Adipolysis Assay Kit

Item No. 10009381

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GENERAL INFORMATION

Materials Supplied

Kit will arrive packaged as a -20°C kit.

Item Number	Item	100 Tests Quantity/Size	Storage
10009948	Adipolysis Assay IBMX Solution (1,000X)	1 vial/100 µl	-20°C
10008979	Insulin Solution (1,000X)	1 vial/1.5 ml	-20°C
10009950	Adipolysis Assay Dexamethasone Solution (1,000X)	1 vial/100 µl	-20°C
10009951	Adipolysis Assay Isoproterenol Solution	1 vial/250 µl	-20°C
10009952	Glycerol Standard Solution	1 vial/250 µl	-20°C
10009953	Free Glycerol Assay Reagent	2 vials	-20°C

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 number (P <10) 3T3-L1 preadipocyte cell line (can be obtained from ATCC)
2. DMEM with 10% fetal bovine serum (FBS)
3. Bovine serum albumin (fatty acid free, low endotoxin)
4. A 12-, 24-, or 96-well plate for induction of adipogenesis and a 96-well plate for the free glycerol assay
5. A 96-well plate reader capable of absorbance measurements at 540 nm

INTRODUCTION

About This Assay

Cayman's Adipolysis Assay Kit provides a complete assay for studying the lipolysis of triglycerides, from the differentiation of adipocyte-like cells to the quantitation of released glycerol. 3T3-L1 cells are differentiated using a common adipogenesis induction procedure to accumulate lipid droplets, and isoproterenol is included as a positive control for release of glycerol from triglycerides. Free glycerol in the cell culture medium is measured by incubation with glycerol kinase, glycerol phosphate oxidase, and horseradish peroxidase in the presence of a colorimetric substrate to generate a chromophore detectable at 540 nm. The amount of glycerol release is correlated with both the amount of stored triglyceride and the degree of lipolysis, making this kit useful for investigators screening compounds affecting either lipid storage or metabolism. The Adipolysis Assay Kit provides enough reagents to assay one 96-well plate of supernatants for glycerol release.

The accumulation of lipid droplets can be alternatively assayed with either the Lipid Droplets Fluorescence Assay Kit (Item No. 500001) or the Adipogenesis Assay Kit (Item No. 10006908), and glycerol release alone can be assayed using the Glycerol Cell-Based Assay Kit (Item No. 10011725).

3T3-L1 Preadipocyte Cell Differentiation

Media Preparation

1. **Induction Medium**

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

   Adipolysis Assay IBMX Solution (1,000X) (Item No. 10009948)

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

   Adipolysis Assay Dexamethasone Solution (1,000X) (Item No. 10009950)

2. **Insulin Medium**

   For a 96-well plate, prepare Insulin Medium by adding 10 µl of Insulin Solution (1,000X) (Item No. 10008979) to 10 ml of DMEM containing 10% FBS. Prepare enough for the duration of the experiment and store at 4°C for up to two weeks.

Induction Procedure

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

1. Seed a 96-well plate with 3 x 10^4 cells/well.
2. Grow preadipocytes to confluence in DMEM containing 10% FBS.
3. Two days post confluence (Day 0), change the medium to either Induction Medium (treatment group) or fresh DMEM containing 10% FBS (control group).
4. Three days after the induction (Day 3) change the medium to Insulin Medium (treatment group) or fresh DMEM containing 10% FBS (control group).
5. Five days after the induction (Day 5) change the medium to fresh Insulin Medium (treatment group) or DMEM containing 10% FBS (control group).
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% FBS (control group) and every three days monitor the degree of differentiation under a microscope for several more days. More than 80% of the cells are usually differentiated by Day 7. Cells may be maintained for up to two weeks after this point for the Adipolysis Assay.

Reagent Preparation

1. **Adipolysis Buffer**

   Dissolve 0.25 g BSA (fatty acid free, low endotoxin) in 50 ml DMEM. Filter through a 0.2 µm filter and store at 4°C.

2. **Free Glycerol Assay Reagent (Item No. 10009953)**

   Free Glycerol Assay Reagent is unstable. Store the unreconstituted Free Glycerol Assay Reagent at -20°C. We recommend that you prepare only the amount you need for your assay. Each vial is to be reconstituted with 5 ml of distilled water and is enough for half of a 96-well plate. The prepared Free Glycerol Assay Reagent should be stable for approximately two weeks stored at 4°C.

3. **Glycerol Standard Solution (Item No. 10009952)**

   The Glycerol Standard Solution is provided at a concentration of 125 µg/ml. To prepare the glycerol standard curve for this assay: obtain six clean test tubes and label them #1 through #6. Aliquot 100 µl of Adipolysis Buffer into tubes #2-#6. Transfer 200 µl of Glycerol Standard Solution (Item No. 10009952) into tube #1. Serially dilute the standard by removing 100 µl from tube #1 and placing it into tube #2; mix thoroughly. Next remove 100 µl from tube #2 and place it into tube #3; mix thoroughly. Repeat it for tubes #4 and #5. Do not add any standard to tube #6. This tube will be your blank.

ASSAY PROTOCOL

Plate Set Up

Each plate should contain a glycerol standard curve and wells containing supernatants from samples treated with and without test compounds. It is recommended that standards be run in duplicate and that each treatment be performed in triplicate.

Performing the Assay

1. Carefully aspirate the culture medium from each well. Replace with 100 µl/well pre-warmed Adipolysis Buffer (prepared above). Incubate at 37°C for four hours.
2. Aspirate the Adipolysis Buffer and replace with fresh, warm Adipolysis Buffer containing test compounds at desired concentrations. The Isoproterenol Solution (Item No. 10009951) may be used as a positive control by diluting the solution as provided 1:1,000 into Adipolysis Buffer to yield a 10 µM solution. Negative controls with buffer alone should also be included.
3. Incubate cells at 37°C for the desired time period. Isoproterenol begins to induce adipolysis within one hour and the amount of detectable free glycerol increases linearly afterwards. Users of the kit must determine the optimal incubation time period for each experimental compound.
4. Collect cell culture supernatants from each well into glycerol-free containers. Samples may be assayed immediately or stored at -20°C.
5. To perform the free glycerol assay, transfer 25 µl of the standards (tubes 1-6) prepared above into a new 96-well plate. We recommend that the standards be run in duplicate.
6. Transfer 25 µl of each supernatant collected in step 4 to the corresponding wells on the new plate. Depending on the experimental compounds being tested, samples may need to be diluted with Adipolysis Buffer prior to addition to the plate in order to fall within the range of the standard curve.
7. Add 100 µl of reconstituted Free Glycerol Assay Reagent per well and incubate for 15 minutes at room temperature.
8. Read absorbance at 540 nm.

ANALYSIS

Data Analysis

Preparation of the Data

Subtract the absorbance reading of the blank wells from all absorbance readings including the rest of the standard curve. This is the corrected absorbance value.

Plotting the Standard Curve

Plot the corrected absorbance as a function of glycerol concentration and determine the equation of the line. See Figure 1, for a typical standard curve.

Determination of Glycerol Concentration

$$\text{Glycerol concentration } (\mu g/ml) = \left[\frac{A_{540} - (\text{y-intercept})}{\text{Slope}} \right] \times \text{Sample dilution}$$

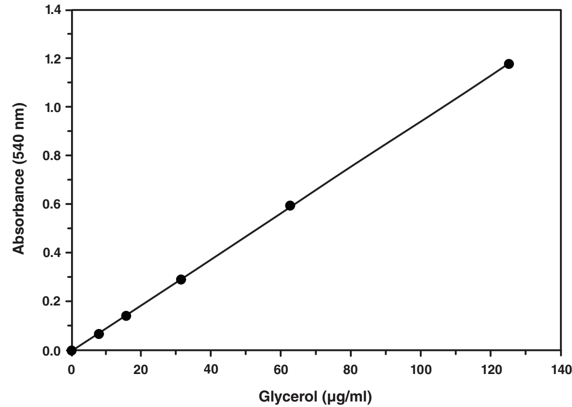

Figure 1. Glycerol standard curve

Performance Characteristics

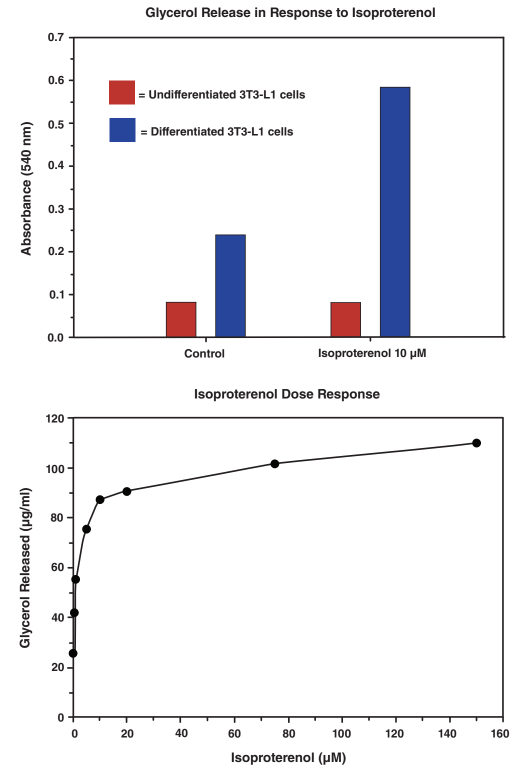

Figure 2. Differentiated 3T3-L1 cells release glycerol upon treatment with isoproterenol. *Top panel:* glycerol release from differentiated and undifferentiated 3T3-L1 cells. *Bottom panel:* isoproterenol dose response curve generated by plotting the isoproterenol concentration *versus* the glycerol released after 24 hours.

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 (P <10) passage number
The absorbance reading of the samples is higher than the range of the standard curve	The free glycerol concentrations of the samples are too high and therefore out of the detection range of the assay	Dilute the samples in culture medium so the concentrations fall within the range of the standard curve

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.

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