Glycerol Cell-Based Assay Kit

Item No. 10011725

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GENERAL INFORMATION

Materials Supplied

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

Item Number	Item	100 Tests Quantity	Storage
10009952	Glycerol Standard Solution	1 vial	-20°C
10009953	Free Glycerol Assay Reagent	2 vials	-20°C
10012670	Chloroquine Positive Control (25 mM)	1 vial	-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. HepG2 cell line (can be obtained from ATCC) and associated cell culture media (other cell lines such as 3T3-L1 pre-adipocytes can also be used)
2. Adjustable pipettes and a repeating pipettor
3. A 12-, 24-, or 96-well plate for culturing cells
4. A spectrophotometer or 96-well plate reader capable of measuring absorbance at 540 nm

INTRODUCTION

Background

In mammals, triglycerides are constantly synthesized from fatty acids and segregated into cytosolic lipid droplets, mainly in adipocytes, as the major energy storage depot. During fasting, triglycerides stored in adipose tissue and liver are hydrolyzed by hormone-sensitive lipase and adipose triglyceride lipase to produce free fatty acids and glycerol. Triglyceride/fatty acid cycling is important in metabolic regulation and heat production, and is highly regulated by enzymes such as phosphenolpyruvate carboxykinase (PEPCK) and lipases. Quantitative changes in the triglyceride/fatty acid cycle have been related to the increased metabolic rate of cachectic patients with esophageal cancer and to metabolic syndrome.[1,2] Abnormal triglyceride accumulation in the form of lipid droplets can occur in adipocytes and/or hepatocytes of obese mammals. *In vitro*, dramatic lipid accumulation can be observed in well-differentiated 3T3-L1 cells, or HepG2 cells treated with steatosis-inducing compounds such as chloroquine. Triglycerides stored in these lipid droplets can be hydrolyzed into free fatty acids and glycerol which are subsequently released into the surrounding environment. The amount of glycerol released into the medium is proportional to the triglyceride/fatty acid cycling rate.

About This Assay

Cayman's Glycerol Cell-Based Assay Kit provides a convenient tool for studying triglyceride/fatty acid cycling and its regulation in adipocytes or hepatocytes. This kit will allow investigators to screen compounds involved in lipid storage and metabolism. Chloroquine is included in the kit as a positive control for screening compounds that induce lipid droplet accumulation and free glycerol release from hepatocytes.

PRE-ASSAY PREPARATION

Treatment of Cells

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

1. Seed a 96-well plate with 10^4 cells/well. Grow cells overnight.
2. The following day, treat cells with experimental compounds or vehicle for 72 hours, or for the period of time used in your typical experimental protocol. Chloroquine, a compound known to induce lipid droplet accumulation, is included in the kit as a positive control. The recommended concentration is 25 µM. A measurable amount of free glycerol is released into the culture medium after 24-48 hours of treatment.
3. Terminate the experiment and examine the effect of experimental compounds on free glycerol release using the assay procedure described on page 8.

Reagent Preparation

1. **Free Glycerol Assay Reagent - (Item No. 10009953)**

   Reconstitute each vial of the Free Glycerol Assay Reagent with 5 ml of distilled water (each vial is sufficient for half of a 96-well plate). The prepared Free Glycerol Assay Reagent should be stable for approximately two weeks at 4°C.

2. **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 eight clean test tubes and label them #1 through #8. Aliquot 100 µl of PBS or water to tubes #2 - #8. Transfer 200 µl of Glycerol Standard Solution 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 for tubes #4-7. Do not add any standard to tube #8. This tube will be your blank for the standard curve.

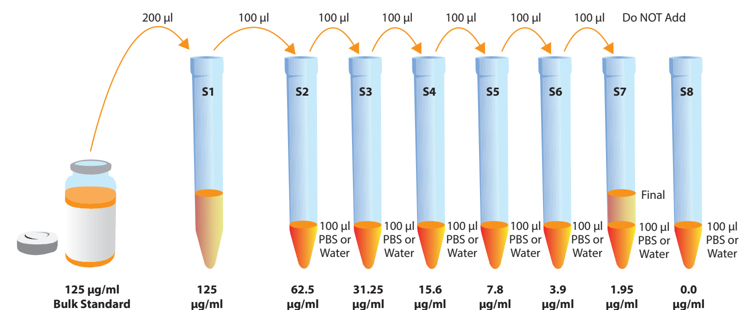

Figure 1. Preparation of the Glycerol Standards

ASSAY PROTOCOL

Plate Set Up

Each plate should contain a glycerol standard curve, wells containing medium only, and wells containing supernatants from samples treated with test compounds or vehicle. We recommend that standards be run in duplicate and that each treatment be performed in triplicate. We suggest that you record the contents of each well on the template sheet provided (see page 14).

Assay Procedure

NOTE: Perform all steps at room temperature.

1. Collect cell culture supernatants from each well and place in glycerol-free containers. Samples may be assayed immediately or stored at 4°C for up to two weeks.
2. To perform the assay, transfer 25 µl of the standards prepared above into a new 96-well plate. We recommend that the standards be run in duplicate.
3. Transfer 25 µl of each supernatant collected in step #1 to triplicate wells on the new plate.
4. Add 100 µl of reconstituted Free Glycerol Assay Reagent per well to the standards and to two of each set of triplicate wells from step #3. Add 100 µl of distilled water to the third well of each sample from step #3. These will be the blanks for your samples.
5. Incubate for 15 minutes at room temperature.
6. Read the absorbance at 540 nm.

ANALYSIS

Calculations

1. Calculate the average absorbance of each standard and sample.
2. Subtract the absorbance value of the standard 8 (0 µg/ml) from all other values (both standards and samples). This is the corrected absorbance.
3. Subtract the corrected absorbance of each sample blank from the absorbance of the corresponding samples. This is the corrected absorbance of each sample.
4. Graph the corrected absorbance values of each standard as a function of the final glycerol concentration. See Figure 2, on page 10, for a typical standard curve.
5. Calculate the amount of glycerol in each sample using the equation obtained from the linear regression of the standard curve by substituting the corrected absorbance values for each sample into the equation.

$$\text{Free glycerol } (\mu g/ml) = \left[\frac{A_{540} - (\text{y-intercept})}{\text{Slope}}\right]$$

Performance Characteristics

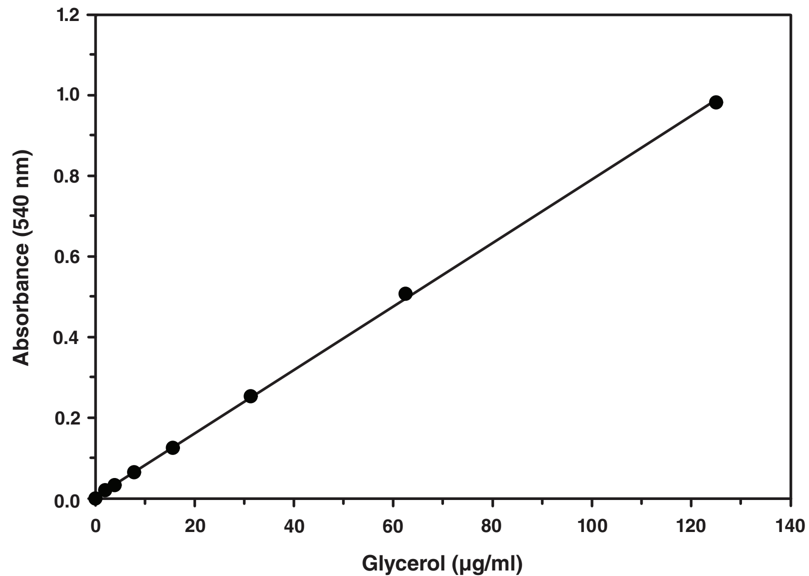

Figure 2. Typical glycerol standard curve

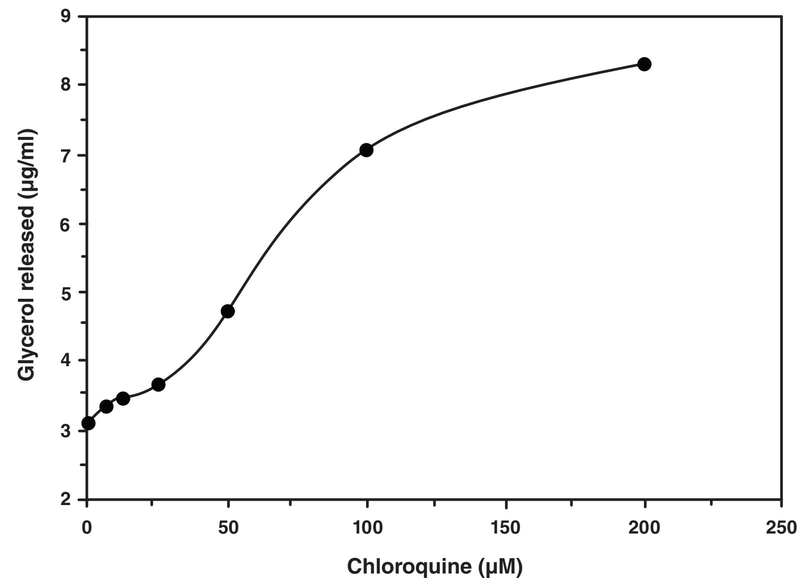

Figure 3. Glycerol release from HepG2 cells treated with chloroquine. HepG2 cells were seeded at a density of 10^4 cells/well in a 96-well plate and grown overnight in a 37°C incubator. The next day, cells were treated with vehicle or different doses of chloroquine for 24 hours. At the end of this incubation, supernatants were collected and analyzed for free glycerol according to the procedure described above.

RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
No difference among different treatments including the positive control	Cell density too high or cells overgrown	Plate cells at a lower density
Cells treated with experimental compound do not release measurable free glycerol	Experimental compounds may have a cytotoxic effect on the cells which results in cell death or the compounds may not cause the release of glycerol from this particular cell line	Lower the concentration of the experimental compounds or test the compounds in a different cell line

References

1. Hanson, R.W. and Reshef, L. Glyceroneogenesis revisited. *Biochimie* **85**, 1199-1205 (2003).
2. Reshef, L., Olswang, Y., Cassuto, H., *et al.* Glyceroneogenesis and the triglyceride/fatty acid cycle. *J. Biol. Chem.* **278(33)**, 30413-30416 (2003).

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
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F												
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H												

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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