ab133131 – Hepatic Lipid Accumulation/ Steatosis Assay Kit

Instructions for Use

For evaluating steatosis risk of drug candidates using Oil Red O to stain neutral lipids in hepatocytes.

View kit datasheet: www.abcam.com/ab133131
(use www.abcam.cn/ab133131 for China, or www.abcam.co.jp/ab133131 for Japan)

This product is for research use only and is not intended for diagnostic use.

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1. Overview

ab133131 provides a convenient tool for evaluating steatosis risk of drug candidates using Oil Red O to stain neutral lipids in hepatocytes. Lipid accumulation can be quantified using a plate reader after the dye is extracted from the lipid droplets. Chloroquine is included in the kit as a positive control for screening pharmaceutical candidates that induce steatosis in hepatocytes.

2. Background

Steatosis, also known as fatty liver, is a pathological process characterized by abnormal accumulation of lipid within cells. There are two distinct patterns of steatosis: macrovesicular and microvesicular. The former is frequently seen in alcohol-induced liver injury, as a complication of metabolic syndrome such as obesity and type II diabetes, and is a marker of the hepatotoxic side effect of certain drugs. Microvesicular steatosis is more commonly related to mitochondrial dysfunction and defects in b-oxidation responsible for fatty liver seen in pregnancy and Reye's syndrome. While simple steatosis may not be associated with significant impairment of liver function, extensive fat accumulation can lead to cirrhosis and even liver failure. Studies on alcohol-induced steatosis revealed a set of transcription factors which are thought to be involved in the process, including SREBP1, PPARα, and Erg-1. The mechanism of non-alcoholic steatosis formation is poorly understood and little

information is available on the pathway(s) responsible for progressive hepatocellular damage following lipid accumulation.

Determining the hepatotoxicity of drug candidates is an essential component of the pharmaceutical discovery process. Steatosis is one of the parameters that is evaluated when determining the hepatoxicity of a drug candidate. *In vitro* liver models, such as the HepG2 cell line, are available for mechanism-based testing of the hepatotoxic effects of drug candidates. Chloroquine is a well known cationic amphiphilic drug that is known to induce steatosis which is often used as a positive control in drug screening for steatosis.

3. Components and Storage

Item	Quantity	Storage
Fixative (10X)	1 x 10 ml	RT
Lipid Droplets Assay Wash Solution	6 x 30 ml	RT
Lipid Droplets Assay Oil Red O Solution	1 x 25 ml	4°C
Lipid Droplets Assay Dye Extraction Solution	1 x 30 ml	RT
Chloroquine Positive Control (25 mM)	1 x 50 µl	4°C
Steatosis Assay Hematoxylin	1 x 30 ml	RT

Materials Needed But Not Supplied

- HepG2 cell line. Other hepatocytes can also be used.
- Adjustable pipettes and a repeat pipettor.
- A 12-, 24-, or 96-well plate for culturing cells.
- A spectrophotometer or 96-well plate reader capable of absorbance measurements between 490 and 520 nm.

4. Pre-Assay Preparation

A. Material Preparation

Fixative (10X)

Prepare a working solution of fixative by diluting the stock solution 1:10 in PBS.

Oil Red O Solution

Prepare a working solution of Oil Red O Solution by diluting the stock solution to 60% in water; that is, mix six parts of stock solution with four parts of distilled water. Filter this solution through a 0.25-0.45 µm syringe filter before use.

5. Assay Protocol

A. Plate Configuration

There is no specific pattern for using the wells on the plate. A 12-, 24-, or 96-well plate can be used. A typical experimental plate will include wells without cells and wells with cells treated with experimental compounds or vehicle. We recommend that each treatment is performed in triplicate.

B. Treatment of Cells

The following protocol is designed for a 96-well plate. For other plate sizes, the volume of medium/solution applied to each well should be adjusted accordingly.

1. Seed a 96-well plate with 10^4 cells/well. Grow cells overnight.

2. The following day, treat cells with experimental compounds or vehicle for 72 hours, or for the period of time used in your typical experimental protocol. Chloroquine, a compound known to induce steatosis, is included in the kit as a positive control. The recommended concentration is 25 µM. A significant induction of lipid droplet accumulation should be observed after 72 hours of treatment at this concentration.

3. Terminate the experiment and examine the effect of experimental compounds on steatosis using the lipid droplet staining procedure described below.

C. Lipid Droplet Staining and Quantification

Note: Perform all steps at room temperature

1. Remove most of the medium from the wells.

2. Add 75 µl of Fixative (prepared on page 4) to each well and incubate for 15 minutes.

3. Wash wells with 100 µl of Wash Solution two times for five minutes each.

4. Let the wells dry completely (placing the plate in a tissue culture hood with the blower turned on will help speed the drying).

5. Add 75 µl of Oil Red O Working Solution (prepared on page 5) to all wells, including the background wells containing no cells, and incubate for 20 minutes.

6. Remove the Oil Red O Solution and wash cells with distilled water several times until the water contains no visible pink color.

7. Wash wells with 100 µl of Wash Solution two times for five minutes each. At this point, microscope images can be taken to visualize pink to red oil droplet staining in cells treated with different compounds.

8. Let the wells dry completely (placing the plate in a tissue culture hood with the blower turned on will help speed the drying). Choose one of the following two options below for analysis of the stained cells.

   Quantification of lipid accumulation: If you want to quantify the accumulation of lipid in your cells, add 100 µl of Dye Extraction Solution to each well. Gently mix for 15-30 min and read the absorbance at 490-520 nm with a 96-well

plate reader. (If using a 12-well plate, add 250 µl of extraction solution to each well, and shake gently on an orbital shaker for 15-30 min, then transfer the extract to a 96-well plate and read OD at 490-520 nm).

OR

Intracellular distribution of lipid droplets: If you prefer to examine the intracellular distribution of lipid droplets, add 50 µl of Steatosis Assay Hematoxylin to each well and immediately wash the cells with tap water. Allow the nuclear staining to develop under tap water for ten minutes. Examine the cells using a light microscope.

6. Data Analysis

A. Quantification of Lipid Accumulation

The following figure shows typical results of lipid accumulation in liver cells following treatment with chloroquine. Your results may vary based on the number of cells plated and your experimental design.

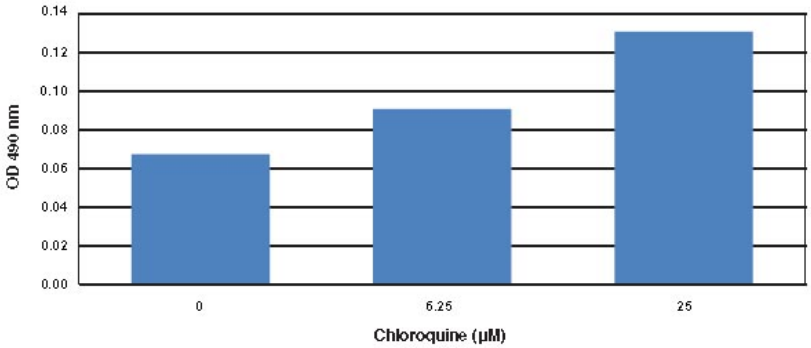

Figure 1. HepG2 cells were seeded at a density of 10^4 cells/well in a 96-well plate and grown in a 37°C incubator overnight. The next day, cells were treated with vehicle or different doses of chloroquine for three days. At the end of this incubation, cells were stained with Oil Red O according to the protocol described above. Lipid accumulation was assessed by extracting the Oil Red O and measuring the absorbance at 490 nm.

B. Intracellular Distribution of Lipid Droplets

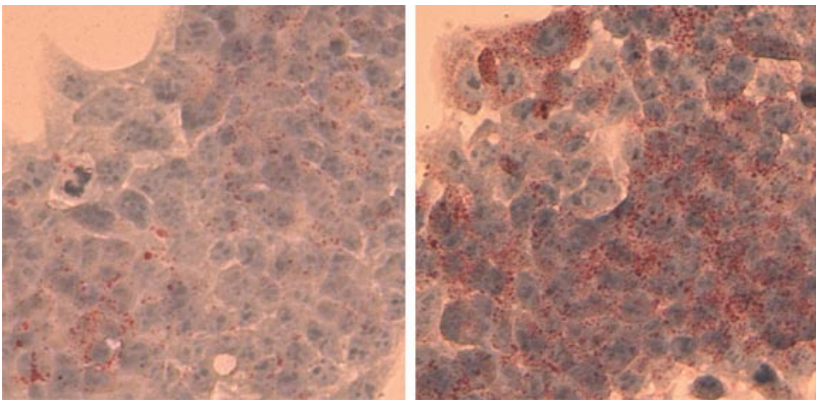

Figure 2. HepG2 cells were seeded at a density of 10^4 cells/well in a 96-well plate and grown in a 37°C incubator overnight. The next day, cells were treated with vehicle or different doses of chloroquine for three days. *Left* p*anel*: HepG2 cells treated with vehicle. There are a few lipid droplets in the cells, appearing as red dots. *Right* p*anel*: HepG2 cells treated with 25 μM chloroquine show significant accumulation of lipid droplets, evident by abundant appearance of red dots visualized by Oil Red O staining.

7. Troubleshooting

Problem	Possible Causes	Recommended Solutions
Cells treated with chloroquine or induction compounds do not form lipid droplets	Cells may not be healthy	Use only healthy cells
High background staining in untreated cells	A. Inadequate washing B. Overgrowth of cells C. Oil Red O Solution contains precipitate	A. Perform washes with Wash Solution until it contains no more pink color B. Make sure that cells are not overgrown C. Filter Oil Red O solution before staining cells

UK, EU and ROW
Email: technical@abcam.com
Tel: +44 (0)1223 696000
www.abcam.com

US, Canada and Latin America
Email: us.technical@abcam.com
Tel: 888-77-ABCAM (22226)
www.abcam.com

China and Asia Pacific
Email: hk.technical@abcam.com
Tel: 400 921 0189 / +86 21 2070 0500
www.abcam.cn

Japan
Email: technical@abcam.co.jp
Tel: +81-(0)3-6231-0940
www.abcam.co.jp