COS-0101

Oil Red O Lipid Staining Kit

(To stain two plates (6- 12-, 24- or 96-well) or five 100 mm culture dishes, Store at room temperature)

Background Information:

Cellular lipids are usually found in the membranes of the cell and its organelles. In those cells where lipid is stored for energy production or as source for more complex structures, it is most often seen as droplets consisting primarily of triglycerides although other neutral lipids may be present. Differentiation of adipocytes into mature cells involves a dramatic accumulation of lipid droplets. There are a number of lipid storage diseases (Gaucher's, Niemann-Pick, Fabry's, etc.) where the accumulation of excess lipids can cause severe pathology. Oil Red O is a lipid-soluble azo dye which preferentially stains neutral lipids red so is ideal for visualization of neutral lipid accumulations. The AkrivisBio Oil Red O Lipid Staining Kit which also includes hematoxylin to counterstain nuclei blue, is an easy, inexpensive way to qualitatively visualize lipid accumulations in cells grown in cell culture under a microscope.

Principle:

1 - Very hydrophobic dye preferentially accumulates with neutral lipids in cells grown in cell culture
2 – After sufficient time has elapsed, lipid accumulations appear as dark red objects.

Kit Contents:

PBS	50 ml	NM	COS-0101A
10% Formalin	24 ml	NM Blue Dot	COS-0101B
Oil Red O	60 mg	NM	COS-0101C
Hematoxylin	24 ml	Amber	COS-0101D
0.22 µm syringe filter	1 ea	-	COS-0101E
10 ml syringe	1 ea	-	COS-0101F

User Supplied Materials:

- Light microscope
- 100% isopropanol

Storage Conditions and Handling:

Store the kit at room temperature.
PBS, Formalin (10%), and Hematoxylin: Ready to use as supplied.
Oil Red O: Add 20 ml 100% isopropanol to the bottle containing the Oil Red O powder, cap tightly and mix well. Allow dissolution to proceed undisturbed for 20 minutes. If tightly capped the solution is good for a year or more.
To make Oil Red O Staining Solution, transfer 6.7 ml to a 15 ml tube and add 4.4 ml of DI H_2O, mix well, and allow to sit for 10 min. Filter the solution with the syringe and filter 15 – 30 min before using. The solution is sufficient for one plate and is stable for about 2 hours.

Lipid Staining Protocol:

For all additions, amount to use per well:

Plate:	96-well	48-well	24-well	12-well	6-well	100 mm dish
	100 µl	200 µl	400 µl	800 µl	1.6 ml	5.5 ml

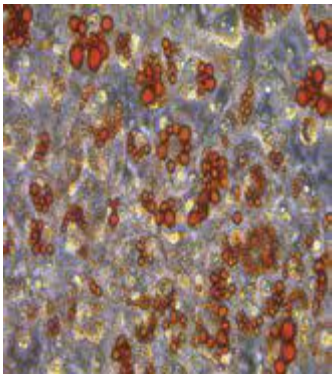
A. Wash: Remove media from cells and gently wash 2X with PBS.

B. Fixation: Add Formalin gently, at the side of each well. Once all wells have been treated, swirl by rotating gently. Incubate for 30-60 min. Prepare 10 ml of 60% isopropanol per plate to be processed. If a semiquantitative treatment is planned (see note below), prepare 40-50 ml per plate to be processed.

C. Staining:
1 - Remove formalin and gently wash cells 2X with DI H_2O.
2 - Add Isopropanol (60%) to each well and incubate for 5 min.
3 - Remove isopropanol and add Oil Red O Working Solution (cover the cells completely). Rotate plate gently and incubate for 10-20 min.
4- Remove Oil Red O solution and wash 2-5X with DI H_2O as needed until excess stain is no longer apparent. Discard unused stain.
5 - Add Hematoxylin and incubate for 1 min. Remove Hematoxylin and wash with DI H_2O 2-5X as needed.

D. View: Keep cells covered with DI H_2O at all times and while viewing under microscope. Lipid droplets appear red and nuclei appear blue.

Note: While Oil Red O staining is not intended to be quantitative, you can get a semi-quantitative measure of lipid content: After staining with Hematoxylin and washing with DI H_2O, (step above) wash 3X, 5 min each, using 60% isopropanol with rocking. Extract Oil Red O with 100% isopropanol (half the amount shown in the table) for 5 min. with gentle rocking. This procedure is not particularly useful for wells smaller than those on a 24 well plate, as smaller wells will give lower signal and there are too many to handle efficiently. The OD can be read at 492 nm.



Cells stained with Oil Red O

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