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Part 4256
Revised February 2024

Gram, Multi-Tissue, Artificial Control Slides – Technical Memo

CONTROL SLIDES:

Part 4256A	**Part 4256B**
10 Slide/Set	98 Slide/Set

PRODUCT SPECIFICATIONS:
Tissue: Gram positive staining rat lung and gram negative staining rat lung.
Fixation: Formalin 10%, Phosphate Buffered (Part 1090).
Section/Glass: Paraffin sections cut at 4 microns on Superfrost™ Plus slides.
Quality Control Stain: Brown-Brenn 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 Gram, Brown-Brenn, Stain Kit:	**Part 9123A**	**Individual Stain Solution**
Solution A: Crystal Violet-Oxalate Stain, Alcoholic	250 ml	Part 10422
Solution B: Iodine, Gram, Aqueous	250 ml	Part 1140
Solution C: Acetone-Alcohol 1:1	250 ml	Part 10016
Solution D: Basic Fuchsin Stain 0.25%, Aqueous	250 ml	Part 1011
Solution E: Tartrazine Stain 0.25%. Acetic Aqueous	250 ml	Part 14016

APPLICATION:

Newcomer Supply Gram, Multi-Tissue, Artificial Control Slides are for the positive histochemical staining of gram positive and gram negative bacteria in separate tissue sections. *Escherichia coli* and *Staphylococcus aureus* are used to produce the positive controls.

PRESTAINING PREPARATION:

1. Heat dry sections in oven according to your laboratory protocol.
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 negative bacteria	Red
Gram positive bacteria	Blue/violet
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, follow manufacturer's recommendation 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*. Gaitheresburg, MD: American Histolabs, 1992. 188-189.
4. Modifications developed by Newcomer Supply Laboratory.