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Part 9119
Revised June 2015

Fat, Oil Red O, Propylene Glycol Stain Kit - Technical Memo

KIT INCLUDES:	**Part 9119A**
Solution A: Propylene Glycol 100%, ACS	250 ml
Solution B: Oil Red O Stain, Propylene Glycol	250 ml
Solution C: Propylene Glycol 85%, Aqueous	250 ml
Solution D: Hematoxylin Stain, Mayer Modified	250 ml

Individual stain solutions may be available for purchase under separate part numbers at www.newcomersupply.com.

Additionally Needed:

Formalin 10%, Phosphate Buffered	Part 1090			
Lithium Carbonate, Saturated Aqueous	Part 12215	or	Scott Tap Water Substitute	Part 1380
Mount-Quick Aqueous	Part 6271A			

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

APPLICATION:

Newcomer Supply Fat, Oil Red O, Propylene Glycol Stain Kit procedure is classified as a physical staining method and is used for identification of fat/lipid in frozen sections.

METHOD:

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

Technique: Frozen sections cut at 8-10 microns mounted on adhesive slides

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

All Newcomer Supply Stain Kits are designed to be used with Coplin jars filled to 40 ml following the staining procedure provided below. Some solutions in the kit may contain extra volumes.

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 place slides in Solution A: Propylene Glycol 100%, ACS for 2-5 minutes.
4. Place slides directly into Solution B: Oil Red O Stain, Propylene Glycol for 1 hour. Agitate occasionally or place Coplin jar on rotator/shaker with continuous gentle agitation.
   - *a. See Procedure Notes #3 and #4.*
5. Differentiate in Solution C: Propylene Glycol 85%, Aqueous with agitation for a minimum of 3 minutes.
6. Rinse gently in two changes of distilled water.
7. Counterstain with Solution D: Hematoxylin Stain, Mayer Modified, for 2-3 minutes, depending on preference of nuclear stain intensity.
8. Wash gently in several changes of tap water.
9. Blue slides in Lithium Carbonate, Saturated Aqueous (12215) or Scott Tap Water Substitute (1380) for 10 dips.
   - *a. See Procedure Note #5.*
10. Wash gently in several changes of tap water.
11. Blot excess water from slide; coverslip with Mount-Quick Aqueous (6271A) mounting medium.
    - *a. See Procedure Note #6.*

RESULTS:

Fat: Bright red
Nuclei: Blue to dark blue

PROCEDURE NOTES:

1. To freeze formalin fixed unprocessed tissue post-fixation: Place specimen in tissue cassette, wash in running water for 5 minutes. Remove tissue from cassette and blot well removing all excess water from tissue. Freeze tissue (fresh or formalin fixed) according to your laboratory protocol.
2. Frozen formalin fixed tissue does not require an additional formalin fixation step.
3. To decrease staining time; preheat Solution B: Oil Red O Stain, Propylene Glycol in a 60ºC oven and decrease incubation time to 7-10 minutes.
4. If a filmy precipitate develops in Solution B: Oil Red O Stain, Propylene Glycol filter with coarse filter paper.
5. The use of a bluing agent in this procedure is an optional step.
6. Use minimal pressure when applying coverslip or fat/lipid staining may be disturbed. To remove trapped air bubbles under coverslip; soak slide in warm water until coverslip can easily be removed. Blot excess water from slide and remount with new coverslip and Mount-Quick Aqueous mounting medium.

REFERENCES:

1. Carson, Freida L., and Christa Hladik. *Histotechnology: A Self-Instructional Text*. 3rd ed. Chicago, Ill.: American Society of Clinical Pathologists, 2009. 184-186.
2. Prophet, Edna B., Bob Mills, Jacquelyn Arrington, and Leslie Sobin. *Laboratory Methods in Histotechnology*. Washington, D.C.: American Registry of Pathology. 1992.178.
3. Sheehan, Dezna C., and Barbara B. Hrapchak. *Theory and Practice of Histotechnology*. 2nd ed. St. Louis: Mosby, 1980. 205.
4. Modifications developed by Newcomer Supply Laboratory.
