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Part 9119
Revised October 2024

Oil Red O 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 Oil Red O Stain Kit procedure is classified as a physical staining method. Oil Red O (ORO) staining is used for identification of fat in frozen tissue sections.

METHOD:

Fixation: Fresh tissue or formalin fixed unprocessed tissue
Technique: Frozen sections cut at 8-10 microns on adhesive slides
  a. *See Procedure Notes #1 and #2.*
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 provided staining procedure. Some solutions in the kit may contain extra volumes.

PRESTAINING PREPARATION:

1. Preheat Solution B: Oil Red O Stain, Propylene Glycol in 60°C oven for a minimum of 30 minutes. Save for Step #7.
   (Skip if using room temperature staining method Step #6.)
2. If a film of precipitate forms on Solution B: Oil Red O Stain, Propylene Glycol, skim the surface with filter paper before use.

STAINING PROCEDURE:

3. Fix frozen sections in Formalin 10%, Phosphate Buffered (1090) for 1 minute.
4. Rinse sections in two changes of distilled water.
5. Blot off water and place in Solution A: Propylene Glycol 100%, ACS for 2-5 minutes with agitation.
6. **Room Temperature ORO Staining:** Place in Solution B: Oil Red O Stain, Propylene Glycol for 1 hour with agitation.
7. **Heated ORO Staining:** Place in preheated Solution B: Oil Red O Stain, Propylene Glycol for 7-10 minutes. Agitate occasionally.
8. Differentiate in Solution C: Propylene Glycol 85%, Aqueous for 3 minutes with agitation.
9. Rinse in two changes of distilled water.
10. Counterstain in Solution D: Hematoxylin Stain, Mayer Modified for 2-3 minutes.
11. Wash in several changes of tap water.
12. **Optional**: Blue slides in Lithium Carbonate, Saturated Aqueous (12215) or Scott Tap Water Substitute (1380) for 10 dips.
13. Wash in several changes of tap water.
14. Blot water from slide; coverslip with Mount-Quick Aqueous (6271A) mounting medium.
    a. *See Procedure Notes #3 and #4.*

RESULTS:

Fat: Bright red
Nuclei: Blue to dark blue

PROCEDURE NOTES:

1. Freeze tissue according to laboratory protocol.
2. To freeze formalin fixed tissue that has not been processed:
   a. *Wash fixed tissue in running water for 5 minutes.*
   b. *Blot excess water from tissue and freeze.*
3. Use minimal pressure with coverslip application to avoid displacement of fat/lipid staining.
4. To remove trapped air bubbles or to recoverslip;
   a. *Soak slide in warm water until coverslip slides off.*
   b. *Blot water from slide.*
   c. *Remount with new coverslip and Mount-Quick Aqueous.*

REFERENCES:

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1. Prophet, Edna B., Bob Mills, Jacquelyn Arrington, and Leslie Sobin. *Laboratory Methods in Histotechnology*. Washington, D.C.: American Registry of Pathology. 1992.178.
2. Sheehan, Dezna C., and Barbara B. Hrapchak. *Theory and Practice of Histotechnology*. 2nd ed. St. Louis: Mosby, 1980. 205.
3. Modifications developed by Newcomer Supply Laboratory.
