Lipid (Oil Red O) Staining Kit

9/14

(Catalog # K580-24; 24 ml; Store at room temperature)

I. **Introduction:**

Lipid droplets are found in all eukaryotic organisms. They function as reservoirs of neutral lipids, primarily triacylglycerol and cholesterol esters. The stored lipids can be used for energy, steroid synthesis, or membrane formation. Lipid droplet accumulation is a normal function in cells. Adipocytes consist predominantly of lipid droplets; however excessive lipid droplet accumulation within cells can be an indicator of metabolic deficiency or pathogenesis. For example, excessive accumulation of lipids in liver cells (steatosis) can lead to cellular dysfunction. At the onset of atherosclerosis, macrophages engulf oxidized-LDL, develop into foam cells, and contribute to artery narrowing. BioVision's Lipid (Oil Red O) Staining Kit allows selective detection of neutral lipids within cultured cells. The kit also includes Hematoxylin to stain nuclei. This kit provides enough reagents to stain two (2) 96-well plates, two (2) 6-well plates, or four (4) 100 mm culture dishes.

II. **Application:**

- Staining of neutral lipids in cells.

III. **Sample Type:**

- Cell culture: adipocytes, hepatocytes, macrophages, etc.

IV. **Kit Contents:**

Components	K580-24	Cap Code	Part Number
PBS	48 ml	NM	K580-24-1
Formalin (10%)	24 ml	NM Green	K580-24-2
Oil Red O	60 mg	NM	K580-24-3
Hematoxylin	24 ml	Amber	K580-24-4

V. **User Supplied Reagents and Equipment:**

- Syringe and 0.2 µm syringe filter or Whatman No. 1 filter paper
- Light microscope
- 100% isopropanol
- 96-well clear plate with flat bottom (optional)
- Multi-well spectrophotometer (ELISA reader) (optional)

VI. **Storage Conditions and Reagent Preparation:**

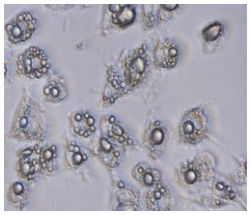
Store kit at room temperature. Read the entire protocol before starting the staining process.

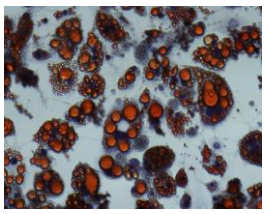
- **PBS, Formalin (10%), and Hematoxylin:** Ready to use as supplied. Stable for 1 year.
- **Oil Red O:** To make Oil Red O Stock Solution, dissolve Oil Red O in 20 ml of 100% isopropanol, mix well and let it sit for 20 min. Stable for 1 year. To make Oil Red O Working Solution, add 3 parts of Oil Red O Stock Solution to 2 parts of dH_2O, mix well, and allow to sit for 10 min. Filter with 0.2 µm syringe filter or Whatman No. 1 paper or equivalent. Prepare 15 min. before use. Working solution is stable for 2 hrs.

VII. **Lipid (Oil Red O) Staining Protocol:**

Note: All components: For 96-well plate, use 100 µl per well; for 24-well plate, use 500 µl per well; for 6-well plate, use 2 ml per well & for 100 mm culture dish, use 6 ml.

1. **Cell Fixing:** Remove media from cells and gently wash 2X with PBS. Add Formalin (10%) to each well and incubate for 30 min. to 1 hr. **Note:** Do not pipet directly onto cells, pipet to the side of well or plate and mix by rotating.
2. **Cell Staining:** Prepare Oil Red O Working Solution as mentioned in step VI. Remove formalin and gently wash cells 2X with dH_2O. Make Isopropanol (60%) by adding 3 parts Isopropanol (100%) to 2 parts of water. Add Isopropanol (60%) to each well and incubate for 5 min. Remove isopropanol and add Oil Red O Working Solution to completely and evenly cover the cells. Rotate plate or dish and incubate for 10-20 min. Remove Oil Red O solution and wash 2-5X with dH_2O as needed until excess stain is no longer apparent. Add Hematoxylin and incubate for 1 min. Remove Hematoxylin and wash with dH_2O 2-5X as needed. Keep cells covered with dH_2O at all times and while viewing under microscope. Lipid droplets appear red and nuclei appear blue. **Note:** Discard used Oil Red O Solution. Re-use of Oil Red O Solution results in poor staining quality.
3. **Quantification (optional):** Oil Red O staining can be measured semi-quantitatively. After staining with Hematoxylin and washing with dH_2O, wash additional 3X with 60% isopropanol. Wash each time for 5 min. with gentle rocking. Extract Oil Red O stain with 100% isopropanol for 5 min. with gentle rocking. It is recommended to use at least 24-well plate, as smaller wells will give low signal. Extract with 50% normal volume (e.g. for 24-well plate, use 250 µl per well) and use 80% of extraction volume to measure. Use 100% isopropanol as background control to subtract the background signal. Read absorbance at 492 nm.

(a) 

(b) 

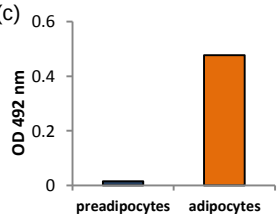
(c) 

Figure: Differentiated 3T3-L1 adipocytes (a) before staining with Oil Red O and (b) after staining with Oil Red O. Neutral lipids are stained red and nuclei are stained blue. (c) Quantification of Oil Red O staining in preadipocytes and adipocytes. Cells were grown in a 24-well plate, stain was extracted in 250 µl isopropanol and 200 µl was used to measure oil Red O stain in a 96-well plate reader at 492 nm. Assay was performed according to the kit protocol.

VIII. RELATED PRODUCTS:

3T3-L1 Differentiation Kit (K579)
Preadipocyte Isolation Kit (K583)
Lipolysis (3T3-L1) Fluorometric Assay Kit (K578)
Adipogenesis Colorimetric/Fluorometric Kit (K610)
Lipolysis (3T3-L1) Colorimetric Assay Kit (K577)
Triglyceride Quantification Colorimetric/Fluorometric Assay Kit (K622)

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