



TECHNICAL MANUAL

Triglyceride-Glo™ Assay

Instructions for Use of Products
J3160 and J3161

Triglyceride-Glo™ Assay

All technical literature is available at: www.promega.com/protocols/
 Visit the web site to verify that you are using the most current version of this Technical Manual.
 E-mail Promega Technical Services if you have questions on use of this system: techserv@promega.com

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1. Description

The Triglyceride-Glo™ Assay^(a) provides a luminescent method for measuring triglycerides in cultured cell lysates and other biological samples such as cell culture medium, serum and tissue homogenates. The assay is ideal for measuring triglyceride accumulation and clearance in normal and pathological conditions. Examples include adipocytes, liver samples or cell culture liver models where excess triglyceride accumulation causes steatosis that progresses to nonalcoholic fatty liver disease (NAFLD) and eventually nonalcoholic steatohepatitis (NASH).

The Triglyceride-Glo™ Assay detects triglyceride levels by measuring glycerol that is released from triglycerides in an enzymatic reaction with a lipase: one molecule of glycerol per molecule of triglyceride. Glycerol is measured in a coupled reaction scheme that links the production of NADH to the activation of a pro-luciferin that produces light with luciferase (Figure 1). The amount of triglyceride is determined from the difference of glycerol measured in the absence (free glycerol) and presence (total glycerol) of lipase.

The Triglyceride-Glo™ Assay is part of a bioluminescent metabolite assay platform that offers rapid and sensitive metabolite detection in many sample types (1–2). The assay can be performed in 96- and 384-well plates where the detection reagents are added directly to samples without the need for organic extraction, making it amenable to high-throughput applications (Figure 2).

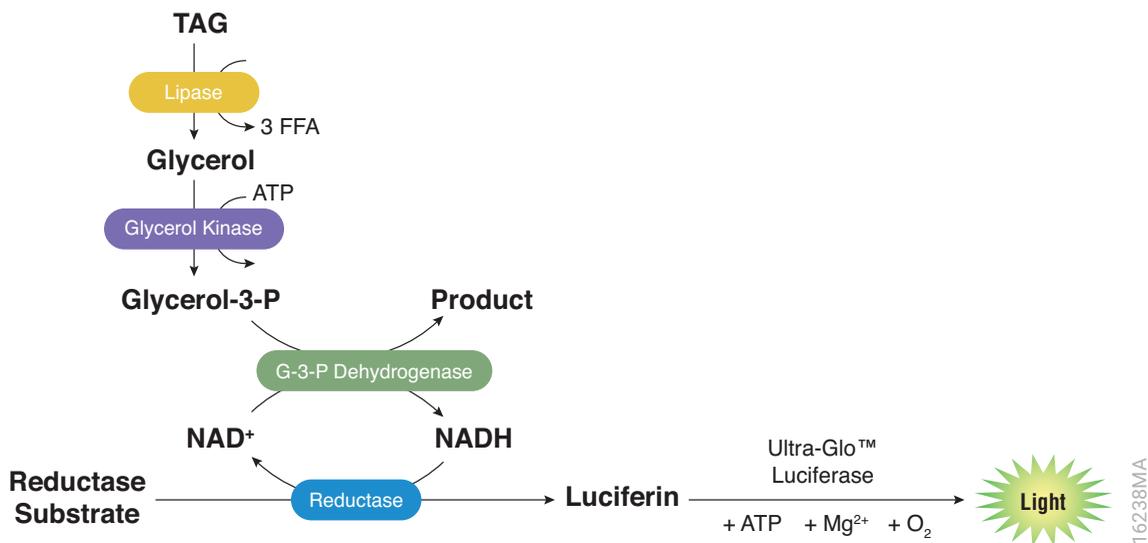


Figure 1. Schematic diagram of the Triglyceride-Glo™ Assay principle. Lipase converts triglyceride (TAG) to glycerol. Glycerol kinase and glycerol-3-phosphate dehydrogenase are used to generate NADH. In the presence of NADH, Reductase enzymatically reduces a pro-luciferin Reductase Substrate to luciferin. 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.

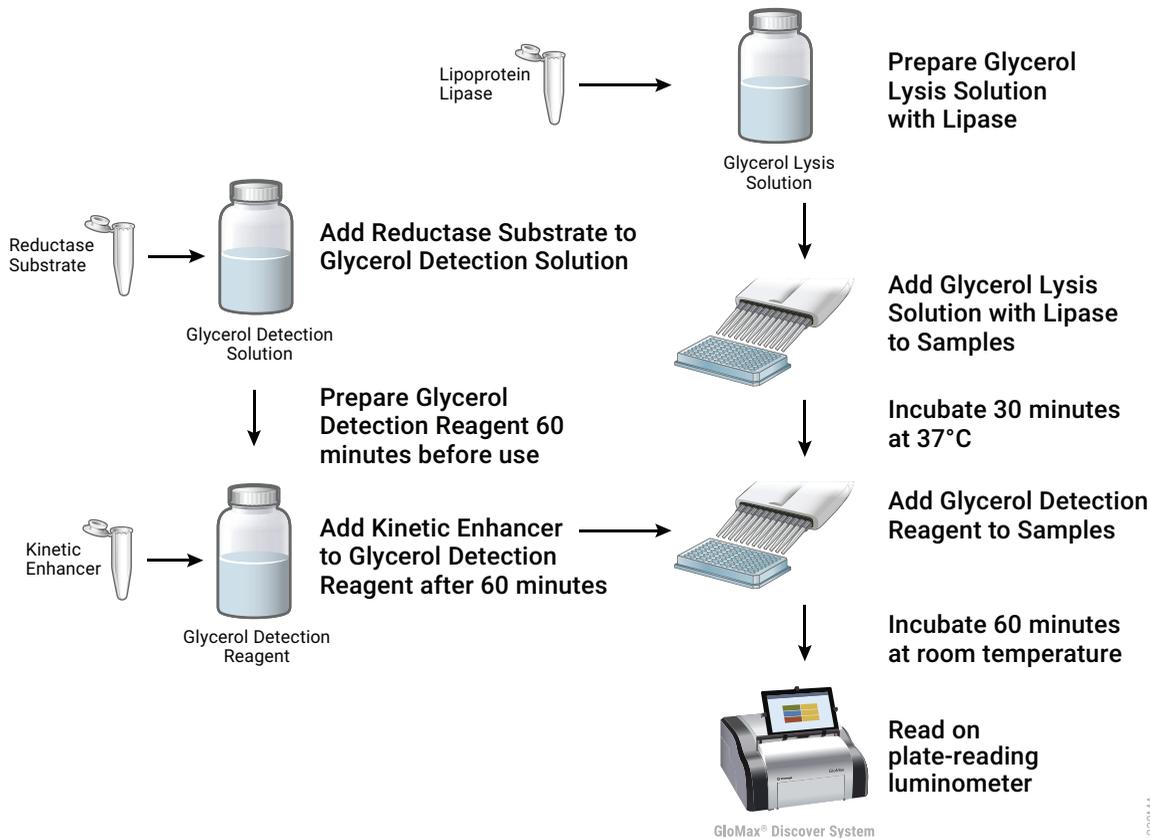


Figure 2. Triglyceride-Glo™ Assay reagent preparation and protocol. The protocol above is for triglyceride detection by measurement of total glycerol. For samples that contain significant amounts of both triglyceride and free glycerol, a second reaction without lipase is required to measure the free glycerol concentration. Measurements of free and total glycerol are performed in separate wells simultaneously so that the amount of triglyceride can be calculated from the difference between free and total glycerol.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT. #
Triglyceride-Glo™ Assay	5ml	J3160

The system contains sufficient reagents to perform 100 reactions in 96-well plates (50µl of sample + 50µl of glycerol detection reagent). Includes:

- 10ml Glycerol Lysis Solution
- 5ml Glycerol Detection Solution
- 55µl Reductase Substrate
- 50µl Kinetic Enhancer
- 500µl Glycerol Standard (20mM)
- 400µl Lipoprotein Lipase

PRODUCT	SIZE	CAT. #
Triglyceride-Glo™ Assay	50ml	J3161

The system contains sufficient reagents to perform 1000 reactions in 96-well plates (50µl of sample + 50µl of glycerol detection reagent). Includes:

- 100ml Glycerol Lysis Solution
- 50ml Glycerol Detection Solution
- 2 × 275µl Reductase Substrate
- 500µl Kinetic Enhancer
- 500µl Glycerol Standard (20mM)
- 4ml Lipoprotein Lipase

Storage Conditions: Store complete kits at less than -65°C. Alternatively, store the Glycerol Detection Solution and Reductase Substrate at less than -65°C, store the Kinetic Enhancer and Lipoprotein Lipase at less than -10°C, and store the Glycerol Lysis Solution and Glycerol Standard at less than +10°C. The kit components can be freeze-thawed three times with no effect on assay performance. As needed, dispense kit components into single-use aliquots to minimize freeze-thaw cycles.

 **Note:** Use personal protective equipment and follow your institution's safety guidelines and disposal requirements when working with biohazardous materials such as cells and cell culture reagents.

3. Measuring Triglyceride

Triglyceride measurement with the Triglyceride-Glo™ Assay requires two steps: 1) triglyceride conversion to glycerol and fatty acids by lipase and 2) glycerol detection with the glycerol detection reagent. Hence, both glycerol released from triglycerides and free glycerol will be measured. If the amount of free glycerol in a sample is known to be insignificant compared to the amount of triglyceride (e.g., intracellularly in mammalian cells), triglyceride levels can be determined directly from a total glycerol measurement in the presence of lipase.

For unknown samples, both free glycerol (without lipase treatment) and total glycerol (with lipase treatment) must be measured.

Since the triglyceride and free glycerol levels can vary significantly in different samples (see Table 2), samples may need to be diluted (<80µM) to fit within the linear range of the assay. Twice as much lysis solution as detection solution has been included in the assay for this purpose.

3.A. Materials to Be Supplied By the User

- PBS
- 96- or 384-well assay plates (opaque white-walled with clear or opaque bottom) compatible with a standard plate reader
- single- and multichannel pipettors, tips and reagent reservoirs
- plate-reading luminometer (e.g., GloMax® Discover, Cat.# GM3000)
- water bath

3.B. Reagent Preparation

This protocol is for a reaction with 50µl of a prepared sample and 50µl of glycerol detection reagent in a 96-well plate. This assay can be adapted to other volumes provided the 1:1 ratio of glycerol detection reagent volume to prepared sample volume is maintained (e.g., 20µl of prepared sample and 20µl of glycerol detection reagent in a 384-well format).



Note: Glycerol is a common laboratory reagent present in many biological formulations (e.g., enzyme storage buffers, medium, FBS) at concentrations significantly higher than the designed linear range of the Triglyceride-Glo™ Assay. For example, 100% glycerol corresponds to a concentration of 13.6M, so even a 0.001% contamination will exceed the upper limit of the assay linearity (80µM). Therefore, care must be taken to reduce the risk of contamination.

1. Thaw all components in a 22°C water bath and mix to ensure homogeneous solutions prior to use. Place the Reductase Substrate, Kinetic Enhancer and Lipoprotein Lipase on ice; all other components can be held at 22°C until use.
2. Determine the amount of reagents necessary for your current experiment. Use reagents on the day they are prepared; do not store prepared reagents for later use.
3. To prepare Glycerol Lysis Solution with lipase, add 80µl of Lipoprotein Lipase per ml of Glycerol Lysis Solution and mix by inversion.
4. To prepare glycerol detection reagent, add 10µl of Reductase Substrate per ml of Glycerol Detection Solution and mix by inversion. Prepare this reagent 1 hour before use in order to minimize the assay background. Hold at room temperature.
5. After 1 hour, add 10µl of Kinetic Enhancer per ml of glycerol detection reagent prepared in Step 4 and mix by inversion.

4. Assay Protocol

This kit includes a 20mM Glycerol Standard for generating standard curves (Figure 3) to confirm that samples are within the linear range of the assay and to calculate triglyceride concentration. If the sample relative light unit (RLU) values fall outside the linear range of the glycerol standard curve, the sample dilutions should be adjusted and re-assayed. It is important to prepare standards in the same buffers used for preparing samples and to follow the same assay protocol. Note that color contributed by the lipoprotein lipase partially quenches the light produced by the assay (Figure 3). Hence, samples with lipase must be compared to standards with lipase. Since the lipase reaction releases one molecule of glycerol per molecule of triglyceride, free and total glycerol can be calculated from the glycerol standards in the presence and absence of lipase; a triglyceride standard is not required.

For each sample, standard or background control, prepare wells for measurement with and without lipase.

4.A. Assaying Medium, Serum or Homogenized Tissue Samples and Controls

1. Dilute samples in Glycerol Lysis Solution to bring their glycerol and/or triglyceride concentrations below 80 μ M. Include glycerol standards and a negative control (assay background). Transfer 25 μ l of sample, standard or background control to a 96-well plate for measurements with and without lipase.
2. Add 25 μ l of Glycerol Lysis Solution with or without lipase to the appropriate wells, shake briefly and incubate for 30 minutes at 37°C.
3. Add 50 μ l of glycerol detection reagent as prepared in Section 3.B to all wells.
4. Shake the plate for 30–60 seconds by hand or at a low rpm on a plate shaker.
5. Incubate at room temperature for 1 hour.
6. Record luminescence using a plate-reading luminometer.
Note: The light signal continues to increase until all glycerol is consumed and the signal plateaus. At any time point the signal is directly proportional to glycerol concentration.
7. Calculate free and total glycerol by comparison of the luminescence of samples and standards under the same conditions, i.e., with and without lipase (See Figure 3).

4.B. Assaying Adherent Cells or 3D Cell Cultures and Controls

1. Remove medium from cells in a 96-well plate for measurements with and without lipase. Wash cells twice with 100 μ l of PBS.
2. Add 50 μ l of Glycerol Lysis Solution with and without lipase to the appropriate wells, shake briefly and incubate for 30 minutes at 37°C.
3. If needed, dilute samples in Glycerol Lysis Solution to bring their glycerol concentrations below 80 μ M. Transfer 50 μ l of diluted samples, standards or background controls with and without lipase to empty wells in a 96-well plate.
4. Add 50 μ l of glycerol detection reagent as prepared in Section 3.B to all wells.
5. Shake the plate for 30–60 seconds by hand or at a low rpm on a plate shaker.
6. Incubate at room temperature for 1 hour.

7. Record luminescence using a plate-reading luminometer.
Note: The light signal continues to increase until all glycerol is consumed and the signal plateaus. At any time point the signal is directly proportional to glycerol concentration.
8. Calculate free and total glycerol by comparison of the luminescence of samples and standards under the same conditions, i.e., with and without lipase (See Figure 3).

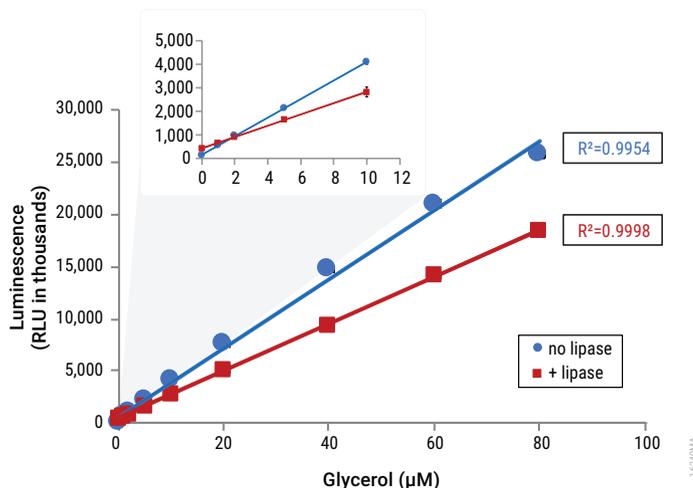


Figure 3. Glycerol standard curves. Dilutions of the provided Glycerol Standard (20mM) were prepared in Glycerol Lysis Solution, beginning with a 2µl aliquot of Glycerol Standard into 498µl of Glycerol Lysis Solution. (Refer to Table 1 for concentrations of Glycerol Standard used in standard curve.) A 50µl aliquot of glycerol detection reagent was added to 50µl of each standard in triplicate and luminescence was read after 1 hour. Concentration was plotted against average RLU at each standard point and a linear curve was fit. The Triglyceride-Glo™ Assay can detect less than 1µM glycerol and has an upper limit of 80µM glycerol. Note that lipase is a colored component that quenches a proportion of the luminescent signal. Therefore, separate standard curves in the presence or absence of lipase must be prepared to calculate sample concentrations in the presence or absence of lipase. See Section 5.A for other ways to convert luminescence into concentration.

4.C. Example Data

Table 1. Glycerol Titration Data in the Presence of Lipase.

Glycerol (μM)	80	60	40	20	10	5	2	1	0
RLU ($\times 10^3$)	18,449	14,144	9,363	5,138	2,823	1,647	927	661	420
Standard Deviation ($\times 10^3$)	371	121	41	22	200	53	8	6	8
Coefficient of Variation	2.0%	0.9%	0.4%	0.4%	7.1%	3.2%	0.8%	0.9%	1.9%
S/B	43.9	33.7	22.3	12.2	6.7	3.9	2.2	1.6	1.0
S/N	2,285.1	1,739.4	1,133.5	598.0	304.6	155.5	64.3	30.5	–

Note: Coefficient of variation is $100 \times \text{standard deviation}/\text{RLU}$. Signal:background ratio (S/B) is mean signal from samples divided by the mean signal from negative controls. Signal:noise ratio (S/N) is the net signal (mean signal minus mean negative control) divided by the standard deviation of the negative control.

Table 2. Recommendations for Different Sample Types.

Sample Type	Expected Triglyceride Concentration in Sample	Preparation Recommendations
Cell culture medium (extracellular)	variable	<ul style="list-style-type: none"> • Dilute medium samples in Glycerol Lysis Solution
Cultured cells (intracellular)	variable	<ul style="list-style-type: none"> • Remove medium from cells and wash with PBS • Add Glycerol Lysis Solution • Further dilution may be necessary
Differentiated adipocytes	100–1,000 μM	<ul style="list-style-type: none"> • Remove medium from cells and wash with PBS • Add Glycerol Lysis Solution • Further dilution after lipase treatment (e.g., 20-fold) will be necessary
Tissues	100s of μM in 25mg/ml mouse liver homogenate	<ul style="list-style-type: none"> • Homogenize tissue with Tissue Tearor or other homogenization device • Add Glycerol Lysis Solution • Further dilution (e.g., 10-fold) may be necessary

5. Example Experiments

5.A. Measuring Triglycerides in Hepatocytes and Mouse Liver Tissue

In the following examples, a single glycerol standard or a glycerol standard spike was used to convert RLU values to triglyceride concentrations. Either approach can be used with any sample type.

Triglyceride concentrations in hepatocytes were measured using a single glycerol standard for converting RLU to glycerol concentration (Figure 4). Signals from hepatocyte samples (sample RLU), a glycerol standard (standard RLU) of known concentration and a negative control (background RLU) containing no glycerol were applied to the following formula:

$$[\text{Glycerol}] \text{ of sample} = \frac{[\text{glycerol standard}] (\mu\text{M}) \times (\text{sample RLU} - \text{background RLU})}{(\text{standard RLU} - \text{background RLU})}$$

Using the data in Figure 4, and considering the samples were diluted 25-fold, this formula yields free and total glycerol concentrations of 4.3 μM and 793 μM , respectively, and the difference of 789 μM is equal to the amount of triglyceride. This is an example of an experiment in which the free glycerol concentration is relatively insignificant and only the total glycerol concentration needs to be determined to quantify triglyceride.

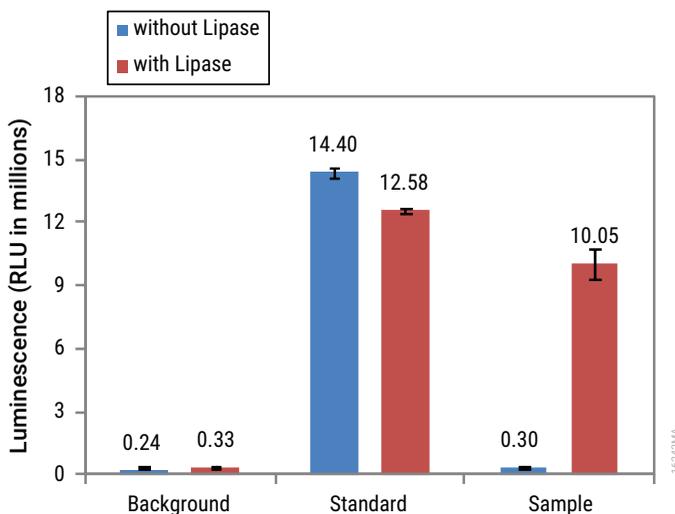


Figure 4. Triglyceride detection in hepatocytes using a single glycerol standard. Hepatocytes (56,000 per well) were maintained in a 96-well plate in standard culture medium. After medium removal, cell washing, lysis and incubation in the absence or presence of lipase, 2 μl aliquots of the hepatocyte samples were diluted into 48 μl of Glycerol Lysis Solution and assayed per the standard protocol. 40 μM and 0 μM of glycerol in Glycerol Lysis Solution in the absence or presence of lipase were assayed as the standard and background controls, respectively. The RLU value (in millions) is listed at the top of each bar.

5.A. Measuring Triglycerides in Hepatocytes and Mouse Liver Tissue (continued)

Triglyceride concentrations in a mouse liver homogenate were measured using a glycerol spike approach (Figure 5). The luminescence of a mouse liver sample in the absence (sample RLU) or presence (sample + spike RLU) of an added amount of glycerol (spike) was converted into glycerol concentration using the luminescence of a negative control (background RLU) containing no glycerol and the following formula:

$$[\text{Glycerol}] \text{ of sample} = \frac{\text{spike} \times (\text{sample RLU} - \text{background RLU})}{(\text{sample} + \text{spike RLU} - \text{sample RLU})}$$

Using the data in Figure 5, and the fact that the homogenate was diluted 20-fold prior to lipase treatment, this formula yields a value of 169 μ M triglyceride in a 25mg/ml mouse liver homogenate. Note that the homogenate itself does not interfere with the assay chemistry because the Δ RLU \pm 10 μ M glycerol is virtually unchanged in the absence (standard RLU – background RLU= 2.17 million RLU) or presence (sample + spike RLU – sample RLU = 2.02 million RLU) of the diluted homogenate.

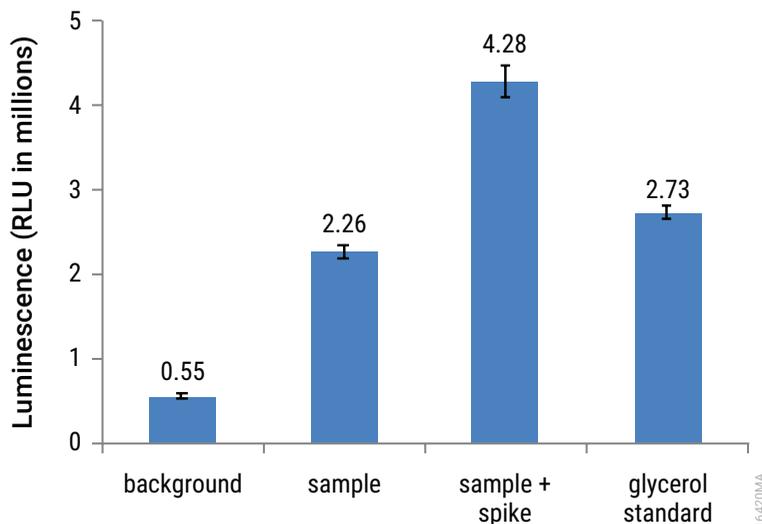


Figure 5. Triglyceride detection in mouse liver using a glycerol spike. Mouse liver (25mg) was placed in 1ml of PBS and homogenized with a Tissue Tearor (BioSpec Cat.# 985370-07) for 30 seconds. A 2.5 μ l aliquot of the homogenate was diluted into 47.5 μ l of Glycerol Lysis Solution with lipase in the presence (sample + spike) or absence (sample) of an additional 10 μ M of glycerol. These diluted samples were incubated for 30 minutes at 37°C and assayed per the standard protocol. 10 μ M and 0 μ M of glycerol in Glycerol Lysis Solution with lipase were assayed as the standard and background controls, respectively. The RLU value (in millions) is listed at the top of each bar.

5.B. Steatosis in Cancer Cells and Human Liver Microtissues

Cultured cells can be used as models for steatosis. BSA-bound fatty acids and lipoproteins stimulate intracellular triglyceride accumulation in both cancer cell lines (Figure 6) and human liver microtissues (Figure 7). In both cases, the intracellular free glycerol levels were insignificant and triglycerides levels were determined directly from total glycerol. Even if basal triglyceride levels are low to undetectable, the stimulated triglyceride accumulation can be significant, with increases over assay background of tenfold or more.

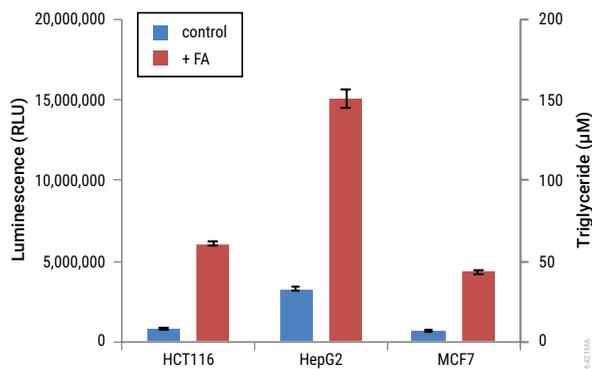


Figure 6. Triglyceride levels in cancer cell lines. Ten thousand cells of three cell lines (HCT116: colon cancer cell line; HepG2: hepatocarcinoma cell line; MCF7: breast cancer cell line) were plated overnight in the absence (control) or presence (+ FA) of 0.3mM linoleic & oleic acid bound to BSA (Sigma Cat.# L9655). After medium removal, cell washing and lipase treatment, 12.5µl aliquots of the treated samples were diluted into 37.5µl of Glycerol Lysis Solution and assayed per the standard protocol.

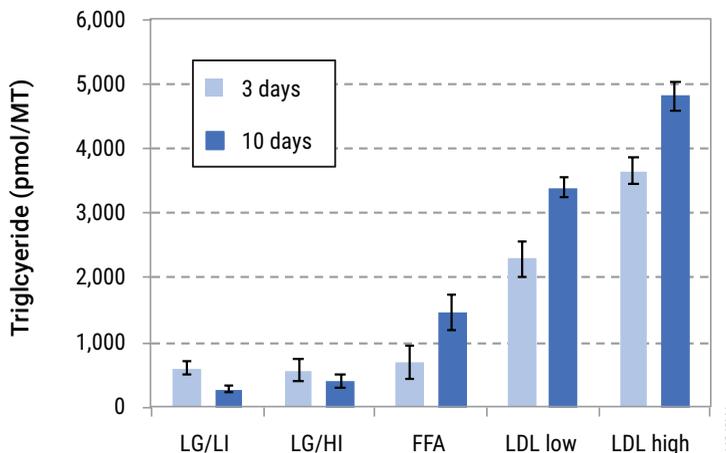


Figure 7. Triglyceride levels in human liver microtissues. 3D InSight™ human Liver Microtissues (MT, InSphero) were incubated for 3 and 10 days in serum-free medium containing either physiological (LG/LI) or supraphysiological (LG/HI) levels of glucose and insulin and supplementation with either free fatty acids bound to BSA (FFA) or low density lipoprotein plasma fraction (LDL). The microtissues were washed twice in PBS and assayed for total glycerol content according to the standard protocol. Values were plotted as concentration of triglyceride per microtissue (MT). The data was provided by InSphero, AG, Zurich, Switzerland.

5.C. Differentiation of Adipocytes: Comparison of Staining to Triglyceride Quantitation

Staining for neutral lipids is a common practice for monitoring changes in triglyceride, so to demonstrate that the Triglyceride-Glo™ Assay provides similar but more quantitative data, we monitored various stages of adipocyte differentiation (Figure 8). In one set of plates, the cells were stained with Oil Red O; in a separate set of plates, the Triglyceride-Glo™ Assay was applied. Both assays show minimal triglyceride in the initial fibroblasts (Figure 8, Panel A) and large amounts of triglyceride in the mature adipocytes (Figure 8, Panel D). Whereas Oil Red O staining provides a qualitative image of lipid accumulation, the Triglyceride-Glo™ Assay provides a quantitative measure (e.g., 70µM at differentiation stage 1; Figure 8, Panel B).

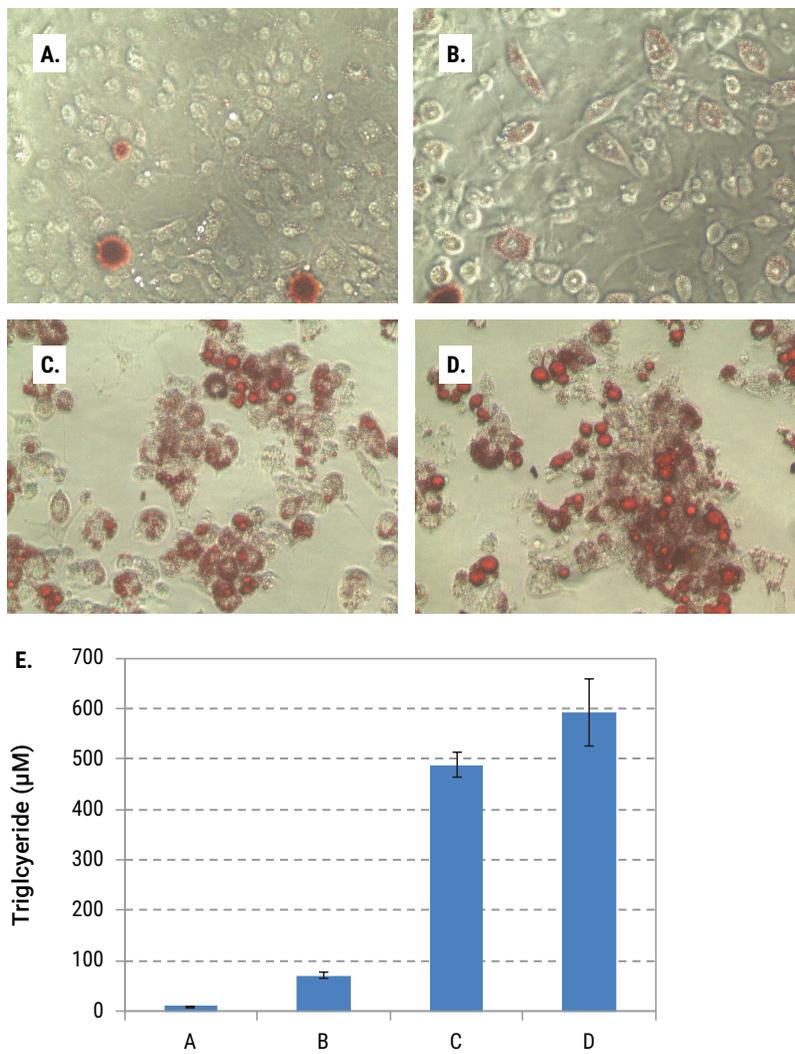


Figure 8. Lipid accumulation during adipocyte differentiation. In parallel 96-well plates, we monitored the lipid accumulation of 3T3L1-MBX cells as they were differentiated from fibroblasts (**Panel A**, day 5) through differentiation stages 1 (**Panel B**, day 12) and 2 (**Panel C**, day 14) to mature adipocytes (**Panel D**, day 21). One set of plates was stained with Oil Red O (**Panels A–D**), and the other was assayed according to the Triglyceride-Glo™ Assay protocol (**Panel E**). (See Section 5.B of the *Glucose Uptake-Glo™ Assay Technical Manual #TM467*, for recommendations on differentiation of fibroblasts into adipocytes.) At each stage of differentiation, after medium removal, cell washing and lipase treatment, 2.5µl aliquots of the treated samples were diluted into 47.5µl of Glycerol Lysis Solution and assayed per the standard protocol. Data are the average of six replicates, and triglyceride was determined by measuring total glycerol.

6. Appendix

6.A. Signal Stability

After addition of the glycerol detection reagent to a sample, the Triglyceride-Glo™ Assay yields a signal that is stable over several hours.

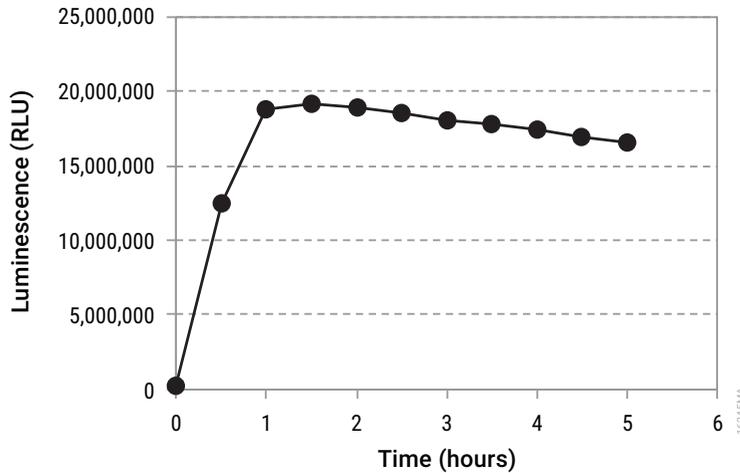


Figure 9. Signal stability of the Triglyceride-Glo™ Assay. An equal volume of glycerol detection reagent was added to 40µM glycerol in Glycerol Lysis Solution and the luminescence was recorded over time. After 5 hours, the signal is >50% of the signal at 1 hour, which is convenient for high-throughput applications.

6.B. Recovery of Triglyceride

One important feature of this assay is that lipoprotein lipase converts triglycerides into glycerol at a 1:1 ratio. Triglycerides are highly insoluble and difficult to titrate in aqueous solution. Thus, to demonstrate conversion, equal amounts of triglyceride and glycerol were placed in glass vials and treated with lipase. Figure 10 shows that triglyceride treated with lipase generates a similar amount of light as an equivalent amount of glycerol.

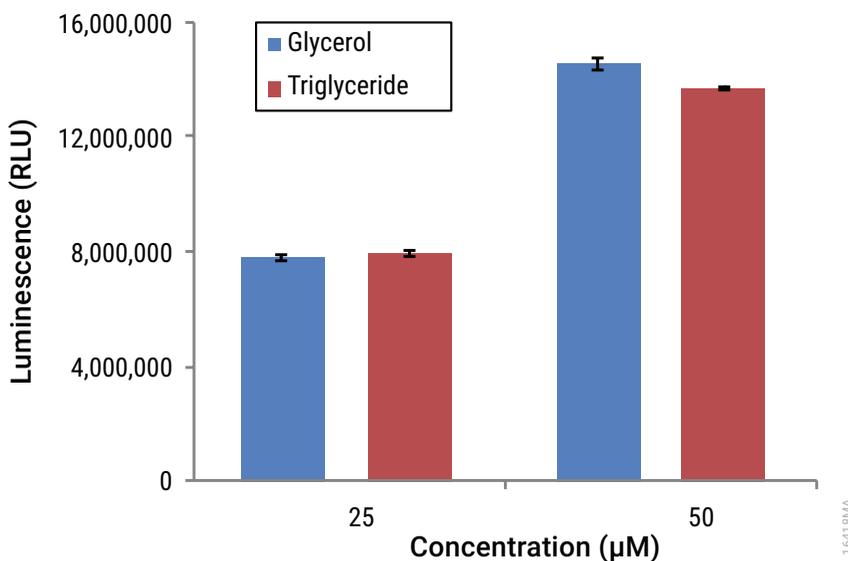


Figure 10. Equimolar conversion of triglyceride into glycerol. Triglyceride (glycerol trioleate) was dissolved in chloroform, different amounts were placed in glass vials and the chloroform was allowed to evaporate overnight. Glycerol Lysis Solution with lipase was added to the dried triglyceride and incubated for 30 minutes at 37°C. Aliquots (50µl) of the lipase-treated triglyceride and of glycerol in Glycerol Lysis Solution with lipase were transferred to a 96-well plate and assayed per the standard protocol.

Another important feature of this assay is the ability to quantitate triglycerides without an organic extraction step. To demonstrate this, triglyceride was measured in adipocytes with the Triglyceride-Glo™ Assay directly or after first extracting the triglycerides with an organic solvent (Figure 11). A similar amount of triglyceride is detected either way, indicating that organic extraction is not required.

6.B. Recovery of Triglyceride (continued)

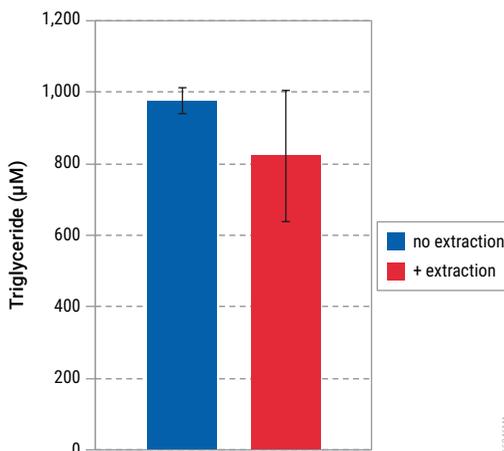


Figure 11. Organic extraction is not required for triglyceride measurement. Adipocytes were differentiated from 3T3L1-MBX fibroblasts in 96-well plates (see Section 5.B of *Glucose Uptake-Glo™ Assay Technical Manual #TM467* for recommendations on differentiation of fibroblasts into adipocytes). After medium removal and cell washing, one set of wells was extracted with methanol, transferred to a glass vial and allowed to evaporate to dryness overnight (red bar); no organic extraction was performed on the other set of wells (blue bar). Glycerol Lysis Solution (50µl) with lipase was added to each set of samples. After a 30 minute incubation at 37°C, 2.5µl aliquots of the treated samples were diluted into 47.5µl of Glycerol Lysis Solution and assayed per the standard protocol. Data are the average of six replicates, and triglyceride was determined by measuring total glycerol.

6.C. Triglyceride Specificity

Triglycerides are the major form of energy storage in mammalian cells, hence the majority of glycerol released after lipase treatment should come from triglycerides. However, any glycerol-containing molecules could potentially contribute to the total glycerol measurement if acted upon by the lipase. While other lipases, such as endothelial lipase or hepatic lipase, can exhibit equivalent or even higher rates of phospholipid versus triglyceride hydrolysis (3), the *Pseudomonas* lipoprotein lipase in the Triglyceride-Glo™ Assay has little to no phospholipid activity (4). Moreover, since the lipase sequentially removes fatty acids from triglycerides, diglycerides and monoglycerides can also contribute to the total glycerol measurement, although as stated above, the triglyceride concentration is typically much higher than the di- or mono-concentrations. Lastly, because of the enzyme combination in our assay, the Triglyceride-Glo™ Assay can also detect glycerol-3-phosphate present in samples; however, the level, similar to intracellular free glycerol, is typically very low.

6.D. Temperature and Reagent Compatibility

The intensity and stability of the luminescent signal is temperature sensitive. For consistent results, equilibrate the reagents and samples to room temperature before use. Avoid the use of DTT and other reducing agents in the samples to be tested. Reducing agents will react with the Reductase Substrate and increase background.

6.E. Plates and Equipment

Most standard plate readers designed for measuring luminescence are suitable for this assay. Some instruments do not require gain adjustment, while others may require optimization of the gain settings to achieve sensitivity and dynamic range. An integration time of 0.5–1 second per well should serve as guidance. For exact instrument settings, consult your instrument manual. For optimum performance, use opaque, white multiwell plates that are compatible with your luminometer (e.g., Corning Costar® Cat. # 3917 96-well or Costar® Cat. # 3570 384-well plates). Luminescence signal is diminished in black plates and increased well-to-well crosstalk is observed in clear plates. The RLU values shown in the figures of this technical manual vary depending on the plates and luminometers used to generate the data. Although relative luminescence output will vary with different instruments, this variation does not affect assay performance.

6.F. Multiplexing and Normalization

The Triglyceride-Glo™ Assay can be multiplexed to normalize for changes in viability and to account for well-to-well variation. If a sample of medium is removed for assay, the remainder of the sample can be assayed with RealTime-Glo™, CellTiter-Fluor™ or CellTiter-Glo® Cell Viability Assays, following the protocols provided with the respective assays. If multiplexing with intracellular triglyceride detection, RealTime-Glo™ and/or CellTiter-Fluor™ reagents can be added to the medium and measured prior to medium removal. To determine if a treatment is toxic to cells, LDH-Glo™ Cytotoxicity Assay may be multiplexed with the Triglyceride-Glo™ Assay. A small (2–5µl) sample of medium can be removed for the LDH-Glo™ Assay to a separate plate, and the remaining cells and medium can be used for triglyceride detection.

6.G. References

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2. Leippe, D. *et al.* (2017) Bioluminescent assays for glucose and glutamine metabolism: High-throughput screening for changes in extracellular and intracellular metabolites. *SLAS Discovery* **22**, 366–77.
3. Griffon, N. *et al.* (2006) Substrate specificity of lipoprotein lipase and endothelial lipase: studies of lid chimeras. *J. Lipid Res.* **47**, 1803–11.
4. Rojas, C. *et al.* (1991) Comparison of the action of lipoprotein lipase on triacylglycerols and phospholipids when presented in mixed liposomes or in emulsion droplets. *Eur. J. Biochem.* **197**, 315–21.
5. *Glucose Uptake-Glo™ Assay Technical Manual #TM467.*

6.H. Related Products

Energy Metabolism Assays

Product	Size	Cat.#
Glycerol-Glo™ Assay	5ml	J3150
Cholesterol/Cholesterol Ester-Glo™ Assay	5ml	J3190
Glucose Uptake-Glo™ Assay	5ml	J1341
Glutamate-Glo™ Assay	5ml	J7021
Glutamine/Glutamate-Glo™ Assay	5ml	J8021
Glucose-Glo™ Assay	5ml	J6021
Lactate-Glo™ Assay	5ml	J5021
NAD(P)H-Glo™ Detection System	10ml	G9061
NAD/NADH-Glo™ Assay	10ml	G9071
NADP/NADPH-Glo™ Assay	10ml	G9081
Mitochondrial ToxGlo™ Assay	10ml	G8000

Other sizes are available.

Viability Assays

Product	Size	Cat.#
RealTime-Glo™ MT Cell Viability Assay	100 reactions	G9711
CellTiter-Glo® 2.0 Assay	10ml	G9241
CellTiter-Glo® Luminescent Cell Viability Assay	10ml	G7570
CellTiter-Glo® 3D Cell Viability Assay	10ml	G9681
CellTiter-Fluor™ Cell Viability Assay	10ml	G6080

Other sizes are available.

Cytotoxicity Assays

Product	Size	Cat.#
LDH-Glo™ Cytotoxicity Assay	10ml	J2380
CellTox™ Green Cytotoxicity Assay	10ml	G8741
CytoTox-Glo™ Cytotoxicity Assay	10ml	G9290
CytoTox-Fluor™ Cytotoxicity Assay	10ml	G9260

Other sizes are available.

Multiplex Viability and Cytotoxicity Assays

Product	Size	Cat. #
MultiTox-Glo Multiplex Cytotoxicity Assay	10ml	G9270
MultiTox-Fluor Multiplex Cytotoxicity Assay	10ml	G9200

Other sizes are available.

Oxidative Stress Assays

Product	Size	Cat. #
ROS-Glo™ H ₂ O ₂ Assay	10ml	G8820
GSH-Glo™ Glutathione Assay	10ml	V6911
GSH/GSSG-Glo™ Assay	10ml	V6611

Other sizes are available.

Detection Instruments

Product	Size	Cat. #
GloMax® Discover System	each	GM3000
GloMax® Explorer System	each	GM3500

7. Summary of Changes

The following changes were made to the 5/23 revision of this document:

1. The legend for Figure 3 was updated.
2. Equations were reformatted and miscellaneous text edits made.
3. The font was updated.

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