Technical Bulletin

Lipid (Oil Red O) Staining Kit

Catalogue Number MAK560

Product Description

Lipid droplets (LDs) are dynamic, ubiquitously present lipid-storage organelles, predominantly present in adipocytes.[1] Triglycerides, neutral lipids, and cholesterol esters stored in LDs are the largest sources of energy. The presence of excess LDs in adipocytes is linked to different stress conditions and pathologies of cells and tissues (such as: Obesity, dyslipidemia, diabetes type 2, diabetic nephropathy, hepatic toxicity, and cancer).

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

nuclei of the cells whereas Oil Red O stains the lipid droplets.

Components

The kit is sufficient for 2 96-well plates.

- Phosphate Buffered Saline 48 mL
  Catalogue Number MAK560A
- Fixation Buffer 10X 2.4 mL
  Catalogue Number MAK560B
- Oil Red O Solution 15 mL
  Catalogue Number MAK560C
- Hematoxylin Solution 24 mL
  Catalogue Number MAK560D

Reagents and Equipment Required but Not Provided

- Double distilled water (ultrapure water)
- 96-well plates, clear, flat bottom.
- Plate reader that can read the wavelength of 492 nm.
- Horizontal Shaker
- Vortex Mixer
- Whatman No. 1 filter paper
- Light microscope
- Isopropanol, 60%
- Isopropanol, 100%
- De-paraffinization kit

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.

Storage/Stability

The product is shipped under ambient conditions. Store at 2-8 °C upon receipt, protected from light.

Preparation Instructions

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

Phosphate buffered saline (PBS): Allow buffer to come to room temperature before use. Ready-to-use solutions.
Store at 2–8 °C.

Oil Red O Stock Solution: Allow solution to come to room temperature before use.

Store at 2-8 °C. See manual on how to prepare Oil Red O Working Solution.

Hematoxylin Solution: Allow buffer to come to room temperature before use. Ready-to-use solution. Store at 2-8 °C.

Procedure

Preparation of Working Solutions

Oil Red O Working Solution: Add 3 parts of Oil Red O Stock Solution (MAK560C) 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.

Fixation Buffer 1X: Add 21.6ml of water to the MAK560B vial. The solution can be stored at Stored at -20 °C.

Sample Type:

- Formalin-fixed histology slides
- Paraffin embedded histology slides (not recommended).
- Cell culture: Adherent or suspension cells.

Protocol for staining of FFPE histology slides:[5,6]

1. Perform de-paraffinization according to the manufacturer's instructions (for fresh histology slides skip that step) of the de-paraffinization kit (not supplied). Be advised that some components may be harmful if inhaled and the procedure should be performed using a well-ventilated chemical hood.

2. Prepare the Oil Red O working solution as explained above.

3. Filter through a Whatman No.1 filter paper (Not supplied) into a plastic tube.

4. Stain the slides with the Oil Red O working solution for 10 minutes.

5. Wash the slides with 60% Isopropanol solution (not supplied) for 5 minutes.

6. Wash the slides with 2 waters changes of ultra-pure water.

7. Stain the slides with Hematoxylin (MAK560D) for 1 minute.

8. Rinse the slides with water for 2-3 minutes.

9. Use aqueous mounting medium (Glycerol-Gelatin, Sigma GG-1, not supplied) preheated to 56 °C and place the glass coverslip on it (Sigma C9802, not supplied).

10. Allow the mounting medium to dry for at least 20 minutes before taking photos with a light microscope.

Protocol for Oil red O staining in cells:[7,8]

Components working concentrations: Use 100 mL/well for a 96 well plate, 500 mL/well for a 24 well plate, 2 mL/well for a 6 well plate, 6 mL for a 100 mm culture dish.

1. Fixation – Remove the medium and gently wash the cells twice with PBS. Add Fixation solution x1 to the cells and incubate for 30 minutes to 1 hour. Note: Do not add for Fixation solution x1 directly onto the cells. Pipette onto the wall and mix by gently rotating.

2. Discard the Fixation solution x1 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.

Note: Keep the cells covered with water to avoid drying.

Semi-quantification of oil-red O staining:[9]

1. Grow cells in 24-well plate.
2. Stain cells with Oil red O according to protocol.
3. Wash cells with 60% isopropanol and rotate for 5 min (x3 times).
4. Extract cells with 250mL/well of 100% isopropanol and rotate for 5 min.
5. Transfer 200mL from each well to read in 96-well plate.
6. Read the absorbance at 492 nm using a plate reader.

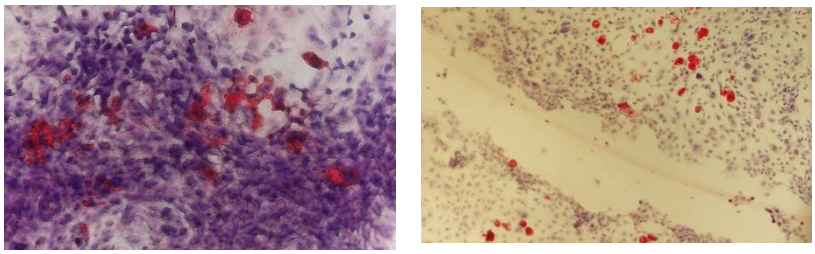
Results

The expected results are red staining of lipids and blue staining of nuclei.

Note: If no oil red o staining is seen in cells using light microscopy:

- Try not to add fixation solution directly onto the cells. Pipette onto the wall and mix by gently rotating.
- Make sure working solutions of fixation solution and Oil red O stain have been prepared according to protocol.

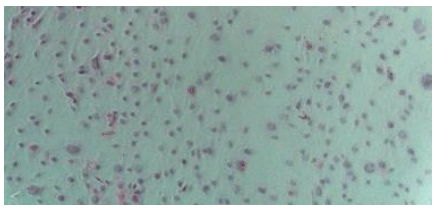
Figure 1:



Differentiated Cells

3T3-L1 (cells in pre adipocyte state) were cultured and differentiated using IBMX-DEX (1µMDEX,0.5Mm IBMX) method to gain adipocyte phenotype. Cells were fixed with fixation solution (MAK560B) and were stained with oil red O solution (MAK560C) and hematoxylin (MAK560D) as seen using light microscopy.

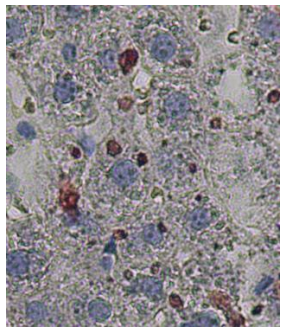
Figure 2:



Undifferentiated cells

3T3-L1 (cells in pre adipocyte state) were cultured Cells and were fixed with fixation solution (MAK560B). Cells were stained with oil red O solution (MAK560C) and hematoxylin (MAK560D) as seen using light microscopy.

Figure 3:



Histology slides of mouse liver X400 magnification 33ms exposure,
Brightfield, Blue- Hematoxylin, Red- Oil Red O

References

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