

## Sudan IV Stain, Herxheimer Alcoholic - Technical Memo

**SOLUTION:** 125 ml  
Sudan IV Stain, Herxheimer Alcoholic Part 1400A

Additionally Needed:

|                                      |            |    |                                      |
|--------------------------------------|------------|----|--------------------------------------|
| Alcohol, Ethyl Denatured, 70%        | Part 10844 |    |                                      |
| Hematoxylin Stain, Mayer Modified    | Part 1202  |    |                                      |
| Lithium Carbonate, Saturated Aqueous | Part 12215 | or | Scott Tap Water Substitute Part 1380 |
| Mount-Quick Aqueous Mounting Medium  | Part 6271A |    |                                      |

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

**APPLICATION:**

Newcomer Supply Sudan IV Stain, Herxheimer Alcoholic procedure is used for identification of fat/lipid in frozen sections. Herxheimer method refers to an acetone/alcohol solvent mixture; the acetone component of this solution may dissolve out small amounts of lipid.

Sudan dyes are a group of fat/lipid soluble solvent dyes, also known as lysochromes. These solvent dyes readily stain fat/lipid due to the fact that the dyes are more soluble in lipid than in the solvents from which they are applied.

**METHOD:**

**Fixation:** Fresh tissue or formalin fixed unprocessed tissue  
a. See Procedure Note #1.

**Technique:** Frozen sections cut at 8 microns on adhesive slides

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

All Newcomer Supply stain procedures are designed to be used with Coplin jars filled to 40 ml following the provided staining procedure.

**STAINING PROCEDURE:**

1. Fix frozen section slides in Formalin 10%, Phosphate Buffered for 1 minute.  
a. See Procedure Note #2.
2. Rinse sections carefully in two changes of distilled water.
3. Rinse in Alcohol, Ethyl Denatured, 70% (10844).
4. Stain in Sudan IV Stain, Herxheimer Alcoholic for 10 minutes.  
a. Keep tightly capped to avoid evaporation.
5. Differentiate quickly in Alcohol, Ethyl Denatured, 70% to remove excess stain.
6. Wash thoroughly in distilled water.
7. Counterstain with Hematoxylin Stain, Mayer Modified (1202) for 2-3 minutes.
8. Wash gently in several changes of tap water.
9. Blue in Lithium Carbonate, Saturated Aqueous (12215) or Scott Tap Water Substitute (1380) for 10 dips.  
a. The use of a bluing agent is optional.
10. Wash gently in several changes of tap water.
11. Blot excess water from slide; coverslip with Mount-Quick Aqueous Mounting Medium.  
a. See Procedure Note #3.

**RESULTS:**

|        |            |
|--------|------------|
| Fat    | Orange/red |
| Nuclei | Blue       |

**PROCEDURE NOTES:**

1. To freeze formalin fixed unprocessed tissue:
  - a. Place specimen in tissue cassette; wash in running water for 5 minutes.
  - b. Remove tissue from cassette; blot well, removing all excess water from tissue.
  - c. Freeze tissue according to laboratory protocol.
2. Frozen formalin fixed tissue does not require additional formalin fixation.
3. Use minimal pressure when applying coverslip or fat/lipid staining may be disturbed. To remove trapped air bubbles or to recover slip;
  - a. Soak slide in warm water until coverslip is easily removed.
  - b. Blot excess water from slide.
  - c. Remount with new coverslip and Mount-Quick Aqueous Mounting Medium.

**REFERENCES:**

1. Culling, C. F. A. *Handbook of Histopathological and Histochemical Techniques: (including Museum Techniques)*. 3rd ed. London: Butterworth, 1974. 359-362.
2. Kiernan, J. A. *Histological and Histochemical Methods: Theory and Practice*. 3rd ed. London, Ontario: Arnold, 2003. 251-254.
3. Lillie, R. D., and Harold Fullmer. *Histopathologic Technic and Practical Histochemistry*. 4th ed. New York: McGraw-Hill, 1976. 565-568.
4. Modifications developed by Newcomer Supply Laboratory.