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	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	RT
600044	Lipid Droplets Assay Wash Solution	6 vials/30 ml	RT
600045	Lipid Droplets Assay Oil Red O Solution	1 vial/25 ml	4°C
600046	Lipid Droplets Assay Dye Extraction Solution	1 vial/30 ml	RT

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

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. Low passage (<P10) 3T3-L1 preadipocyte cells (can be obtained from ATCC)
2. Medium: DMEM + 10% Fetal Bovine Serum (FBS)
3. Phosphate buffered saline, pH 7.2 (PBS)
4. 96-well plate for culturing cells and induction of adipogenesis
5. 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.[1] 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.[1,2] 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.[3]

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 PPARγ that regulate adipogenesis.[3] The increase in C/EBP and PPARγ expression cooperatively drives the expression of genes that are necessary for the generation and maintenance of the adipogenic phenotype. [1,4,5] These genes include fatty acid binding protein 4 (aP2), phosphoenolpyruvate carboxykinase (PEPCK), 11β-HSD1, fatty acid synthase (FAS), acyl CoA carboxylase, Glut4, and the insulin receptor.[4,6,7] 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

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 (Item No. 10008978)

   1,000X 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 µl of 1,000X 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 x 10^4 cells/well.
2. Grow preadipocytes to confluency in medium (DMEM containing 10% FBS).
3. Two days post confluence (Day 0), change the medium to either induction medium (treatment group) or fresh medium alone (control group).
4. Three days after the induction (Day 3) change to insulin medium (treatment group) or fresh medium alone (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 medium alone.
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 medium alone (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. 600044)**

3. **Lipid Droplets Assay Oil Red O Working Solution (Item No. 600045, 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 µm syringe filter before use.

4. **Lipid Droplets Assay Dye Extraction Solution (Item No. 600046)**

   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 (see page 10) 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.

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 differentaition that you obtain.

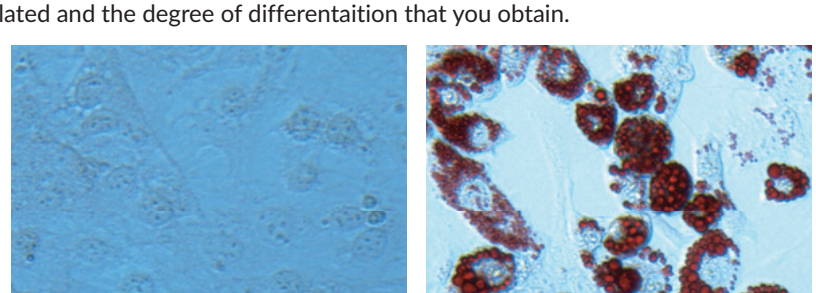

Figure 1: *Panel A:* non-differentiated 3T3-L1 cells were not stained by Oil Red O Solution. *Panel B:* more than 80% of preadipocytes were differentiated four days after weaning the cells from induction medium to insulin medium. Lipid droplet accumulation in the differentiated cells can be visualized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

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	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

References

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2. Spiegelman, B.M. and Flier, J.S. Adipogenesis and obesity: Rounding out the big picture. *Cell* **87**, 377-389 (1995).
3. Tong, Q. and Hotamisligil, G.S. Molecular mechanisms of adipocyte differentation. *Reviews in Endocrine & Metabolic Disorders* **2**, 349-355 (2001).
4. Prusty, D., Park, B.-H., Davis, K.E., *et al.* Activation of MEK/ERK signaling promotes adipogenesis by enhancing peroxisome proliferator-activated receptor γ (PPARγ) and C/EBPα gene expression during the differentiation of 3T3-L1 preadipocytes. *J. Biol. Chem.* **277(48)**, 46226-46232 (2002).
5. Vidal-Puig, A., Jimenez-Linan, M., Lowell, B.B., *et al.* Regulation of PPARγ gene expression by nutrition and obesity in rodents. *J. Clin. Invest.* **97**, 2553-2561 (1996).
6. Scherer, P.E., Lisanti, M.P., Baldini, G., *et al.* Induction of caveolin during adipogenesis and association of GLUT4 with caveolin-rich vesicles. *J. Cell Biol.* **127**, 1233-1243 (1994).
7. Thompson, G.M., Trainor, D., Biswas, C., *et al.* A high-capacity assay for PPARγ ligand regulation of endogenous aP2 expression in 3T3-L1 cells. *Anal. Biochem.* **330**, 21-28 (2004).

NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.