

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

KIT INCLUDES:

Solution A: Propylene Glycol 100%, ACS	Part 9119A
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, 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 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:

- Fix frozen section slides in Formalin 10%, Phosphate Buffered (1090) for 1 minute.
a. See Procedure Note #2.
- Rinse sections carefully in two changes of distilled water.
- Blot off excess water and place slides in Solution A: Propylene Glycol 100%, ACS with agitation for 2-5 minutes.
- 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.
- Differentiate in Solution C: Propylene Glycol 85%, Aqueous with agitation for a minimum of 3 minutes.
- Rinse gently in two changes of distilled water.
- Counterstain with Solution D: Hematoxylin Stain, Mayer Modified, for 2-3 minutes.
- Wash gently in several changes of tap water.
- Optional:** Blue slides in Lithium Carbonate, Saturated Aqueous (12215) or Scott Tap Water Substitute (1380) for 10 dips.
- Wash gently in several changes of tap water.
- Blot excess water from slide; coverslip with Mount-Quick Aqueous (6271A) mounting medium.
a. See Procedure Note #5.

RESULTS:

Fat: Bright red
Nuclei: Blue to dark blue

PROCEDURE NOTES:

- To freeze formalin fixed unprocessed tissue:
 - Place fixed specimen in tissue cassette, wash in running water for 5 minutes.
 - Remove tissue and blot excess water from tissue.
 - Freeze tissue (fresh or formalin fixed) according to your laboratory protocol.
- Frozen formalin fixed tissue does not require additional fixation.
- To decrease staining time; preheat Solution B: Oil Red O Stain, Propylene Glycol in a 60°C oven and incubate for 7-10 minutes.
- If filmy precipitate forms on Solution B: Oil Red O Stain, Propylene Glycol, skim the surface with filter paper before use.
- Use minimal pressure when applying coverslip or fat/lipid staining may be disturbed. To remove trapped air bubbles or to recoverslip;
 - Soak slide in warm water until coverslip is easily removed.
 - Blot excess water from slide.
 - Remount with new coverslip and Mount-Quick Aqueous mounting medium.

REFERENCES:

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