Rev. 1

Lipid Accumulation/ Steatosis Colorimetric Assay Kit (SCA)

Catalog #8758
500 Tests in 96-well plate

Product Description

Hepatic steatosis is characterized by the excessive accumulation of fat, primarily in hepatocytes, leading to the formation of lipid droplets in the cytoplasm and are visible under a microscope. The ScienCell Steatosis Colorimetric Assay Kit (SCA) provides a convenient solution for evaluating the potential risk of steatosis associated with drug candidates. It utilizes Oil Red O to selectively stain neutral lipids within cells. Lipid accumulation can be quantified using a plate reader after extracting the dye from the lipid droplets. The kit includes oleic acid as a positive control, a commonly used substance in drug screening for steatosis. Additionally, this kit can be used to quantify lipid droplet formation in other cell types, such as adipocytes.

Kit Components

Cat. #	Product Name	Quantity	Storage
8758a	Oil Red O	50 ml	Room Temperature
8758b	Fixative	50 ml	Room Temperature
8758c	Dye Extraction Solution	50 mL	Room Temperature
8758d	Oleic Acid (200 mM)	200 µL	-20ºC

Materials Supplied by User

Whatman Paper
Funnel
Deionized H_2O (diH_2O)
Dulbecco's Phosphate Buffered Saline (DPBS) - Cat. #0303
0.2 µm syringe-driven filter unit – optional

Quality Control

Oil Red O is tested to ensure it effectively stains cellular lipid droplets, inducing a distinct red coloration (See figure 1).

Product Use

This kit is applicable to cell culture samples. SCA is for research use only and is not approved for human or animal use, or for application in *in vitro* diagnostic procedures.

Shipping

8758a, 8758b and 8758c are shipped at room temperature. 8758d is shipped on gel ice. Upon receipt 8758d must be stored at -20.

Procedure

A. Preparation of Working Solution

1. Dilute Oil Red O solution 3:2 using deionized H_2O to make Oil Red O working solution.

   Example: 3mL Oil Red O + 2mL deionized H_2O

2. Place a piece of Whatman paper inside the funnel and filter the Oil Red O working solution. Alternatively, a syringe filter unit can be used in place of a Whatman paper/funnel system to filter the Oil Red O working solution.
3. The working solution must be used within 24 hours post filtration.

B. Plate preparation

1. Plate the optimal number of cells in a 12-, 24-, or 96-well plate. A typical experimental plate should include wells without cells and wells with cells treated with experimental compounds or vehicle. We recommend performing each treatment in triplicate.
2. Incubate cells overnight at 37°C in the cell culture incubator.

C. Treatment of cells

1. After overnight incubation, treat the cells with either the vehicle or experimental compounds for the specified duration in your experimental protocol. The kit includes oleic acid (200 mM) as a positive control, recommended to be applied to the cells at a final concentration of 200 µM. Seventy-two hours after treatment, a significant increase in lipid droplet accumulation is expected in hepatocytes.

   Note: Oleic acid is provided in an ethanol solution. To remove the solvent, evaporate the ethanol and promptly introduce the solvent of your preference, or alternatively, use aqueous buffers. Oleic acid exhibits a solubility of 100 mg/ml in DMSO and approximately 100 µg/ml in PBS. Before conducting biological experiments, it is advisable to further dilute the stock solution in aqueous buffers and isotonic saline. Storing the aqueous solution for more than one day is not recommended.

2. Assess the impact of experimental compounds on steatosis using the lipid droplet staining procedure outlined below.

D. Cell or Tissue Fixation and Staining

1. Wash cells or tissue sections once in 1X DPBS. Volume varies depending on sample vessel. Enough solution should be used to completely cover the sample.
2. Fix cells or tissue sections using the provided fixative solution (Cat. #8758b) at room temperature for 15 minutes. Fixation time should be empirically determined for individual user samples.
3. Remove fixative and wash sample 3X with diH_2O.
4. Remove diH_2O and pipette Oil Red O working solution.
5. Incubate for 15 minutes at room temperature.
6. Remove Oil Red O working solution and wash 5X with diH_2O or until the diH_2O is clear.
7. Samples are now ready for imaging under microscope and lipid droplets will appear red (Figure 1).

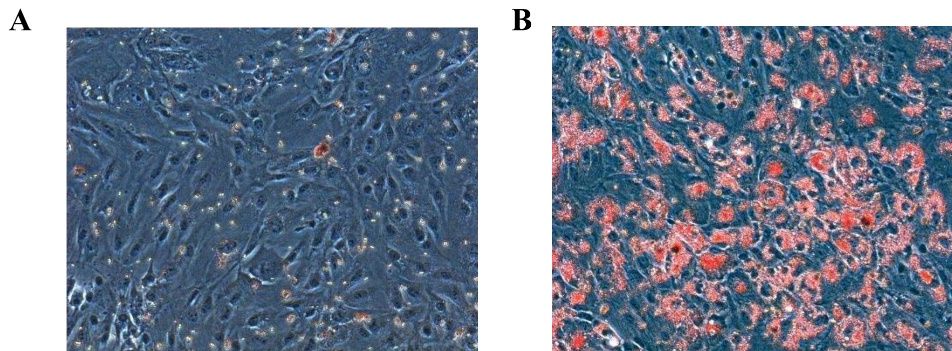

Figure 1*- Hepatocytes were initially seeded at a density of 25000 cells/cm^2 and incubated overnight at 37°C. Subsequently, the cells were either left untreated (A) or subjected to treatment with 200 µM oleic acid (B). The treated cells exhibited a significant accumulation of lipid droplets, as evidenced by the abundant presence of red dots observed through Oil Red O staining(B).*

E. Quantification of Lipid Accumulation

1. If using 96-well plate, add 100 µL of Dye Extraction Solution to each well including wells without cells, serving as the blank. Incubate for 15-30 minutes on an orbital shaker.
2. Read the absorbance at 510 nm using a 96-well plate reader.

   Note: If using a 12-well plate, add 250 µL of extraction solution to each well, incubate for 15-30 minutes on an orbital shaker. Transfer 100 µl of eluate from each well and create a duplicate by filling another set of wells on the plate a 96-well plate. Read the absorbance at 510 nm.
3. Deduct the absorbance of the wells from the blank and plot the results (Figure 2)

Note: Your results may vary based on the number of cells plated and your experimental design.

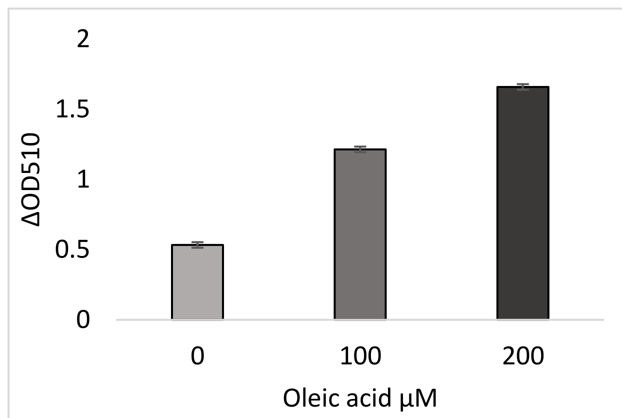

Figure 2*- Hepatocytes were initially seeded at a density of 25000 cells/cm^2 and incubated overnight at 37°C. Then, cells were either left untreated (A) or subjected to treatment with different oleic acid doses for 72 hours. Next, cells were stained with Oil Red O following the protocol above, and after extracting the dye from the lipid droplets lipid accumulation was assessed by measuring absorbance at 510 nm.*

Caution: Improper handling of certain components in this product may pose a health hazard. Take appropriate precautions, including wearing protective clothing and eyewear. Dispose of properly. See SDS for further information.