ab133102 – Adipogenesis Assay Kit (Cell-Based)

Instructions for Use

For the study of induction and inhibition of adipogenesis in adherent cells.

View kit datasheet: www.abcam.com/ab133102
(use www.abcam.cn/ab133102 for China, or
www.abcam.co.jp/ab133102 for Japan)

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

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1. Overview

ab133102 Adipogenesis Assay Kit provides the reagents required for studying the induction and inhibition of adipogenesis in the established 3T3-L1 model using the adipogenesis induction procedure. This kit can also be used to screen drug candidates involved in adipogenesis. The classic Oil Red O staining for lipid droplets is used in this kit as an indicator of the degree of adipogenesis and can be quantified with a plate reader after the dye is conveniently extracted from the lipid droplet.

2. Background

Obesity is a growing concern worldwide and has reached epidemic proportions in the United States. It is a risk factor in many major chronic diseases that afflict our society, including cardiovascular disease, diabetes mellitus, and cancer. In recent years, numerous studies have focused on identifying the mechanism of development of obesity, which is a process of either increasing the number of fat cells (fat cell hyperplasia) or enlargement of fat cells with each cell carrying greater amounts of fat (fat cell hypertrophy), or both. The ability to regulate the cell cycle and differentiation of adipocytes are key in the development and physiology of obesity and also in the origin of cancer. Understanding of these processes is critical to a

rational approach to the treatment of obesity and cancer.

Adipose tissue plays an important role in energy homeostasis. Mammals have both white adipose tissue (WAT) which stores excess energy as triglyceride in lipid droplets, and brown adipose tissue (BAT) which utilizes lipids to generate heat in a process known as thermogenesis. Adipocytes are derived from multipotent mesenchymal precursor cells that commit to preadipocytes and then either remain dormant or proceed to become differentiated adipocytes. Two model systems that are frequently used to study the differentiation process are: adipocytes isolated from stromal-vascular cells, and adipocytes differentiated from immortalized preadipocyte cell lines.

3T3-L1 cells are a well-characterized cell line often used to study the differentiation of adipocytes. 3T3-L1 cells have been used extensively to investigate insulin-induced glucose uptake and mechanisms of obesity development. This model system has greatly advanced the understanding of the molecular basis and signaling pathways of adipogenesis. During terminal differentiation, the fibroblast-like preadipocytes undergo a series of morphological and biochemical changes to eventually accumulate lipid droplets. These in vitro differentiated adipocytes share similar morphology with adipocytes in vivo. Extensive studies using this model of adipogenesis have revealed many factors including the C/EBP family of transcription factors and the nuclear receptor PPAR gamma that regulate adipogenesis. The increase in C/EBP and PPAR gamma

expression cooperatively drives the expression of genes that are necessary for the generation and maintenance of the adipogenic phenotype. These genes include fatty acid binding protein 4 (aP2), phosphoenolpyruvate carboxykinase (PEPCK), 11ß-HSD1, fatty acid synthase (FAS), acyl CoA carboxylase, Glut4, and the insulin receptor. This list is expected to expand as new discoveries arise from studies using this model.

3. Components and Storage

Item	Quantity	Storage
Cell-Based Assay IBMX Solution (1,000X)	1 x 500 µl	-20°C
Adipogenesis Assay Insulin Solution (1,000X)	1 x 1.5 ml	-20°C
Adipogenesis Assay Dexamethasone Solution (1,000X)	1 x 500 µl	-20°C
Lipid Droplets Assay Fixative (10X)	1 x 10ml	RT
Lipid Droplets Assay Wash Solution	6 x 30ml	RT
Lipid Droplets Assay Oil Red O Solution	1 x 25ml	4°C
Lipid Droplets Assay Dye Extraction Solution	1 x 30ml	RT

Materials Needed But Not Supplied

- 3T3-L1 preadipocyte cell line (can be obtained from ATCC) and associated tissue culture media, Dulbecco's Modified Eagles Medium (DMEM).

- Calf Serum.

- Fetal Bovine Serum.

- Phosphate buffered saline, pH 7.2 (PBS).

- Adjustable pipettes and a repeat pipettor.

- A 12-, 24-, or 96-well plate for culturing cells and induction of adipogenesis.

- A spectrophotometer or 96-well plate reader capable of absorbance measurements at 490 nm and 540 nm.

4. Assay Protocol

A. 3T3-L1 Preadipocite Cell Differentiation

Materials needed:

1. 1,000X Isobutylmethylxanthine Solution in DMSO

2. 1,000X Insulin Solution

3. 1,000X Dexamethasone Solution in DMSO

Plate Configuration

There is no specific pattern for using the wells on the plate. A 12-, 24-, or a 96-well plate can be used. A typical experimental plate will include wells without cells, wells with cells treated with or without differentiation induction media, and wells with cells treated with differentiation induction media together with any compound to be tested for its role in adipogenesis. We recommend that each treatment is performed in triplicate.

Media Preparation

1. **Induction Medium** For a 96-well plate, prepare induction medium by adding 10 μl of the following to 10 ml of DMEM containing 10% FBS:

   1,000X Cell-Based Assay IBMX Solution

   1,000X Adipogenesis Assay Insulin Solution

   1,000X Dexamethasone Solution in DMSO

   For a 96-well plate, prepare insulin medium by adding 10 μl of 1,000X Adipogenesis Assay Insulin Solution to 10 ml of DMEM containing 10% FBS.

2. **Insulin Medium** For a 96-well plate, prepare insulin medium by adding 10 μl of 1,000X Adipogenesis Assay Insulin Solution to 10 ml of DMEM containing 10% FBS.

Differentiation Procedure

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

1. Seed a 96-well plate with 3 x 104 cells/well.

2. Grow preadipocytes to confluency in DMEM containing 10% calf serum.

3. Two days post confluence (Day 0), change the medium to either induction medium (treatment group) or fresh DMEM containing 10% calf serum (control group).

4. Three days after the induction (Day 3) change to insulin medium (treatment group) or fresh DMEM containing 10% calf serum (control group).

5. Five days after induction (Day 5) change the medium in the treatment group to fresh insulin medium and in the control group to fresh DMEM containing 10% calf serum.

6. Monitor visible accumulation of lipid droplets under a microscope two days later (Day 7). If more differentiation is desired, change medium to fresh insulin medium (treatment group) or DMEM containing 10% calf serum (control group) and monitor the degree of differentiation under a microscope for several more days. More than 80% of the cells are usually differentiated by Day 7.

B. **Lipid Droplet Staining and Quantification**

Materials needed

1. Lipid Droplets Assay Fixative (10X):

   Prepare a working solution by adding the whole vial to 90 ml of PBS, pH 7.2.

2. Lipid Droplets Assay Wash Solution.

3. Lipid Droplets Assay Oil Red O Working Solution:

    Prepare a working solution by diluting the stock solution to 60% in water; that is, 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.

4. Lipid Droplets Assay Dye Extraction Solution:

    The following protocol is designed for a 96-well plate. For other size plates, the volume of solution to add to each well should be adjusted accordingly. After dye extraction solution is added and the plate gently shaken on an orbital shaker, the extract can be transferred to a 96-well plate and absorbance measured using a plate reader or to glass tubes and read with a spectrophotometer.

Staining procedure (Perform all steps at room temperature):

1. Remove most of the medium from the wells.

2. Add 75 µl of diluted Lipid Droplets Assay Fixative 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 under a blowing hood will help speed the drying).

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

6. Remove all 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 droplets staining in differentiated cells.

8. Let the wells dry completely (placing the plate under a blowing hood will help speed the drying).

9. 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 extraction to a 96-well plate and read O.D. at 490-520 nm).

5. Data Analysis

A. Cell Staining

An example of typical staining of differentiated adipocytes obtained using this kit is shown in the figure below. Your results may vary based on the number of cells plated and the degree of differentaition that you obtain.
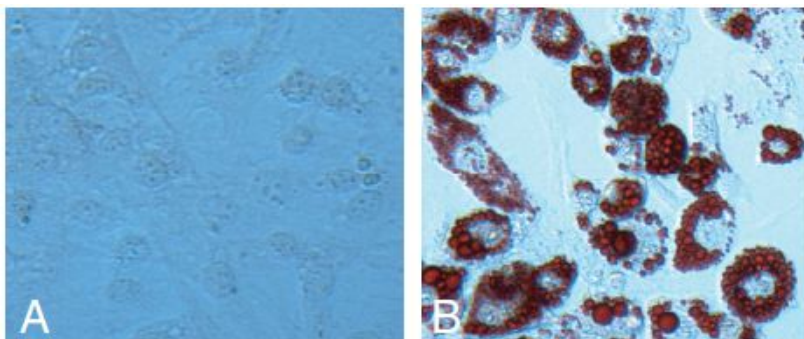

Figure 1. Panel A: Non-differentiated 3T3-L1 cells were not stained by Oil Red O Solution. Panel B: more than 80% of preadipocytes were differentiated four days after weaning the cells from induction medium to insulin medium. Lipid droplet accumulation in the differentiated cells can be visualized by Oil Red O Solution staining.

B. Absorbance Measurements

The absorbance reading at 492 nm, from a well of differentiated cells in a 96-well plate, is typically around 0.2-0.4 and in a 12-well plate the absorbance is around 0.7-1.0; the absorbance is usually around 0.05 in a well of control, non-differentiated cells.

6. Troubleshooting

Problem	Possible Causes	Recommended Solutions

Cells treated with induction medium do not form lipid droplets	Cells are from a late passage and may have lost the capacity to differentiate	Use cells at a low passage number
High background of staining in untreated cells	A. Inadequate washes. B. Differentiation of un-induced 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 grown to confluence to increase the staining difference between treated and control C. Filter Oil Red O before staining

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