Monitoring Lipolysis Using the Glycerol-Glo™ Assay and GloMax® Discover System

Promega Corporation

Materials Required:

- Adipocyte cells (e.g., 3T3-L1 MBX fibroblasts that have been differentiated into adipocytes; refer to *Glucose Uptake-Glo™ Assay Technical Manual* #TM467 for differentiation protocol)
- Tissue culture equipment and reagents
- Phosphate-buffered saline (PBS)
- Isoproterenol (Sigma Cat.# I6504)
- Insulin (Sigma Cat.# I9278)
- Glycerol-Glo™ Assay Kit (Cat.# J3150)
- 96-well plate with opaque walls and clear or opaque bottom (e.g., Costar® Cat.# 3917)
- GloMax® Discover System (Cat.# GM3000)

Assay Media Composition:

- 98% RPMI (Gibco Cat.# 22400)
- 2% fatty acid-free BSA (Sigma Cat.# A7030)
- 5µM triacsin C (Sigma Cat.# T4540)

Caution: We recommend the use of gloves, lab coats and eye protection when working with these or any chemical reagents.

Protocols: *GloMax® Discover System Technical Manual* #TM397 and *Glycerol-Glo™ Assay Technical Manual* #TM599 are available at: **www.promega.com/protocols/**

Introduction

In adipose tissue, lipid metabolism is primarily regulated by hormone-mediated signaling cascades. The role of the hormone insulin is to dually inhibit lipolysis and promote lipogenesis. However, in metabolic disease states such as diabetes and obesity, these functions of insulin can be disrupted. Developing safe and effective treatments for metabolic diseases depends on assays that can measure lipolysis and lipogenesis reliably and accurately.

To study lipid metabolism and its regulation, researchers commonly measure the end products of lipolysis or lipogenesis pathways. In the case of lipolysis, a triacylglyceride molecule is metabolized into three free fatty acids and one glycerol molecule that are secreted from the cell. The amount of glycerol secreted into the media is measurable and is used as a proxy for lipolysis in cells.

The Glycerol-Glo™ Assay is well-suited for monitoring lipolysis via glycerol release in cultured adipocytes. The assay works by correlating glycerol molecules to light production (Figure 1). First, a coupled reaction catalyzes glycerol to generate NADH. Next, a reductase enzyme uses NADH to convert a proluciferin reductase substrate into luciferin. Finally, Ultra-Glo™ rLuciferase oxidizes luciferin to produce visible light. The amount of light produced can be measured using a luminometer such as the GloMax® Discover. In this way, the Glycerol-Glo™ Assay offers rapid and sensitive glycerol detection. It uses a simple add-and-read protocol and can be performed in 96- or 384-well plates, making it amenable to both low- and high-throughput adipocyte cell culture applications.

The GloMax® Discover plate reader makes data collection from the Glycerol-Glo™ Assay fast, accurate and easy with a pre-programmed instrument protocol. GloMax® plate readers provide excellent luminescence sensitivity and low well-to-well cross-talk to maximize data output. Visualization and analysis of the data is also simple, with all RLU values compiled in a table for computing statistics and plotting. Here, we present an example protocol to monitor glycerol release from adipocytes following drug treatment using the Glycerol-Glo™ Assay and the GloMax® Discover System.

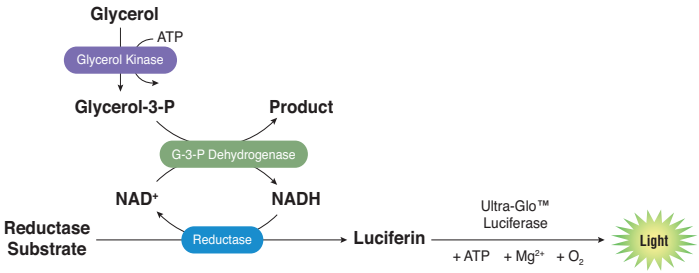

Figure 1. Schematic diagram of the Glycerol-Glo™ Assay principle. Glycerol kinase and glycerol-3-phosphate dehydrogenase are used to generate NADH. In the presence of NADH, reductase enzymatically reduces a proluciferin Reductase Substrate to luciferin. Luciferin is detected in a luciferase reaction using Ultra-Glo™ Luciferase and ATP, and the amount of light produced is proportional to the amount of glycerol in the sample.

Glycerol-Glo™ Assay Protocol

Note: For more detailed instructions, see the *Glycerol-Glo™ Assay Technical Manual* #TM599. The following protocol is written for 96-well format, but the assay is scalable and also performs robustly in 384-well format.

A. Treat Adipocytes and Collect Media Samples

1. Prepare adipocytes cells (e.g., 3T3-L1 MBX fibroblasts that have been differentiated into adipocytes) by removing media and washing cells twice with 100µl of PBS.
2. Prepare Assay Media (RPMI with 2% fatty acid-free BSA and 5µM triacsin C).

   Note: This media promotes extracellular glycerol accumulation. BSA binds fatty acids and triacsin C inhibits lipogenesis.
3. Dilute insulin and/or isoproterenol in Assay Media according to desired treatment concentrations.

   Note: These drugs affect the lipolysis pathway in adipocytes. Insulin inhibits lipolysis and isoproterenol promotes lipolysis.
4. Add 100µl Assay Media with insulin and/or isoproterenol to the adipocyte cells according to desired treatment configuration(s).
5. Incubate cells at 37°C.
6. Collect 25µl samples of media supernatant at desired timepoints during the incubation. Store samples at 4°C or –20°C until ready to assay.

B. Perform Glycerol-Glo™ Assay

1. Prepare Glycerol-Glo™ Assay Reagents and glycerol standards (0–80µM).
2. Arrange the media samples to be tested alongside the glycerol standards in an opaque-walled 96-well plate, with each having a final volume of 25µl.

   Note: The upper limit for linear detection in this assay is 80µM glycerol. Samples above this concentration must be diluted in Glycerol Lysis Solution prior to assay for accurate results.
3. Add 25µl of Glycerol Lysis Solution to each sample or standard. The final volume in each well should be 50µl. Shake briefly to mix.
4. Add 50µl Glycerol Detection Solution to each well. Shake gently for 30–60 seconds.
5. Incubate for 1 hour at room temperature.
6. Record luminescence of samples and standards using GloMax® Discover System.
7. Calculate glycerol concentration of media samples by comparing their luminescence signal to that of known glycerol standards.

Example Data

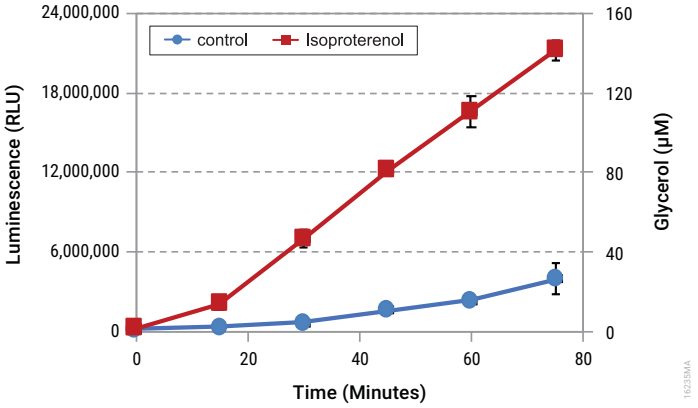

Figure 2. Glycerol release from adipocytes upon isoproterenol treatment. Adipocytes differentiated from 3T3-L1 MBX cells were treated with 10µM isoproterenol (red squares) or a control with no isoproterenol (blue circles). At the indicated timepoints, media samples were taken from each well. Samples were tested alongside glycerol standards using the Glycerol-Glo™ Assay. The experiment was performed in triplicate, and average luminescence was plotted with error bars representing standard deviation. Glycerol release indicates that lipolysis is occurring in the adipocytes.

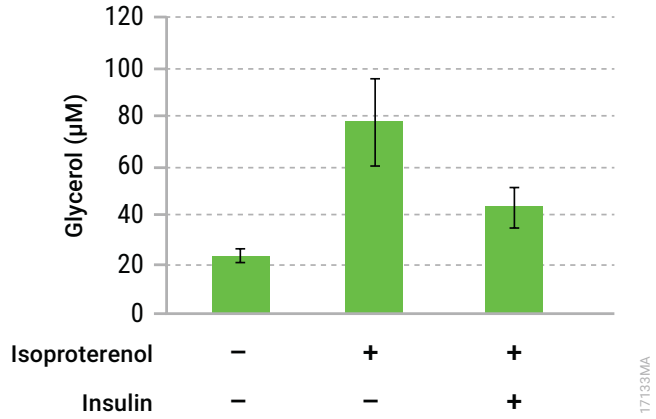

Figure 3. Insulin-mediated inhibition of glycerol release in adipocytes. Adipocytes differentiated from 3T3-L1 MBX cells were treated with combinations of isoproterenol (25nM) and insulin (150nM). After 90 minutes of treatment, a sample of the media was removed from each well. Samples and glycerol standards were assayed using the Glycerol-Glo™ Assay. Each condition was tested in triplicate, and the error bars represent standard deviation. Glycerol release from adipocytes is a proxy for lipolysis. Here, isoproterenol-stimulated lipolysis was inhibited twofold by insulin.

Conclusion

The Glycerol-Glo™ Assay provides excellent signal response for glycerol release in cell culture (Figures 2 and 3). The assay sensitivity and superior signal-to-background ratio make the Glycerol-Glo™ Assay a simple and effective multiwell plate assay for measuring lipolysis in cultured adipocytes.

The GloMax® Discover System

The GloMax® Discover System offers superior sensitivity and dynamic range and limited well-to-well cross talk. The instrument was developed and optimized with Promega cell and gene reporter assays and may be integrated into low- and medium-throughput automation workflows. The GloMax® Discover System allows flexible use of filters to measure fluorescence intensity, filtered luminescence, BRET, FRET and UV-visible absorbance for a wide variety of laboratory applications. The instrument is operated by an integrated Tablet PC, which provides quick and easy navigation through the control options. Exporting your results is made seamless with a variety of options, including exporting data to your local network.

Ordering Information

Product	Size	Cat.#
Glycerol-Glo™ Assay	5 ml	J3150
	50ml	J3151
GloMax® Discover System	1 each	GM3000