

Safranin O Stain 0.25%, Aqueous for Gram Staining of Smears

Technical Memo

SOLUTION: 500 ml
Safranin O Stain 0.25%, Aqueous Part 1360A

Additionally Needed:
Crystal Violet Stain 1%, Aqueous, Brown-Hopps Part 1041
Iodine, Gram, Aqueous Part 1140
Acetone, ACS Part 10014

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

APPLICATION:

Newcomer Supply Safranin O Stain 0.25%, Aqueous provides the preferred counterstain for Gram staining of microbiology smears. The Gram Stain technique, is used for differential staining of gram-positive and gram-negative bacteria in both smears and tissue sections.

METHOD:

Technique: Flat staining rack method.

Solutions: All solutions are manufactured by Newcomer Supply, Inc.

STAINING PROCEDURE:

1. Prepare within an accepted time frame, a well-made smear(s) per laboratory protocol, with a focus on uniform distribution of material.
2. Allow slides to thoroughly air-dry before staining.
3. Fix slides according to laboratory protocol.
 - a. See Procedure Note #1.
4. Place slides on flat staining rack suspended over sink.
 - a. See Procedure Note #2.
5. Flood fixed smears with Crystal Violet Stain 1%, Aqueous, Brown-Hopps (1041) for 45-60 seconds.
6. Drain off Crystal Violet Stain and rinse well in distilled water.
7. Mordant smears in Iodine, Gram, Aqueous (1140) for 45-60 seconds.
8. Rinse well in running tap water to remove excess iodine.
9. Blot one slide at a time and individually decolorize in Acetone (10014) until color stops running off the smear; 5-10 seconds.
 - a. See Procedure Note #3.
10. Quickly rinse in distilled water to remove excess acetone.
11. Counterstain in Safranin O Stain 0.25%, Aqueous for 30-60 seconds.
 - a. See Procedure Note #4.
12. Wash well in distilled water.
13. Allow slides to air-dry.
14. Examine microscopically with oil immersion.

RESULTS:

Gram-positive bacteria	Blue to blue/black
Gram-negative bacteria	Pink to red
Background	Yellow

PROCEDURE NOTES:

1. Gentle heat or methanol are both accepted methods of smear fixation.
2. Do not allow smears to dry out at any point during staining procedure.
3. To decolorize slower, dip slides in Alcohol, Ethyl Denatured, 95% (10842) in Coplin jar for 10-60 seconds or until color stops running off the smear.
4. For enhanced counterstaining:
 - a. Safranin O Stain 0.25%, Aqueous 10 ml
 - b. 95% Ethyl Alcohol (10842) 1 ml
 - c. Combine; mix well and stain for 30-60 seconds.

REFERENCES:

1. Kidd, Larry. "Histology vs. Microbiology – The Gram Stain the Easy Way." *The Journal of Histotechnology* 7.2 (1984): 85-86.
2. Lillie, R. D., and Harold Fullmer. *Histopathologic Technic and Practical Histochemistry*. 4th ed. New York: McGraw-Hill, 1976. 726-727.
3. McPherson, Richard and Matthew Pincus. *Henry's Clinical Diagnosis and Management by Laboratory Methods*. 22nd ed. Philadelphia: Elsevier Saunders, 2011. 1080.
4. Sheehan, Dezna C., and Barbara B. Hrapchak. *Theory and Practice of Histotechnology*. 2nd ed. St. Louis: Mosby, 1980. 235.
5. Modifications developed by Newcomer Supply Laboratory.