

# **Adipogenesis Assay Kit**

Item No. 10006908

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## GENERAL INFORMATION

### Materials Supplied

Kit will arrive packaged as a -20°C kit. For best results, remove components and store as stated below.

Item Number	Item	100 Tests Quantity/Size	Storage
10008978	Cell-Based Assay IBMX Solution (1,000X)	1 vial/500 µl	-20°C
10008979	Adipogenesis Assay Insulin Solution (1,000X)	1 vial/1.5 ml	-20°C
10008980	Adipogenesis Assay Dexamethasone Solution (1,000X)	1 vial/500 µl	-20°C
10008981	Lipid Droplets Assay Fixative (10X)	1 vial/10 ml	Room Temperature
10008982	Lipid Droplets Assay Wash Solution	2 vials/250 ml	Room Temperature
10008983	Lipid Droplets Assay Oil Red O Solution	1 vial/60 ml	4°C
10008984	Lipid Droplets Assay Dye Extraction Solution	1 vial/50 ml	Room Temperature

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 975-3999. We cannot accept any returns without prior authorization.



**WARNING:** This product is for laboratory research use only: not for administration to humans. Not for human or veterinary diagnostic or therapeutic use.

## Precautions

Please read these instructions carefully before beginning this assay.

For research use only. Not for human or diagnostic use.

## If You Have Problems

### Technical Service Contact Information

**Phone:** 888-526-5351 (USA and Canada only) or 734-975-3888

**Fax:** 734-971-3641

**Email:** techserv@caymanchem.com

**Hours:** M-F 8:00 AM to 5:30 PM EST

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

## Storage and Stability

This kit will perform as specified if stored as directed and used before the expiration date indicated on the outside of the box.

## Materials Needed But Not Supplied

1. 3T3-L1 preadipocyte cell line (can be obtained from ATCC) and associated tissue culture media, Dulbecco's Modified Eagles Medium (DMEM; GibcoBRL-Item No. 11965-084)
2. Calf Serum (GibcoBRL-Item No. 16170-078)
3. Fetal Bovine Serum (FBS) (GibcoBRL-Item No. 10437-028)
4. Phosphate buffered saline, pH 7.2 (PBS)
5. Adjustable pipettes and a repeat pipettor
6. A 12-, 24-, or 96-well plate for culturing cells and induction of adipogenesis
7. A spectrophotometer or 96-well plate reader capable of absorbance measurements at 490 nm and 540 nm

## INTRODUCTION

### Background

Obesity is a growing concern worldwide and has reached epidemic proportions in the United States.<sup>1</sup> 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.<sup>1,2</sup> 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.<sup>3</sup>

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.<sup>3</sup> 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.<sup>1,4,5</sup> These genes include fatty acid binding protein 4 (aP2), phosphoenolpyruvate carboxykinase (PEPCK), 11 $\beta$ -HSD1, fatty acid synthase (FAS), acyl CoA carboxylase, Glut4, and the insulin receptor.<sup>4,6,7</sup> This list is expected to expand as new discoveries arise from studies using this model.

## About This Assay

Cayman's 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.

## ASSAY PROTOCOL

### 3T3-L1 Preadipocyte Cell Differentiation

#### Materials

- 1,000X Isobutylmethylxanthine Solution in DMSO (IBMX, Item No. 10008978).
- 1,000X Insulin Solution (Item No. 10008979).
- 1,000X Dexamethasone Solution in DMSO (Item No. 10008980).

#### 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. We suggest you record the contents of each well on the template sheet provided (see page 16).

#### Media Preparation

##### 1. Induction Medium

For a 96-well plate, prepare induction medium by adding 10  $\mu$ l of the following to 10 ml of DMEM containing 10% FBS:

1,000X Cell-Based Assay IBMX Solution (Item No. 10008978)

1,000X Adipogenesis Assay Insulin Solution (Item No. 10008979)

1,000X Adipogenesis Assay Dexamethasone Solution (Item No. 10008980)

##### 2. Insulin Medium

For a 96-well plate, prepare insulin medium by adding 10  $\mu$ l of 1,000X Adipogenesis Assay Insulin Solution (Item No. 10008979) 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  $3 \times 10^4$  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.

## Lipid Droplet Staining and Quantification

### Materials Needed

1. **Lipid Droplets Assay Fixative (10X) - (Item No. 10008981)**  
Prepare a working solution by adding the whole vial to 90 ml of PBS, pH 7.2.
2. **Lipid Droplets Assay Wash Solution (Item No. 10008982)**
3. **Lipid Droplets Assay Oil Red O Working Solution (Item No. 10008983, stock)**  
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  $\mu\text{m}$  syringe filter before use.
4. **Lipid Droplets Assay Dye Extraction Solution (Item No. 10008984)**  
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.

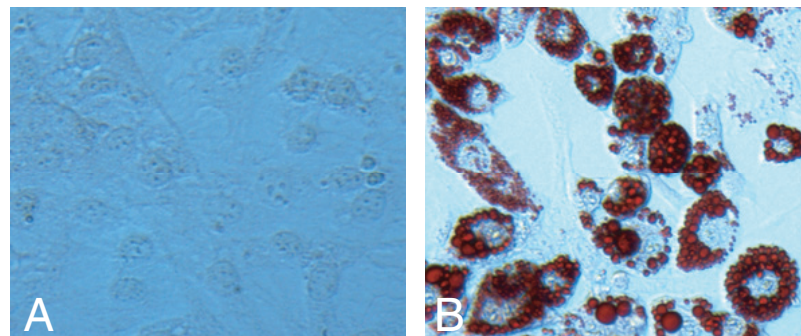
## Staining procedure (Perform all steps at room temperature):

1. Remove most of the medium from the wells.
2. Add 75  $\mu$ l of diluted Lipid Droplets Assay Fixative (see page 9) to each well and incubate for 15 minutes.
3. Wash wells with 100  $\mu$ l 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  $\mu$ l 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  $\mu$ l 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  $\mu$ l 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  $\mu$ l 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).

## PERFORMANCE CHARACTERISTICS

### 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 differentiation 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.

### 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.

## RESOURCES

### 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	<p>A. Inadequate washes.</p> <p>B. Differentiation of un-induced cells</p> <p>C. Oil Red O Solution contains precipitate</p>	<p>A. Perform washes with Wash Solution until it contains no more pink color</p> <p>B. Make sure that cells are grown to confluence to increase the staining difference between treated and control</p> <p>C. Filter Oil Red O before staining</p>

### References

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- Thompson, G.M., Trainor, D., Biswas, C., *et al.* A high-capacity assay for PPAR $\gamma$  ligand regulation of endogenous aP2 expression in 3T3-L1 cells. *Anal. Biochem.* **330**, 21-28 (2004).

### Related Products

Adipolysis Assay Kit - Item No. 10009381  
 Adiponectin (human) EIA Kit - Item No. 500641  
 Adiponectin (human) EIA Kit (HS) - Item No. 10007619  
 Adiponectin (mouse) EIA Kit - Item No. 10007620  
 Cholesterol Cell-Based Detection Assay Kit - Item No. 10009779  
 ChREBP Cell-Based Translocation Assay Kit - Item No. 10010060  
 Coenzyme A Assay Kit - Item No. 700440  
 Ghrelin (human acylated) EIA Kit - Item No. 10006306  
 Ghrelin (human unacylated) EIA Kit - Item No. 10008952  
 Ghrelin (rat acylated) EIA Kit - Item No. 10006307  
 Ghrelin (rat unacylated) EIA Kit - Item No. 10008953  
 Glucose Assay Kit - Item No. 10009582  
 JC-1 Mitochondrial Membrane Potential Assay Kit - Item No. 10009172  
 Leptin Receptor (human) EIA Kit - Item No. 10007608  
 Leptin (mouse/rat) EIA Kit - Item No. 10007609  
 Phagocytosis Assay Kit (IgG FITC) - Item No. 500290  
 Progranulin (human) EIA Kit - Item No. 500940  
 Progranulin (mouse) EIA Kit - Item No. 500950  
 Resistin (human) EIA Kit - Item No. 10007610  
 SREBP-2 Cell-Based Translocation Assay Kit - Item No. 10009239  
 Thyroid-Stimulating Hormone EIA Kit - Item No. 500740

## Warranty and Limitation of Remedy

Cayman Chemical Company makes **no warranty or guarantee** of any kind, whether written or oral, expressed or implied, including without limitation, any warranty of fitness for a particular purpose, suitability and merchantability, which extends beyond the description of the chemicals hereof. Cayman **warrants only** to the original customer that the material will meet our specifications at the time of delivery. Cayman will carry out its delivery obligations with due care and skill. Thus, in no event will Cayman have **any obligation or liability**, whether in tort (including negligence) or in contract, for any direct, indirect, incidental or consequential damages, even if Cayman is informed about their possible existence. This limitation of liability does not apply in the case of intentional acts or negligence of Cayman, its directors or its employees.

Buyer's **exclusive remedy** and Cayman's sole liability hereunder shall be limited to a refund of the purchase price, or at Cayman's option, the replacement, at no cost to Buyer, of all material that does not meet our specifications.

Said refund or replacement is conditioned on Buyer giving written notice to Cayman within thirty (30) days after arrival of the material at its destination. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver by Buyer of all claims hereunder with respect to said material.

For further details, please refer to our Warranty and Limitation of Remedy located on our website and in our catalog.

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## NOTES

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