholic
Solution B: Iodine, Gram, Aqueous
Solution C: Acetone-Alcohol 1:1
Solution D: Basic Fuchsin Stain 0.25%, Aqueous
Solution E: Tartrazine Stain 0.25%, Acetic Aqueous

Part 9123A

250 ml
250 ml
250 ml
250 ml
250 ml

COMPLIMENTARY POSITIVE CONTROL SLIDES: Enclosed are two complimentary unstained positive control slides 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 bottle labels.

APPLICATION:

Newcomer Supply Gram, Brown-Brenn Modified Stain Kit is a simple and rapid procedure for differential staining of gram-positive and gram-negative bacteria with a tartrazine counterstain.

METHOD:

Fixation: Formalin 10%, Phosphate Buffered (Part 1090)
Technique: Paraffin sections cut at 4 microns and smears.
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 provided staining procedure. Some solutions may contain extra volumes.

PRESTAINING PREPARATION:

1. If necessary, heat dry tissue sections/slides in oven.
2. Filter Solution A: Crystal Violet-Oxalate Stain, Alcoholic.

STAINING 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. Stain in freshly filtered Solution A: Crystal Violet-Oxalate Stain, Alcoholic (Step #2) for 1 minute.
5. Rinse well in distilled water.
6. Mordant in Solution B: Iodine, Gram, Aqueous for 1 minute.
7. Rinse well in distilled water, removing excess iodine.
8. Decolorize in Solution C: Acetone-Alcohol 1:1 until blue stops running; 7-10 dips.
9. Rinse well in distilled water.
10. Place in Solution D: Basic Fuchsin Stain 0.25%, Aqueous for 90 seconds.
11. Rinse well in distilled water.
12. Dip once in Solution C: Acetone-Alcohol 1:1.
13. Counterstain in Solution E: Tartrazine Stain 0.25%, Acetic Aqueous for 5-15 seconds.

14. Rinse well in distilled water.
15. Dehydrate in two changes of 100% ethyl alcohol, 5 dips each. Clear in three changes of xylene, 10 dips each; coverslip with compatible mounting medium.
 - a. Do not use 95% alcohol in the dehydration step.

RESULTS:

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

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. If using a xylene substitute, closely follow the manufacturer's recommendations for deparaffinization and clearing steps.

REFERENCES:

1. Bancroft, John D., and Marilyn Gamble. *Theory and Practice of Histological Techniques*. 6th ed. Oxford: Churchill Livingstone Elsevier, 2008. 312-313.
2. Brown, J.H., and L. Brenn. "A Method for the Differential Staining of Gram Positive and Gram Negative Bacteria in Tissue Sections". *Bulletin of The Johns Hopkins* 48.2 (1931): 69-73.
3. Luna, Lee G. *Histopathologic Methods and Color Atlas of Special Stains and Tissue Artifacts*. Gaithersburg, MD: American Histolabs, 1992. 188-189.
4. Modifications developed by Newcomer Supply Laboratory.