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Product Information

Lipid (Oil Red O) Staining Kit

Catalog Number **MAK194**
Store at Room Temperature

TECHNICAL BULLETIN

Product Description

Lipid droplets (LDs) are dynamic, ubiquitously present lipid-storage organelles, predominantly present in the adipocytes. Triglycerides, neutral lipids, and cholesterol esters stored in LDs are the largest sources of energy. The presence of excess LDs in adipocytes results in obesity and obesity-linked pathologies such as dyslipidemia and diabetes type 2.[1,2]

The Lipid (Oil Red O) Staining Kit is suitable for selective staining and detection of neutral lipids in cultured cells. Hematoxylin included in the kit stains the nuclei of the cells.

Components

The kit is sufficient to stain two 96 well plates, two 6 well plates, or four 100 mm culture dishes.

PBS Catalog Number MAK194A	48 mL
Formalin (10%) Catalog Number MAK194B	24 mL
Oil Red O Catalog Number MAK194C	60 mg
Hematoxylin Catalog Number MAK194D	24 mL

Reagents and Equipment Required but Not Provided

- Whatman® No. 1 filter paper
- Light microscope
- Isopropanol, 60%
- Isopropanol, 100%

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents.

PBS, Formalin (10%), and Hematoxylin – Ready-to-use solutions. Stable for 1 year.

Oil Red O Stock Solution – Reconstitute with 20 mL of 100% isopropanol. Mix well and leave undisturbed for 20 minutes to make the Oil Red O Stock Solution. The Oil Red O Stock Solution is stable for 1 year.

Oil Red O Working Solution – Add 3 parts of Oil Red O Stock Solution to 2 parts of water. Mix well and leave undisturbed for 10 minutes. Filter through Whatman No. 1 filter paper. The Oil Red O Working Solution is stable for 2 hours and must be prepared 15 minutes before use.

Storage/Stability

The kit is shipped at ambient temperature and storage at room temperature is recommended.

Procedure

Note: All components:
Use 100 µL/well for a 96 well plate
2 mL/well for a 6 well plate
6 mL for a 100 mm culture dish

Cell Staining

1. Fixation – Remove the medium and gently wash the cells twice with PBS. Add Formalin (10%) to the cells and incubate for 30 minutes to 1 hour.
   Note: Do not add formalin directly onto the cells. Pipette onto the wall and mix by gently rotating.

2. Discard the formalin and wash the cells twice using water. Add 60% isopropanol to the cells and incubate for 5 minutes.

3. Discard 60% isopropanol and cover the cells evenly with Oil Red O Working Solution. Rotate the plate or dish, and incubate for 10–20 minutes.

4. Discard the Oil Red O solution and wash the cells 2–5 times with water until no excess stain is seen.

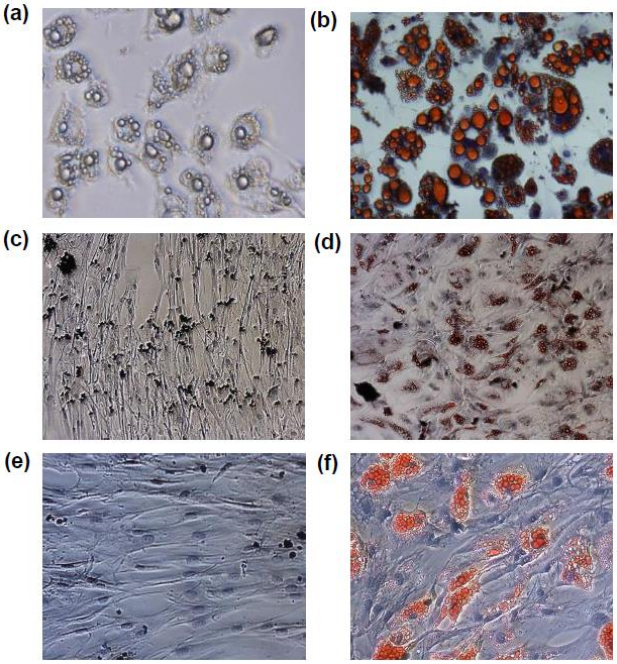
5. Add Hematoxylin to the cells and incubate for 1 minute. Discard Hematoxylin and wash the cells 2–5 times with water.

6. Cover the cells with water and view under microscope. Lipid droplets appear red and nuclei appear blue.
   Notes: Keep the cells covered with water to avoid drying.

   Reuse of Oil Red O Working Solution is not recommended as it results in poor staining quality.

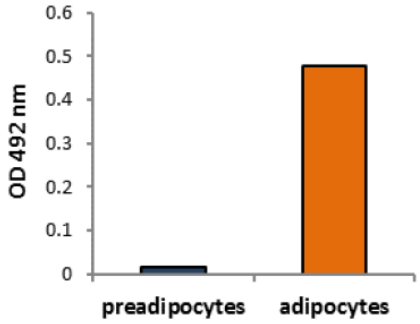
Results

Figures (a-f).
Differentiated 3T3-L1 adipocytes



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. Oil Red O staining on undifferentiated cells (c and e) at 10× and 20× magnifications and differentiated cells (d and f) at 10× and 20× magnifications, respectively.

Figure (g).
Quantification of Oil Red O staining



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.

References

1. Krahmer, N., et al., Balancing the fat: lipid droplets and human disease. EMBO Mol. Med., **5**, 905–915 (2013).
2. Konige, M., et al., Role of adipose specific lipid droplet proteins in maintaining whole body energy homeostasis. Biochim. Biophys. Acta, **1842**, 393–401 (2013).

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