



TECHNICAL MANUAL

# Glucose Uptake-Glo™ Assay

Instructions for Use of Products  
**J1341, J1342 and J1343**

# Glucose Uptake-Glo™ Assay

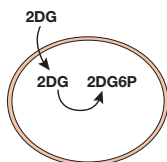
All technical literature is available at: [www.promega.com/protocols/](http://www.promega.com/protocols/)  
 Visit the web site to verify that you are using the most current version of this Technical Manual.  
 E-mail Promega Technical Services if you have questions on use of this system: [techserv@promega.com](mailto:techserv@promega.com)

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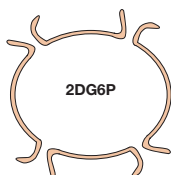
## 1. Description

The Glucose Uptake-Glo™ Assay is a nonradioactive, plate-based, homogeneous bioluminescent method for measuring glucose uptake in mammalian cells based on the detection of 2-deoxyglucose-6-phosphate (2DG6P). When 2-deoxyglucose (2DG) is added to cells, it is transported across the membrane and rapidly phosphorylated in the same manner as glucose. However, enzymes that further modify glucose-6-phosphate (G6P) cannot modify 2DG6P, and thus this membrane-impermeable analyte accumulates in the cell. After a brief period of incubation, an acid detergent solution (Stop Buffer) is added to lyse cells, terminate uptake and destroy any NADPH within the cells. A high-pH buffer solution (Neutralization Buffer) is then added to neutralize the acid. A Detection Reagent containing glucose-6-phosphate dehydrogenase (G6PDH), NADP<sup>+</sup>, Reductase, Ultra-Glo™ Recombinant Luciferase and pro-luciferin substrate is added to the sample wells. G6PDH oxidizes 2DG6P to 6-phosphodeoxygluconate and simultaneously reduces NADP<sup>+</sup> to NADPH. The Reductase uses NADPH to convert the pro-luciferin to luciferin, which is then used by Ultra-Glo™ Recombinant Luciferase to produce a luminescent signal that is proportional to the concentration of 2DG6P.

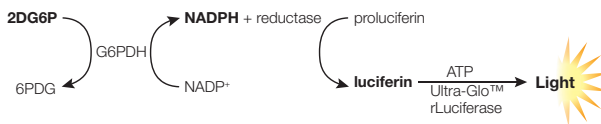
**Step 1.** Add 2DG to cells.



**Step 2.** Add Stop and Neutralization Buffers to end reactions, lyse cells and eliminate cellular NADPH.

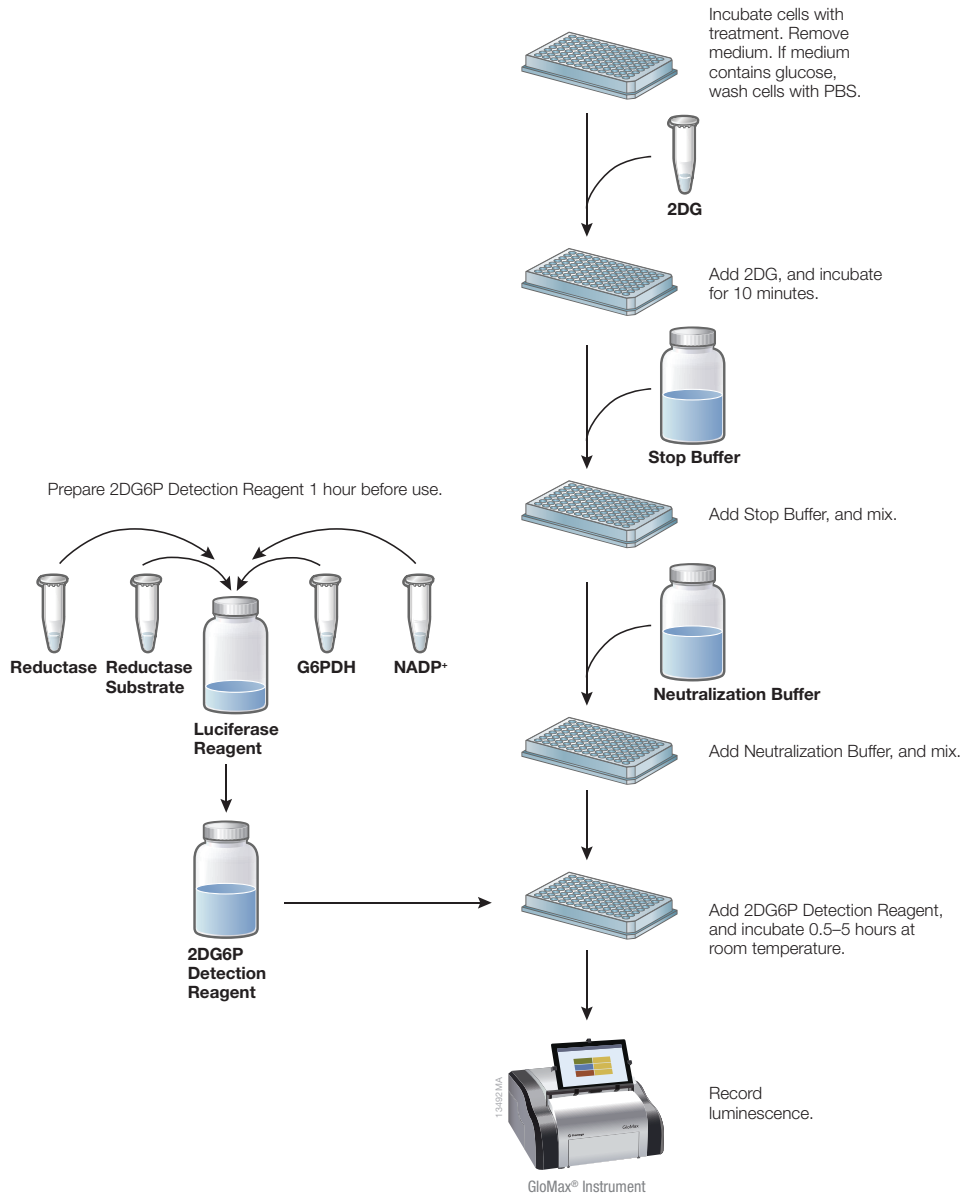


**Step 3.** Add 2DG6P Detection Reagent.



2DG = 2-deoxyglucose  
2DG6P = 2-deoxyglucose-6-phosphate  
G6PDH = glucose-6-phosphate dehydrogenase

**Figure 1. Schematic diagram of the Glucose Uptake-Glo™ Assay technology.** 2-deoxyglucose (2DG) is transported into cells and phosphorylated to produce 2-deoxyglucose-6-phosphate (2DG6P). Adding Stop Buffer stops 2DG transport, lyses cells, and inactivates proteins. The acid is neutralized by the Neutralization Buffer before the addition of the 2DG6P Detection Reagent. The glucose-6-phosphate dehydrogenase (G6PDH) within the 2DG6P Detection Reagent oxidizes 2DG6P to 6-phosphodeoxygluconate (6PDG) and reduces NADP<sup>+</sup> to NADPH. The reductase uses NADPH to convert the pro-luciferin to luciferin, which is then used by Ultra-Glo™ Recombinant Luciferase to produce light.



**Figure 2. Schematic diagram of the Glucose Uptake-Glo™ Assay protocol and reagent preparation.**

## 2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
<b>Glucose Uptake-Glo™ Assay</b>	<b>5ml</b>	<b>J1341</b>

The system contains sufficient reagents to perform 50 reactions in 96-well plates (50µl of sample + 25µl of Stop Buffer + 25µl of Neutralization Buffer + 100µl of 2DG6P Detection Reagent). Includes:

- 5ml Luciferase Reagent
- 15ml Stop Buffer
- 15ml Neutralization Buffer
- 50µl NADP<sup>+</sup> (20mM)
- 125µl Glucose-6-Phosphate Dehydrogenase (G6PDH)
- 25µl Reductase
- 55µl Reductase Substrate
- 250µl 2-deoxyglucose (2DG, 100mM)
- 50µl 2DG6P Standard (1mM)

PRODUCT	SIZE	CAT.#
<b>Glucose Uptake-Glo™ Assay</b>	<b>10ml</b>	<b>J1342</b>

The system contains sufficient reagents to perform 100 reactions in 96-well plates (50µl of sample + 25µl of Stop Buffer + 25µl of Neutralization Buffer + 100µl of 2DG6P Detection Reagent). Includes:

- 10ml Luciferase Reagent
- 15ml Stop Buffer
- 15ml Neutralization Buffer
- 100µl NADP<sup>+</sup> (20mM)
- 250µl Glucose-6-Phosphate Dehydrogenase (G6PDH)
- 55µl Reductase
- 55µl Reductase Substrate
- 250µl 2-deoxyglucose (2DG, 100mM)
- 50µl 2DG6P Standard (1mM)

PRODUCT	SIZE	CAT.#
<b>Glucose Uptake-Glo™ Assay</b>	<b>50ml</b>	<b>J1343</b>

The system contains sufficient reagents to perform 500 reactions in 96-well plates (50µl of sample + 25µl of Stop Buffer + 25µl of Neutralization Buffer + 100µl of 2DG6P Detection Reagent). Includes:

- 50ml Luciferase Reagent
- 15ml Stop Buffer
- 15ml Neutralization Buffer
- 500µl NADP<sup>+</sup> (20mM)
- 1.25ml Glucose-6-Phosphate Dehydrogenase (G6PDH)
- 275µl Reductase
- 55µl Reductase Substrate
- 250µl 2-deoxyglucose (2DG, 100mM)
- 50µl 2DG6P Standard (1mM)

**Storage Conditions:** Store all components below –65°C. Alternatively, store the Reductase Substrate below –65°C and all other components at –30°C to –10°C. Do not freeze-thaw the kit components more than three times.

### 3. General Considerations

#### 3.A. Glucose in the Assay Buffer

PBS is typically used as the assay buffer for cell washing and initiation of uptake with 2DG, but this can be replaced with similar cell-compatible buffers. Such buffers may include low millimolar amounts of calcium and/or magnesium salts, different buffering agents (e.g., HEPES instead of phosphate), or even BSA (e.g., 0.5%).

However, it is critical that the assay buffer does not include glucose since it is the largest source of interference for this assay. Glucose is a poor substrate for G6PDH, but the millimolar levels typically found in media can compete with micromolar levels of 2DG6P from cells. We recommend a PBS wash prior to performing the assay for media that contain glucose. The assay can be initiated by adding concentrated 2DG (e.g., 5 $\mu$ l of 10mM 2DG to a 50 $\mu$ l sample) without first removing the medium if there is no glucose present. Be aware that although other carbohydrate sources (e.g., galactose) may not interfere with the G6PDH in the Glucose Uptake-Glo™ Assay, they may interfere with glucose transporters, and hence medium removal and/or washing with PBS may still be required.

#### 3.B. Uptake Time

The time allowed for glucose uptake (i.e., the time between adding 2DG and adding Stop Buffer) is an important consideration. If this time is too short, the signal may be too low to distinguish from the assay background. If this time is too long, changes in the luminescent signal will no longer be proportional to the rate of glucose uptake. For example, Figure 9 in Section 6.B shows that the signal at 10 minutes for 10,000 HCT116 cells is significantly greater than the assay background but still within the linear range of the assay.

It is also important to ensure that the number of cells per sample is within the linear range of the assay for a given uptake time. We typically see greater than a threefold signal-to-background ratio with 5,000 cells, but can measure a higher cell quantity as long as the total amount of 2DG6P produced during the assay is less than 30 $\mu$ M. See Figure 10 in Section 6.B for more information.

#### 3.C. 2-Deoxyglucose Concentration

Our standard recommendation for initiating glucose uptake is a solution of 1mM 2DG for 10 minutes. Higher than 1mM 2DG typically provides little benefit. A lower concentration of 2DG is acceptable, however we recommend 100 $\mu$ M as the lower limit since less input of 2DG means less output of 2DG6P, and a corresponding decrease in the signal of the assay.

#### 3.D. Biological Response vs. Assay Sensitivity

Although bioluminescence can offer great sensitivity, the Glucose Uptake-Glo™ Assay is limited by the cell's biological response. If the difference between stimulated and nonstimulated pathways is small (e.g., the twofold increase in glucose uptake observed when L6 myotubes are stimulated with insulin; see Figure 5), this cannot be improved with any assays. An assay with a good signal above background can more readily detect small responses, but it cannot make the response larger.

### **3.D. Biological Response vs. Assay Sensitivity (continued)**

One of the keys to maximizing a biological response is optimizing the growth and differentiation of cells. In particular, changes in glucose uptake in response to insulin in both fat and muscle cells can be very sensitive to handling conditions. See Section 5 for example protocols for the growth and differentiation of two of the more common insulin responsive cell types.

### **3.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 0.3–1 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® Cat.# 3903). Luminescence signal is diminished in black plates and increased well-to-well cross talk 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 should not affect assay performance.

## **4. Measuring Glucose Uptake**

The following protocol is for cells prepared in a 96-well plate. For other plate formats, see Section 4.D. Multiple steps recommend shaking to encourage homogeneous mixing. This can be done with a circular motion by hand or with a short interval (e.g., 10 seconds) on a plate shaker.

### **Materials to be Supplied by the User**

- phosphate-buffered saline (PBS, e.g., Sigma Cat.# D8537 or GIBCO Cat.# 14190) or other cell compatible glucose-free buffer
- 96-well assay plates (white or clear bottom, e.g., Corning® Cat.# 3903)
- luminometer (e.g., GloMax® Discover Cat.# GM3000)

**Note:** Be sure to prepare 2DG6P Detection Reagent 1 hour before use to minimize assay background.

### **4.A. Reagent Preparation**

1. Thaw all components in a room temperature water bath. Once thawed, keep the Luciferase Reagent, Stop Buffer, and Neutralization Buffer at room temperature; all other components should be placed on ice.

**Note:** Be sure to mix thawed components to ensure homogenous solutions prior to use.

- To prepare the 2DG6P Detection Reagent, add components to the Luciferase Reagent in the relative volumes listed below.

<b>Component</b>	<b>Per Reaction</b>	<b>Per 10ml</b>
Luciferase Reagent	100µl	10ml
NADP <sup>+</sup>	1µl	100µl
G6PDH	2.5µl	250µl
Reductase	0.5µl	50µl
Reductase Substrate	0.0625µl	6.25µl



**Note:** After preparation, allow the 2DGP Detection Reagent to equilibrate at room temperature for 1 hour before use to minimize assay background.

- Dilute 2-deoxyglucose from 100mM to 1mM in PBS or other cell compatible glucose-free buffer.
- Components can be refrozen, but the 2DG6P Detection Reagent should be used on the day it is prepared. We recommend to prepare only the amount of reagent needed per assay.

#### **4.B. Protocol**

- Treat cells as desired (see Section 5 for examples on how to handle certain cell types). Remove medium and wash with 100µl PBS if glucose is present.

**Note:** To most efficiently remove glucose from the cell culture, we recommend slow removal of the medium and the PBS using a pipettor.

- Add 50µl of the prepared 1mM 2DG per well, shake briefly, and incubate 10 minutes at room temperature. The optimal number of cells and incubation time will vary with different cell types (see Section 3.B). If the medium does not contain glucose, a concentrated aliquot (above 1mM) of 2DG can be added directly to the cells without medium removal (e.g., 5µl of 10mM 2DG to a 50µl sample).
- Add 25µl of Stop Buffer and shake briefly.
- Add 25µl of Neutralization Buffer and shake briefly.
- Add 100µl of 2DG6P Detection Reagent and shake briefly. If fewer dispensing steps are desired, the Neutralization Buffer may be added to the 2DG6P Detection Reagent just prior to assay, and the combination can be added in a volume of 125µl.

**Note:** Be sure to prepare 2DG6P Detection Reagent 1 hour before use to minimize assay background.

- Incubate for 0.5–5 hours at room temperature.
- Record luminescence using a 0.3–1 second integration on a luminometer. If you are using a GloMax<sup>®</sup> instrument, you can record luminescence by selecting the “Glucose Uptake-Glo<sup>™</sup> protocol.”



#### 4.C. Assay Controls

A number of control reactions can be performed to measure the background of the assay. Control reactions can confirm that the assay is generating a net signal above background. If the net signal is comparable to the background, the background can be subtracted from the signal to more correctly represent fold changes under given experimental conditions.

Each of the recommendations listed below create conditions where no 2DG6P accumulates.

1. Omit 2DG: Adding PBS without 2DG provides no substrate for transport.
2. Add the Stop Buffer prior to 2DG: The acidic detergent solution can disrupt membranes and inactivate kinases before addition of 2DG, so no 2DG6P can be produced.
3. Incubate cells with a glucose transporter inhibitor: Adding an inhibitor (e.g., 50 $\mu$ M cytochalasin B for 5 minutes) before performing the assay prevents transport of 2DG inside the cells.

#### 4.D. Different Plate Formats for the Glucose Uptake-Glo™ Assay

The general protocol in Section 4.B is for cells plated in a 96-well plate. The initial steps can be scaled for different plate formats and still be assayed in a 96-well plate in order to minimize the amount of 2DG6P Detection Reagent needed.

Number of Wells	Culture ( $\mu$ l)	1mM 2DG in PBS ( $\mu$ l)	Stop Buffer ( $\mu$ l)	Neutralization Buffer ( $\mu$ l)	2DG6P Detection Reagent ( $\mu$ l)
6	2,000	1,000	500		
12	1,000	500	250		Transfer 75 $\mu$ l of each sample to a 96-well plate and continue with regular protocol
24	500	250	125		
48	200	100	50		
96	100	50	25	25	100
384	20	10	5	5	20

## 5. Additional Protocols for Differentiating Cells and Multiplexing Assays

### 5.A. Composition of Media

Medium	Component	Final Concentration	Catalog Number
Maintenance Medium (MM)	DMEM	97%	ATCC #30-2002
	FBS	3%	ATCC #30-2020
	Antibiotic-antimycotic	1X	Life Technologies #15240
Differentiation Medium I (DM-I)	DMEM	97%	ATCC #30-2002
	FBS	3%	ATCC #30-2020
	Antibiotic-antimycotic	1X	Life Technologies #15240
	Insulin	1µg/ml	Sigma #I9278
	Isobutylxanthine	0.5mM	Sigma #I5879
	Dexamethasone	1µM	Sigma #D4902
	Rosiglitazone	2µM	Sigma #R2408
Differentiation Medium II (DM-II)	DMEM	97%	ATCC #30-2002
	FBS	3%	ATCC #30-2020
	Antibiotic-antimycotic	1X	Life Technologies #15240
	Insulin	1µg/ml	Sigma #I9278

### 5.B. 3T3L1-MBX Fibroblasts: Differentiation into Adipocytes

The following is an example protocol for the differentiation of 3T3L1-MBX fibroblasts into adipocytes followed by the Glucose Uptake-Glo™ Assay. Other protocols may be followed, but we've found this protocol to be very reproducible. The IC<sub>50</sub> shown in Figure 3 is similar to results shown in the literature. The images in Figure 4 are an example of what adipocytes look like after reaching maturity.

#### Notes:

- Because of the numerous cell handling steps, 1X antibiotic-antimycotic (Life Technologies, Cat. # 15240) is included in all the growth and differentiation media for these cells.
- We have used this protocol with 3T3L1 and 3T3L1-MBX cells and obtained good results with both cell types. However, we have observed a larger and more reproducible insulin response with the 3T3L1-MBX cells. 3T3L1-MBX fibroblasts are grown in DMEM + 10% fetal bovine serum and passaged less than 10 times. These cells should never be allowed to become confluent or they may begin differentiating in an uncontrolled fashion. Maintaining subconfluent cells at a low passage number is critical both when preparing for differentiation and when generating stocks of cells.

### **5.B. 3T3L1-MBX Fibroblasts: Differentiation into Adipocytes (continued)**

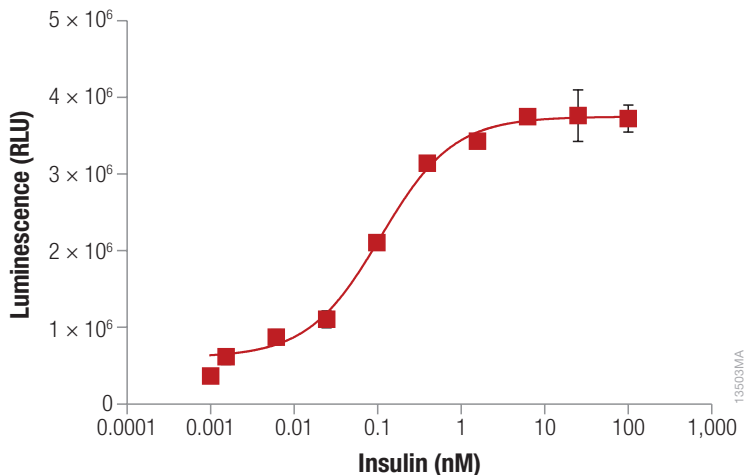
To differentiate 3T3L1-MBX cells:

1. On Day 1, thaw a 1ml vial of low passage number 3T3L1-MBX cells and combine with 9ml of Maintenance Medium (MM). Centrifuge the cells at  $200 \times g$  for 10 minutes and aspirate the liquid medium. Resuspend the cell pellet in 11ml of MM. Plate the cells at 20,000 cells per 100 $\mu$ l in a 96-well plate. Grow the cells to confluency at 37°C in 5% CO<sub>2</sub> with medium replacement every 2 days. Because of the weak adherence of these cells during differentiation, cells should be plated on collagen coated plates (Corning, Cat.# 356650). Medium removal and addition should be performed at the slowest pipetting speeds possible.
2. On Day 5, replace the medium with 100 $\mu$ l Differentiation Medium I (DM-I). Continue to replace the DM-I every 2 days.
3. On Day 12, replace the medium with 100 $\mu$ l Differentiation Medium II (DM-II).
4. On Day 14, replace the medium with 100 $\mu$ l of MM. Continue to replace the MM every 2 days.

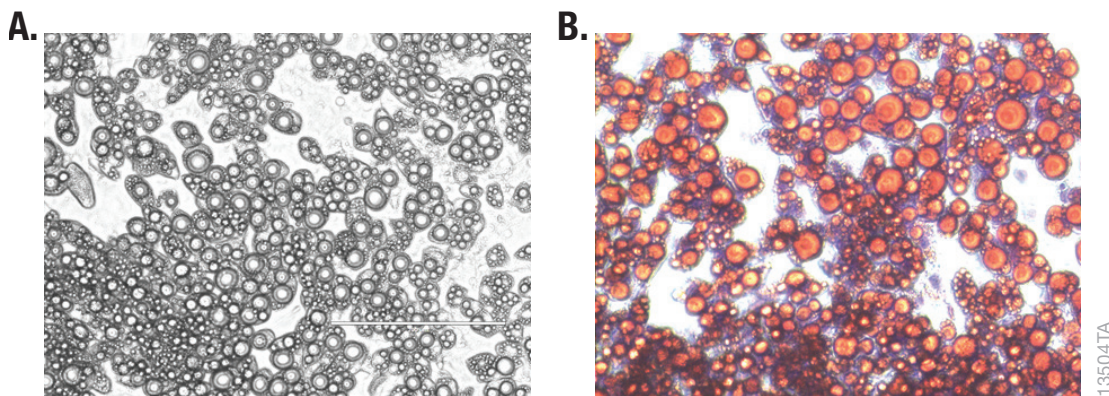
**Note:** The adipocytes are mature approximately 8 days after insulin removal. We recommend measuring any insulin responses between 8–11 days.

To assay 3T3L1-MBX adipocytes:

1. One day before the assay, replace the medium with 100 $\mu$ l MM without serum.
2. On the day of the assay, replace the medium with 100 $\mu$ l DMEM without serum or glucose (Life Technologies, Cat.# 11966) containing a range of insulin concentrations. Incubate for 1 hour at 37°C in 5% CO<sub>2</sub>.
3. Remove the medium and add 50 $\mu$ l of 2DG (1mM) in PBS and incubate for 10 minutes at 25°C.
4. Add 25 $\mu$ l of Stop Buffer and briefly shake the sample.
5. Add 25 $\mu$ l of Neutralization Buffer and shake briefly.
6. Add 100 $\mu$ l of 2DG6P Detection Reagent, shake briefly and incubate for 1 hour at 25°C.
7. Record luminescence with 0.3–1 second integration on a luminometer.



**Figure 3. Insulin titration of 3T3L1-MBX adipocytes.** The cells were plated at 20,000 cells per 100 $\mu$ l in a 96-well plate. They were grown to confluency at 37°C in 5% CO<sub>2</sub> with medium replacement every 2 days. Insulin response is best measured between 8–11 days. The EC<sub>50</sub> shown above is similar to results shown in the literature for other assays.



**Figure 4. Images of mature 3T3L1-MBX adipocytes.** Panel A shows a black and white phase contrast image, while Panel B shows a color image to highlight the oil red staining of the lipid droplets, indicating cell maturity. The adipocytes are mature approximately 8 days after insulin removal.

### 5.C. L6 Myoblasts: Differentiation into Myotubes

The following is an example protocol for the differentiation of L6 myoblasts into myotubes and the assay of the mature myotubes. As with adipocyte differentiation, other protocols may be followed, but we've found this protocol to be very reproducible. The twofold stimulation in Figure 5 may not seem large, but it matches the maximum stimulation observed with other assays. Note that as myotubes age, their basal signal increases, so to maximize the insulin response, it is best to assay them soon after they reach maturity.

L6 myoblasts are grown in DMEM + 10% fetal bovine serum and passaged less than 10 times. These cells should never be allowed to become confluent or they may begin differentiating in an uncontrolled fashion. Maintaining subconfluent cells at a low passage number is critical both when preparing for differentiation and when generating stocks of cells.

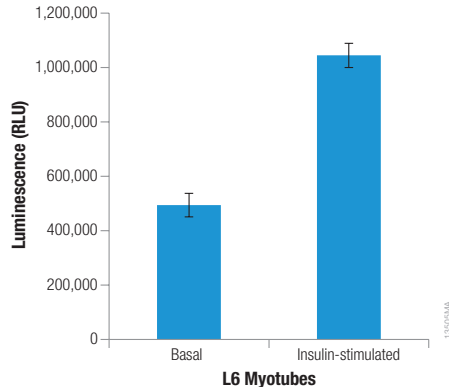
**Note:** Because of the numerous cell handling steps, 1X antibiotic-antimycotic (Life Technologies, Cat. # 15240) is included in all the growth and differentiation media of these cells.

To differentiate L6 myoblasts:

1. On Day 1, plate 5,000 cells per 100 $\mu$ l in DMEM + 10% fetal bovine serum in a 96-well plate. Replace medium every 2–3 days.
2. On Day 5, remove media and initiate differentiation by adding 100 $\mu$ l DMEM + 2% horse serum. Replace medium daily. The myotubes reach maturity after 3 days of low serum.

To assay L6 myotubes:

1. One day before the assay, remove the medium and add 100 $\mu$ l DMEM without serum.
2. On the day of the assay, replace the medium with 100 $\mu$ l DMEM  $\pm$  1 $\mu$ M insulin without serum or glucose and incubate for 1 hour at 37°C in 5% CO<sub>2</sub>.
3. Remove the medium and add 50 $\mu$ l of 0.1mM 2DG in PBS. Incubate for 30 minutes at 25°C.
4. Add 25 $\mu$ l of Stop Buffer and briefly shake the sample.
5. Add 25 $\mu$ l of Neutralization Buffer and shake briefly.
6. Add 100 $\mu$ l of 2DG6P Detection Reagent and shake briefly. Incubate for 1 hour at 25°C.
7. Record luminescence with 0.3–1 second integration on a luminometer.



**Figure 5. Insulin stimulation of L6 myotubes.** L6 myotubes were assayed after 3 days of serum starvation following the protocol in Section 5.C. The insulin-stimulated cells exhibited twice the glucose uptake as basal cells.

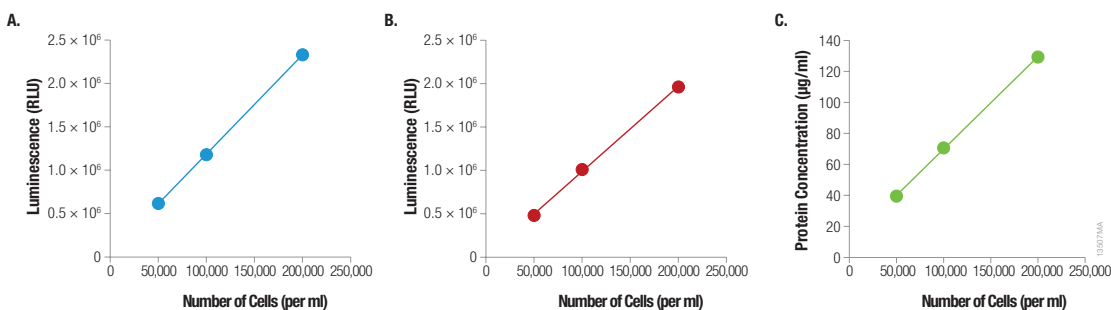
#### 5.D. Multiplexing Cancer Cells with the Glucose Uptake-Glo™ and CellTiter-Glo® Assays

The following is an example of how to assay cells grown in a 12-well plate, including how to multiplex the CellTiter-Glo® Assay and protein quantitation with the Glucose Uptake-Glo™ Assay. Each assay is ultimately measured in separate wells, but they all begin from the same plate of cells.

1. Plate 50,000, 100,000 and 200,000 HCT116 cells at 1ml per well in a 12-well plate and grow overnight.
2. Remove medium from each well and wash with 1ml PBS.
3. Add 500µl of 1mM 2DG in PBS to each well and incubate for 10 minutes at room temperature.
4. Add 250µl Stop Buffer to each well and mix briefly.
5. To measure glucose uptake:
  - a) Transfer 75µl from each sample to a 96-well assay plate.
  - b) Add 25µl Neutralization Buffer and mix briefly.
  - c) Add 100µl 2DG6P Detection Reagent and mix briefly. Incubate for 1 hour at 25°C.
  - d) Record luminescence with 0.3–1 second integration on a luminometer.
6. To measure cell viability:
  - a) Transfer 10µl from each sample to a 96-well assay plate.
  - b) Add 200µl CellTiter-Glo® Reagent (Cat. # G9241) and mix briefly. Incubate for 10 minutes at 25°C.
  - c) Record luminescence with 0.3–1 second integration on a luminometer.

### 5.D. Multiplexing Cancer Cells with the Glucose Uptake-Glo™ and CellTiter-Glo® Assays (continued)

7. To measure the protein concentration:
  - a) Prepare protein assay reagent by adding 1g of Pierce Ionic Detergent Compatibility Reagent (IDCR, Cat.# 22663) per 20ml of Pierce 660nm Protein Assay Reagent (Cat.# 22660).
  - b) Prepare dilutions of BSA from 62.5–500µg/ml for a standard curve to calculate concentration from the measured absorbance. Transfer 10µl of each dilution to a 96-well assay plate.
  - c) Transfer 10µl from each cell sample to a 96-well plate.
  - d) Add 150µl of protein assay reagent and mix briefly. Incubate for 5 minutes at 25°C.
  - e) Record the absorbance at 660nm on a spectrophotometer.



**Figure 6. Multiplexing the CellTiter-Glo® Assay and protein quantitation with the Glucose Uptake-Glo™ Assay.** All assays were performed from the same 12-well plate of HCT116 cells using the above protocol. **Panel A** shows results with the Glucose Uptake-Glo™ Assay, **Panel B** shows results with the CellTiter-Glo® Assay, and **Panel C** shows results with Pierce 660nm Protein Assay Reagent + IDCR. The results indicate that as cell number increases, metabolic activity increases as seen by an increase in glucose uptake, ATP concentration and protein concentration, respectively.

### 5.E. Adipocytes Multiplexed with RealTime-Glo™ Assay

The following example demonstrates how multiplexing the RealTime-Glo™ Assay with the Glucose Uptake-Glo™ Assay can separate immediate effects on glucose uptake from global effects on cell health. In adipocytes, insulin induces translocation of glucose transporters to the cell surface, and thus increases glucose uptake above basal levels. Cytochalasin B is a glucose transporter inhibitor, and thus it decreases glucose uptake. LY294002 is a phosphatidylinositol 3-kinase (PI3K) inhibitor, and because PI3K is an essential insulin signaling enzyme, LY294002 decreases glucose uptake relative to insulin alone. We can show that the changes in glucose uptake are not having significant effects on cell health by measuring the cell viability with RealTime-Glo™ prior to measuring glucose uptake.

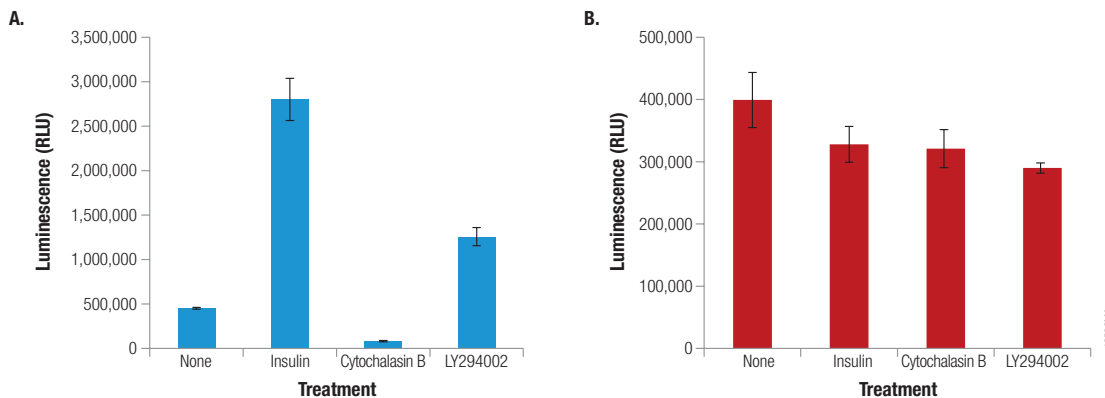
To differentiate 3T3L1-MBX adipocytes:

1. On Day 1, thaw a 1ml vial of low passage number 3T3L1-MBX cells and combine with 9ml of Maintenance Medium (MM). Centrifuge the cells at  $200 \times g$  for 10 minutes and aspirate the liquid medium. Resuspend the cell pellet in 1 ml of MM. Plate the cells at 20,000 cells per 100 $\mu$ l in a 96-well plate. Grow the cells to confluency at 37°C in 5% CO<sub>2</sub> with medium replacement every 2 days. Because of the weak adherence of these cells during differentiation, cells should be plated on collagen coated plates (Corning, Cat.# 356650). Medium removal and addition should be performed at the slowest pipetting speeds possible.
2. On Day 5, replace the medium with 100 $\mu$ l Differentiation Medium I (DM-I). Continue to replace the DM-I every 2 days.
3. On Day 12, replace the medium with 100 $\mu$ l Differentiation Medium II (DM-II).
4. On Day 14, replace the medium with 100 $\mu$ l of MM. Continue to replace the MM every 2 days.  
**Note:** The adipocytes are mature approximately 8 days after insulin removal. We recommend measuring any insulin responses between 8–11 days.
5. One day before the assay, replace the medium with 100 $\mu$ l MM (see Section 5.A) without serum.

To assay 3T3L1-MBX adipocytes:

1. On the day of the assay, replace the medium with 100 $\mu$ l DMEM without serum or glucose, containing 1X RealTime-Glo™ Reagent (Cat.# G9711) and  $\pm$  50 $\mu$ M cytochalasin B or LY294002. Incubate for 30 minutes at 37°C in 5% CO<sub>2</sub>.
2. Add 10 $\mu$ l of DMEM  $\pm$  10 $\mu$ M insulin and incubate for 1 hour at 37°C in 5% CO<sub>2</sub>.
3. To measure viability, record the luminescence with 0.3–1 second integration on a luminometer.
4. Remove the medium and add 50 $\mu$ l of 1mM 2DG in PBS. Incubate for 10 minutes at 25°C
5. Add 25 $\mu$ l of Stop Buffer and briefly shake the sample
6. Add 25 $\mu$ l of Neutralization Buffer and briefly shake the sample.
7. Add 100 $\mu$ l of 2DG6P Detection Reagent and briefly shake the sample. Incubate 1 hour at 25°C.
8. To measure glucose uptake, record the luminescence with 0.3–1 second integration on a luminometer.





**Figure 7. Multiplexing the RealTime-Glo™ Assay and Glucose Uptake-Glo™ Assay.** Panel A shows the results of the Glucose Uptake-Glo™ Assay under various conditions, where “None” indicates the basal level of glucose uptake. Panel B shows the results of the RealTime-Glo™ Assay under those same conditions. Although there are dramatic changes in glucose uptake (Panel A), there are minimal changes in viability (Panel B).

## 6. Appendix

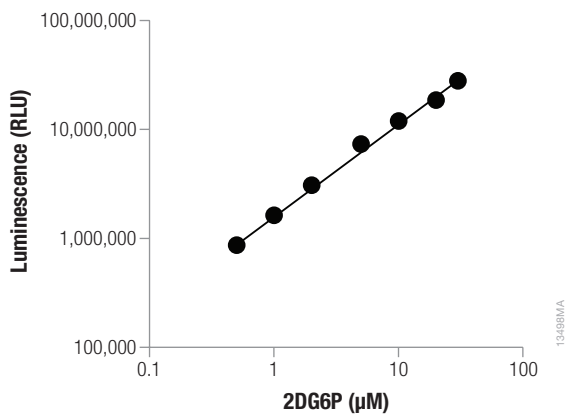
### 6.A. Calculating the Glucose Uptake Rate

To simply monitor changes in glucose uptake, data can be left in the form of relative light units (RLU). To calculate the rate of glucose uptake, however, a standard curve will need to be created to convert RLU into 2DG6P concentration (see Figure 8). The following information is needed to calculate the rate of glucose uptake: the time of uptake, the number of cells in the sample, and the amount of 2DG6P that accumulated. The calculation is performed as follows:

$$\text{Rate of glucose uptake} = ([2\text{DG6P}] \times (\text{volume of sample})) \div ((\text{number of cells}) \times (\text{time of uptake}))$$

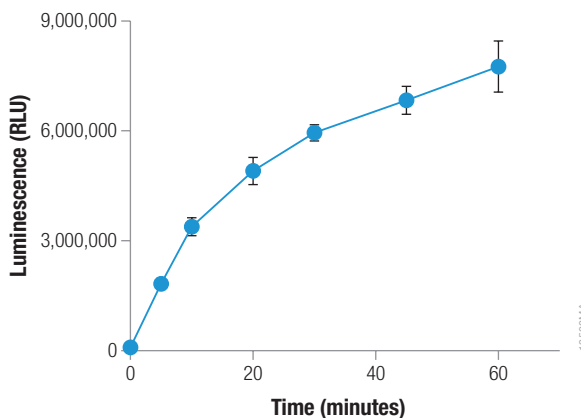
For example, an experiment may determine that 20,000 HCT116 colon cancer cells in a volume of 50 $\mu$ l accumulated 11.7 $\mu$ M 2DG6P in 10 minutes. This corresponds to 3fmol/cell/min, a value comparable to results reported for the radioactive assay.

**Note:** This assay will also detect glucose-6-phosphate since glucose-6-phosphate dehydrogenase has greater specificity for glucose-6-phosphate than for 2-deoxyglucose-6-phosphate. However, because glucose-6-phosphate does not accumulate in the cell, its concentration is typically low and does not interfere with detection of 2-deoxyglucose-6-phosphate.

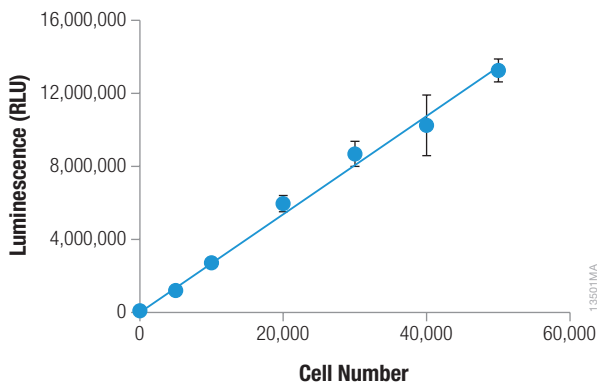


**Figure 8. 2DG6P standard curve.** The assay was initiated with 0.5–30 μM 2DG6P in PBS in a volume of 50 μl and continued with Steps 3–6 of the protocol in Section 4.B. Following the completion of the Glucose Uptake-Glo™ Assay, luminescence was measured using the GloMax® Discover System. Use the standard curve to calculate the rate of glucose uptake (see Section 4.C).

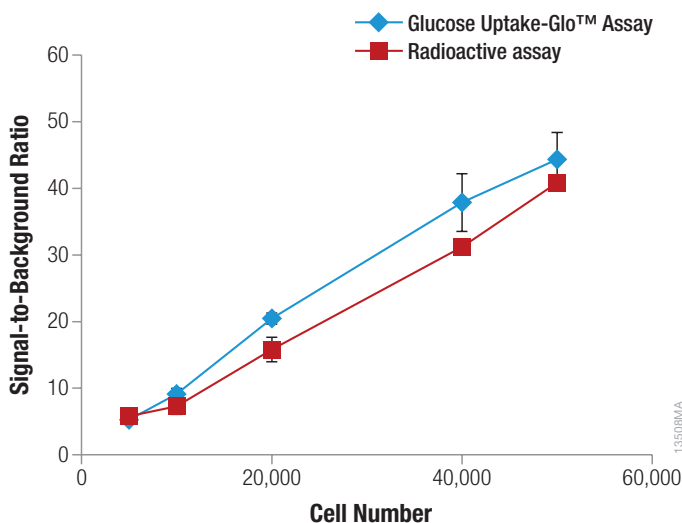
### 6.B. Additional Data



**Figure 9. Luminescence over time.** Ten thousand HCT116 colon cancer cells were plated overnight in a volume of 100 μl, washed with 100 μl PBS, and then incubated with 50 μl of 1 mM 2DG for different lengths of time before addition of the Stop Buffer.



**Figure 10. Luminescence from varying cell numbers.** Different numbers of HCT116 colon cancer cells were plated overnight in a volume of 100µl, washed with 100µl PBS, and then incubated with 50µl of 1mM 2DG for 10 minutes before addition of the Stop Buffer. A linear correlation between RLU and cell number was observed for all cancer cell lines tested: HCT116, K562, HEK293, MCF7, U2OS, HeLa and HepG2.



**Figure 11. Comparison of a radioactive method and Glucose Uptake-Glo™ Assay.** The signal-to-background ratios from varying amounts of HCT116 colon cancer cells for the Glucose Uptake-Glo™ Assay (diamonds) and the standard radioactive method (squares) are comparable. For both methods, different amounts of cells were plated in a volume of 100µl per well in a 96-well plate for 24 hours.

## 6.C. Related Products

### Metabolism Assays

Product	Size	Cat.#
NAD(P)H-Glo™ Detection System	10ml	G9061
NAD/NADH-Glo™ Assay	10ml	G9071
NADP/NADPH-Glo™ Assay	10ml	G9081

Other sizes are available.

### Oxidative Stress Assays

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

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
CellTiter-Blue® Cell Viability Assay	20ml	G8080

Other sizes are available.

### Cytotoxicity Assays

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

Other sizes are available.



## 6.C. Related Products (continued)

### 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.

### Mechanism-Based Viability and Cytotoxicity Assays

Product	Size	Cat.#
ApoTox-Glo™ Triplex Assay	10ml	G6320
ApoLive-Glo™ Multiplex Assay	10ml	G6410

Other sizes are available.

### Apoptosis Assays

Product	Size	Cat.#
Caspase-Glo® 3/7 Assay	10ml	G8091
Caspase-Glo® 8 Assay	10ml	G8201
Caspase-Glo® 9 Assay	10ml	G8211
Apo-ONE® Homogeneous Caspase-3/7 Assay	10ml	G7790
RealTime-Glo™ Annexin V Apoptosis and Necrosis Assay	100 assays	JA1011

Other sizes are available.

### Inflammation Assay

Product	Size	Cat.#
Caspase-Glo® 1 Inflammasome Assay	10ml	G9951
	5 × 10ml	G9952

### Mitochondrial Toxicity

Product	Size	Cat.#
Mitochondrial ToxGlo™ Assay	10ml	G8000
	100ml	G8001

## Cytochrome P450 Cell-Based Assays

<b>Product</b>	<b>Size</b>	<b>Cat.#</b>
P450-Glo™ CYP1A2 Induction/Inhibition Assay	10ml	V8421
P450-Glo™ CYP3A4 Assay with Luciferin-IPA	10ml	V9001
P450-Glo™ CYP2C9 Assay	10ml	V8791
P450-Glo™ CYP2B6 Assay	10ml	V8321

Other sizes are available.

## Detection Instrumentation

<b>Product</b>	<b>Size</b>	<b>Cat.#</b>
GloMax® Discover System	each	GM3000
GloMax® Explorer System	each	GM3500

## 7. Summary of Changes

The following changes were made to the 4/21 revision of this document:

1. Updated the table of contents.
2. Corrected the numbering of protocol steps in Section 5.D.
3. Added Section 3.E.
4. Updated the cover.

<sup>(a)</sup>U.S. Pat. No. 9,273,343 and other patents pending.

<sup>(b)</sup>European Pat. No. 1131441 and Japanese Pat. No. 4520084.

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