

Gram Positive & Gram Negative Bacteria, Artificial Control Slides - Technical Memo

CONTROL SLIDES:	Part 4255A	Part 4255B
	10 Slide/Set	98 Slide/Set

PRODUCT SPECIFICATIONS:

Tissue: Positive 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:

Solution A: Crystal Violet-Oxalate Stain, Alcoholic	Part 9123A	Individual Stain Solution
Solution B: Iodine, Gram, Aqueous	250 ml	Part 10422
Solution C: Acetone-Alcohol 1:1	250 ml	Part 1140
Solution D: Basic Fuchsin Stain 0.25%, Aqueous	250 ml	Part 10016
Solution E: Tartrazine Stain 0.25%, Acetic Aqueous	250 ml	Part 1011
		Part 14016

APPLICATION:

Newcomer Supply Gram Positive & Gram Negative Bacteria, Artificial Control Slides are for the positive histochemical staining of gram positive and gram negative bacteria in a single tissue section. *Escherichia coli* and *Staphylococcus aureus* purchased from Remel Microbiology Products are used to produce the positive controls.

PRESTAINING PREPARATION:

- Heat dry sections in oven according to your laboratory protocol.
- Filter Solution A: Crystal Violet-Oxalate Stain, Alcoholic.

STAINING PROCEDURE:

- 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.
 - See Procedure Notes #1 and #2.
- Stain in freshly filtered Solution A: Crystal Violet-Oxalate Stain, Alcoholic (Step #2) for 1 minute.
- Rinse well in distilled water.
- Mordant in Solution B: Iodine, Gram, Aqueous for 1 minute.
- Rinse well in distilled water, removing excess iodine.
- Decolorize in Solution C: Acetone-Alcohol 1:1 until blue stops running; 7-10 dips.
- Rinse well in distilled water.
- Place in Solution D: Basic Fuchsin Stain 0.25%, Aqueous for 90 seconds.
- Rinse well in distilled water.
- Dip once in Solution C: Acetone-Alcohol 1:1.
- Counterstain in Solution E: Tartrazine Stain 0.25%, Acetic Aqueous for 5-15 seconds.
- Rinse well in distilled water.
- 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.
 - Do not use 95% alcohol in the dehydration step.

RESULTS:

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

PROCEDURE NOTES:

- Drain slides after each step to prevent solution carry over.
- Do not allow sections to dry out at any point during procedure.
- If using a xylene substitute, follow manufacturer's recommendation for deparaffinization and clearing steps.

REFERENCES:

- Bancroft, John D., and Marilyn Gamble. *Theory and Practice of Histological Techniques*. 6th ed. Oxford: Churchill Livingstone Elsevier, 2008. 312-313.
- 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.
- Luna, Lee G. *Histopathologic Methods and Color Atlas of Special Stains and Tissue Artifacts*. Gaithersburg, MD: American Histolabs, 1992. 188-189.
- Modifications developed by Newcomer Supply Laboratory.

