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Part 1401
Revised November 2019

Sudan Black B Stain, Propylene Glycol - Technical Memo

SOLUTIONS:	**125 ml**	**250 ml**
Sudan Black B Stain, Propylene Glycol	Part 1401A	Part 1401B

Additionally Needed:

Formalin 10%, Phosphate Buffered	Part 1090
Propylene Glycol 100%, ACS	Part 13391
Propylene Glycol 85%, Aqueous	Part 133912
Nuclear Fast Red Stain, Kernechtrot	Part 1255
Mount-Quick Aqueous Mounting Medium	Part 6271A

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

APPLICATION:

Newcomer Supply Sudan Black B Stain, Propylene Glycol procedure is used for identification of fat/lipid in frozen sections. Sudan black B is a lipid soluble solvent dye that readily stains neutral fats and phospholipids.

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 8-10 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 (1090) for 1 minute.
   - *a. See Procedure Note #2.*
2. Rinse sections carefully in two changes of distilled water.
3. Blot off excess water and dehydrate slides in Propylene Glycol 100%, ACS (13391) for 10-15 minutes.
4. Place directly into Sudan Black B Stain, Propylene Glycol for 30 minutes to 1 hour. Agitate occasionally or place Coplin jar on rotator/shaker with continuous gentle agitation.
   - *a. See Procedure Note #3.*
5. Differentiate in Propylene Glycol 85%, Aqueous (133912) for 3 minutes with agitation.
6. Rinse gently in distilled water.
7. Counterstain in Nuclear Fast Red Stain, Kernechtrot (1255) for 5 minutes.
   - *a. Shake solution well before use; do not filter.*
8. Wash gently in several changes of tap water.
   - *a. See Procedure Note #4.*
9. Blot excess water from slide; coverslip with Mount-Quick Aqueous (6271A) Mounting Medium.
   - *a. See Procedure Note #5.*

RESULTS:

Fat	Blue-black
Nuclei	Red

PROCEDURE NOTES:

1. To freeze formalin fixed unprocessed tissue:
   - *a. Place specimen in tissue cassette, wash in running tap 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. To decrease staining time; preheat Sudan Black B Stain, Propylene Glycol in a 60ºC oven; stain for 3-10 minutes.
4. Wash well after Nuclear Fast Red Stain, Kernechtrot to avoid cloudiness in coverslipped sections.
5. Use minimal pressure when applying coverslip or fat/lipid staining may be disturbed. To remove trapped air bubbles or to recoverslip;
   - *a. Soak slide(s) 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. Bancroft, John D., and Marilyn Gamble. *Theory and Practice of Histological Techniques*. 6th ed. Oxford: Churchill Livingstone Elsevier, 2008. 192-193.
2. Carson, Freida L., and Christa Hladik. *Histotechnology: A Self-Instructional Text*. 3rd ed. Chicago, Ill.: American Society of Clinical Pathologists, 2009. 185-186.
3. Luna, Lee G. *Histopathologic Methods and Color Atlas of Special Stains and Tissue Artifacts*. Gaitheresburg, MD: American Histolabs, 1992. 464-465.
4. Sheehan, Dezna C., and Barbara B. Hrapchak. *Theory and Practice of Histotechnology*. 2nd ed. St. Louis: Mosby, 1980. 204-205.
5. Modifications developed by Newcomer Supply Laboratory.