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Part 1277
Revised September 2015

Oil Red O Stain, Isopropanol - Technical Memo

SOLUTION:	**250 ml**	**500 ml**
Oil Red O Stain, Isopropanol	Part 1277A	Part 1277B

Additionally Needed:

Alcohol, Isopropyl ACS, 100%	Part 12094
Formalin 10%, Phosphate Buffered	Part 1090
Hematoxylin Stain, Mayer Modified	Part 1202
Lithium Carbonate, Saturated Aqueous	Part 12215
Mount-Quick Aqueous Mounting Medium	Part 6271A

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

APPLICATION:

Newcomer Supply Oil Red O Stain, Isopropanol procedure, classified as a physical staining method, 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 procedures are designed to be used with Coplin jars filled to 40 ml following the staining procedure provided below.

STAINING PROCEDURE:

1. Prepare fresh Working Oil Red O Isopropanol Solution; combine and mix well.
   - *a. Oil Red O Stain, Isopropanol 30 ml*
   - *b. Distilled Water 20 ml*
   - *c. Cover solution and allow to stand at room temperature for 10 minutes; filter prior to use.*
   - *d. Solution is stable for 1-2 hours.*
2. Prepare Alcohol, Isopropanol, 60%:
   - *a. Alcohol, Isopropyl ACS, 100% 60 ml*
   - *b. Distilled Water 40 ml*
   - *c. See Procedure Note #2.*
3. Fix frozen section slides in Formalin 10%, Phosphate Buffered for 1 minute.
   - *a. See Procedure Note #3.*
4. Rinse sections carefully in two changes of distilled water.
5. Rinse in Alcohol Isopropyl, 60%.
6. Stain in filtered Working Oil Red O Isopropanol Solution for 10 to 15 minutes.
7. Rinse in fresh Alcohol Isopropyl, 60%.
8. Wash thoroughly in distilled water.
9. Counterstain with Hematoxylin Stain, Mayer Modified for 2-3 minutes, depending on preference of nuclear stain intensity.
10. Wash gently in several changes of tap water.
11. Blue slides in Lithium Carbonate, Saturated Aqueous; 5 to 10 dips.
12. Wash gently in several changes of tap water.
13. Blot excess water from slide; coverslip with Mount-Quick Aqueous Mounting Medium.
    - *a. See Procedure Note #4.*

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. Isopropyl alcohol must be used; do not substitute with another grade of alcohol.
3. Frozen formalin fixed tissue does not require an additional formalin fixation step.
4. 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 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. Lillie, R. D., and Harold Fullmer. Histopathologic Technic and Practical Histochemistry. 4th ed. New York: McGraw-Hill, 1976. 567.
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.