TECHNICAL MANUAL

Glucose-Glo™ Assay

Instructions for Use of Products
J6021 and J6022

3/17
TM494

Glucose-Glo™ Assay

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1. Description

The Glucose-Glo™ Assay is a bioluminescent assay for rapid, selective and sensitive detection of glucose in biological samples. The assay couples glucose oxidation and NADH production with a bioluminescent NADH detection system (1,2) (Figure 1). Glucose dehydrogenase uses glucose and NAD^+ to produce NADH. In the presence of NADH a pro-luciferin Reductase Substrate is converted by Reductase to luciferin that is then used by Ultra-Glo™ Recombinant Luciferase to produce light.

When Glucose Detection Reagent, containing glucose dehydrogenase, NAD^+, Reductase, Reductase Substrate and Luciferase, is added to a sample containing glucose at a 1:1 ratio, the enzyme-coupled reactions start and run simultaneously (Figure 2). The luminescent signal is proportional to the amount of glucose in the sample and increases until all glucose is consumed, at which point a stable luminescent signal is achieved (Figure 3, Table 1).

The Glucose-Glo™ Assay is a versatile system that is amenable to higher-throughput formats (3) and compatible with many sample types (Figure 2). Samples may require upfront sample processing, including dilutions, to fit into the linear range of the assay. They may also require inactivation of endogenous enzyme activity/deproteinization and NAD(P)H degradation (Section 3.C, Table 2). To simplify sample processing, methods for rapid enzyme inactivation and NAD(P)H degradation are provided that are compatible with 96- and 384-well plates and do not require sample centrifugation or spin columns.

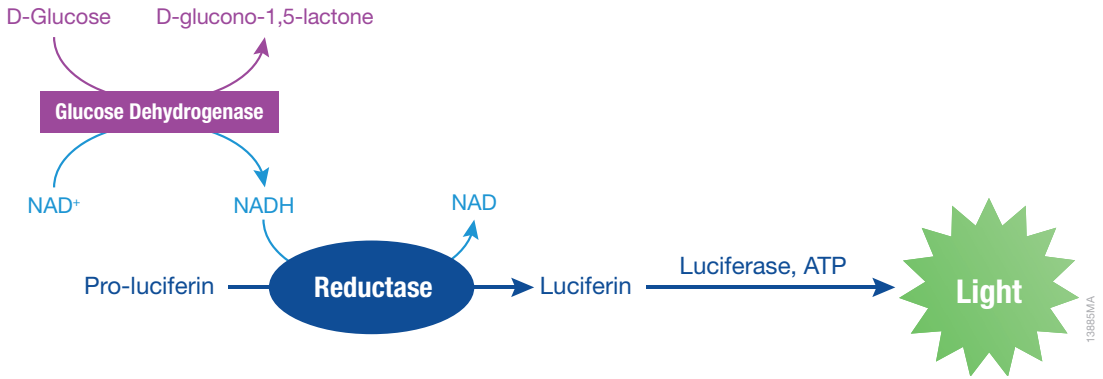

Figure 1. Schematic diagram of the Glucose-Glo™ Assay principle. Glucose dehydrogenase catalyzes the oxidation of glucose with concomitant reduction of NAD^+ to NADH. In the presence of NADH, Reductase enzymatically reduces a pro-luciferin Reductase Substrate to luciferin. Luciferin is detected in a luciferase reaction using Ultra-Glo™ rLuciferase and ATP, and the amount of light produced is proportional to the amount of glucose in the sample.

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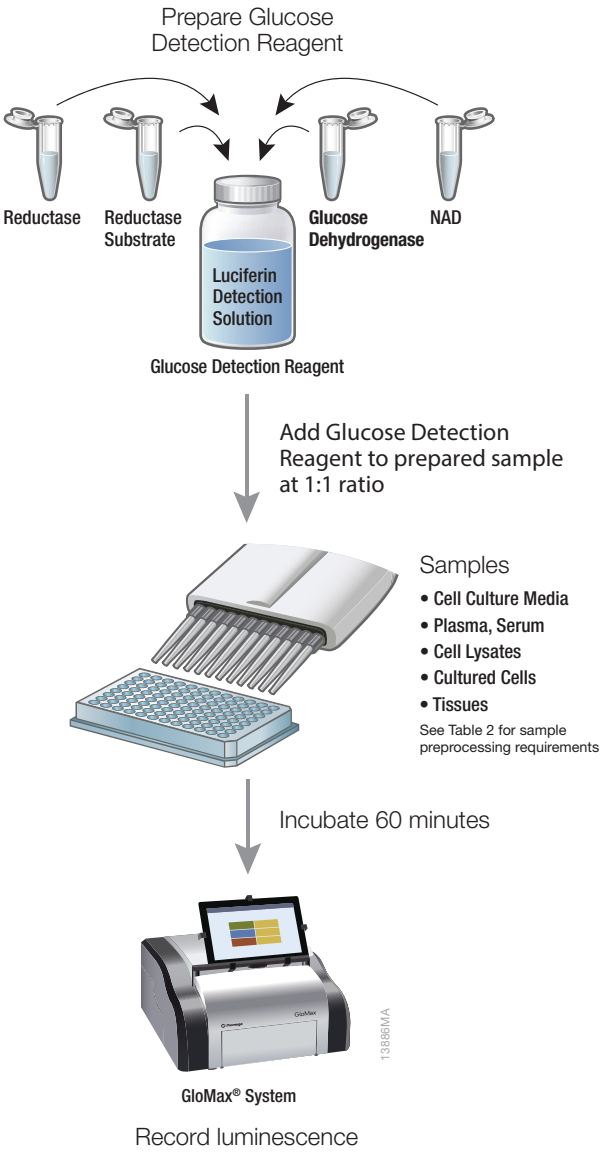

Figure 2. Glucose-Glo™ Assay reagent preparation and protocol.

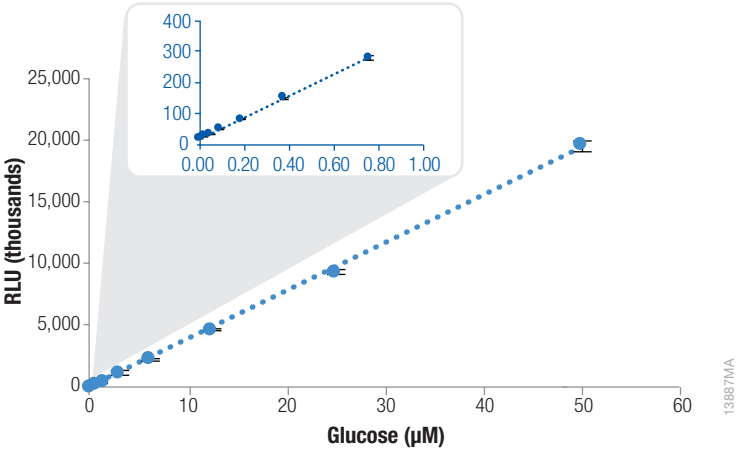

Figure 3. Glucose titration curve. Twofold serial dilutions of glucose were prepared in PBS, starting from 50µM. Aliquots of the prepared standards (50µl) were transferred to a 96-well plate and the assay was performed following the protocol in Section 3.B. Data represent the average of four replicates from readings using a GloMax® Luminometer.

Table 1. Glucose Titration Data

Glucose, µM	0	0.0031	0.0122	0.0488	0.1953	0.7813	3.125	12.5	50
Ave. RLU (thousands)	15	16	19	30	78	278	1,127	4,650	19,416
St. Dev. (thousands)	0.192	0.135	0.210	0.643	2.03	10.18	31.52	90.3	380
CV	1%	1%	1%	2%	3%	4%	3%	2%	2%
S/B	1.0	1.1	1.3	2	5	18	74	303	1,267
S/N	0	4	20	79	327	1,366	5,783	24,115	100,949

Note: Average relative light unit (RLU) and standard deviation values are in thousands. Signal-to-background (S/B) was calculated by dividing the mean signal from samples by the mean signal from negative controls. Signal-to-noise (S/N) was calculated by dividing the net signal (mean signal minus mean negative control) by the standard deviation of the negative control. The table represents data from four-fold serial dilutions of glucose.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
Glucose-Glo™ Assay	**5ml**	**J6021**

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

- 5ml Luciferin Detection Solution
- 55µl Reductase
- 55µl Reductase Substrate
- 200µl Glucose Dehydrogenase
- 30µl NAD
- 50µl Glucose (10mM)

PRODUCT	SIZE	CAT.#
Glucose-Glo™ Assay	**50ml**	**J6022**

The system contains sufficient reagents to perform 1,000 reactions in 96-well plates (50µl of sample + 50µl of Glucose Detection Reagent). Includes:

- 50ml Luciferin Detection Solution
- 275µl Reductase
- 275µl Reductase Substrate
- 2 × 1ml Glucose Dehydrogenase
- 275µl NAD
- 50µl Glucose (10mM)

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

3. Measuring Glucose

Materials to be Supplied by the User

- phosphate-buffered saline (PBS, e.g., Sigma Cat.# D8537 or Gibco Cat.# 14190) or other compatible buffer
- 96-well assay plates (white or clear bottom, e.g., Corning Cat.# 3903 or 3912)
- luminometer (e.g., GloMax® Discover Cat.# GM3000)

3.A. Reagent Preparation

This protocol is for a reaction with 50µl of sample and 50µl of Glucose Detection Reagent in a 96-well plate. The assay can be adapted to other volumes provided the 1:1 ratio of Glucose Detection Reagent volume to sample volume is maintained (e.g., 12.5µl of sample and 12.5µl Glucose Detection Reagent in a 384-well format).

1. Thaw all components. Once thawed, equilibrate the Luciferin Detection Solution to room temperature; all other components should be placed on ice. Be sure to mix thawed components to ensure homogeneous solutions prior to use.
2. Prepare Glucose Detection Reagent as shown in the table below. The amount of Glucose Detection Reagent to prepare per reaction is for a 96-well plate format using 50µl of prepared sample. Prepare the amount of reagent needed for your experiment, factoring in that some volume may be lost during pipetting.

Component	Per Reaction	Per 5ml
Luciferin Detection Solution	50µl	5ml
Reductase	0.25µl	25µl
Reductase Substrate	0.25µl	25µl
Glucose Dehydrogenase	2.0µl	200µl
NAD	0.25µl	25µl

3. Mix by gently inverting five times.

   Note: Return unused Luciferin Detection Solution, Reductase, NAD, and Glucose Dehydrogenase to storage at less than −65°C or −30°C to −10°C. Return unused Reductase Substrate to storage at less than −65°C protected from light. Do not store unused Glucose Detection Reagent.

3.B. Protocol

Upfront sample processing may be required. See Section 3.C for guidelines on preparing your specific sample type, including cell culture media, cell lysates, tissues, or plasma and sera.

1. Transfer 50µl of sample or glucose control into a 96-well plate. Include a negative control (buffer only) for determining assay background.
2. Add 50µl of Glucose Detection Reagent prepared as described in Section 3.A.
3. Shake the plate for 30–60 seconds.
4. Incubate for 60 minutes at room temperature.

   Note: The light signal continues to increase until all glucose is consumed and the signal plateaus. At any time point the signal is directly proportional to the glucose concentration.
5. Record luminescence using a plate-reading luminometer as directed by the luminometer manufacturer.

3.C. Sample Processing

The glucose concentration in standard culture medium can range from 5–25mM and will decrease to micromolar levels as cells grow and consume glucose. Thus, the glucose concentration in the medium can vary significantly, depending on cell density and sampling time. Upon uptake into cells, glucose is rapidly phosphorylated by hexokinase and, therefore, the intracellular glucose levels will be transient and variable. Other factors, such as the presence of dehydrogenases and reduced NAD(P)H dinucleotides in the samples, can affect the Glucose-Glo™ Assay signal and background. Upfront sample processing, such as dilution to fit into the linear range and/or enzyme inactivation (deproteinization), may be required to avoid these issues.

Table 2 provides examples of glucose concentration ranges in samples and suggestions for sample preparation. Section 4 provides example protocols for processing various sample types.

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Table 2. Recommendations for Sample Processing

Recipes for Inactivation and Neutralization Solutions are provided in Table 3, Section 4.B.

Sample Type	Glucose Concentration in Sample	Processing Recommendations
Cell culture medium (extracellular)	50µM to 7mM[1]	• Dilute medium sample 100- to 250-fold in PBS
Cell lysates (intracellular)	Transient; glucose is rapidly phosphorylated to glucose-6-phosphate upon uptake into cells	• Remove media from cells, wash with PBS and add fresh PBS • Add Inactivation Solution (half of sample volume) • Add Neutralization Solution (same volume as Inactivation Solution)
Cells in culture (extracellular + intracellular)	Up to 50µM[2]	• Cells in medium or PBS • Add Inactivation Solution (1/8 of sample volume) • Add Neutralization Solution (the same volume as Inactivation Solution)
Tissues	75µM if 3mg of liver tissue is homogenized in 1ml	• Tissues in homogenization buffer • Add Inactivation Solution (1/8 of sample volume)[3] • Add Neutralization Solution (the same volume as Inactivation Solution)
Plasma and serum	3mM–7mM	• Dilute plasma or serum samples 500-fold in PBS

[1] We do not recommend media containing high glucose (25mM).

[2] Applicable for short incubation times (1–2 hours) when total glucose concentration is within the linear range of the assay.

[3] Homogenizing tissue in buffer containing Inactivation Solution is the preferred method. If needed, Inactivation Solution can be added immediately after homogenization.

4. Example Protocols and Data for Various Sample Types

4.A. Cell Culture Medium

The Glucose-Glo™Assay can be used to measure changes in glucose concentration in mammalian cell culture medium. Standard cell culture media contain 5–25mM glucose. As growing cells consume glucose, the concentration of glucose in the medium will decrease depending on cell type, cell density and time. To confidently detect changes in concentration, at least 10% of the glucose has to be consumed by the cells. We recommend using a medium containing no more than 7mM glucose and sampling the medium after culturing the cells for at least 8 hours.

Samples of the medium will require dilution into the linear range of the Glucose-Glo™ Assay. Medium containing 5mM glucose must be diluted at least 100-fold into PBS to fit into the linear range of the Glucose-Glo™ assay. If cells are grown in media with higher glucose concentrations, the samples will have to be diluted accordingly.

An example showing measurement of glucose consumption by adherent lung carcinoma A549 cells is shown in Figure 4. Suspension cells can also be assayed; Figure 5 shows data with bone marrow leukemia K562 cells.

1. Plate 5,000–50,000 cells per well in a 96-well plate. Include control wells consisting of medium only.
2. Collect a sample of the medium at experimental time points by removing 2–5µl into 98–95µl PBS.
3. Proceed to Step 4 or freeze collected samples at –20°C until you are ready to perform the assay. Make sure the samples are well sealed. For example, collect the samples into a 96-well plate that is sealed with an adhesive plate sealer and a plastic plate lid.
4. On the day of the assay, thaw the samples and further dilute them 2- to 4-fold in PBS (final dilution 100-fold). Transfer 50µl to a 96-well assay plate.
5. Add 50µl of Glucose Detection Reagent prepared as described in Section 3.A.
6. Shake the plate for 30–60 seconds to mix.
7. Incubate for 60 minutes at room temperature.
8. Record luminescence.

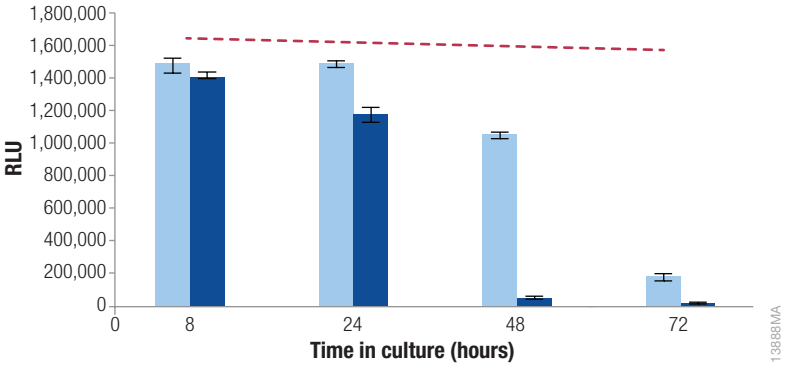

Figure 4. Glucose consumption by A549 cells. A549 cells were plated at 5,000 (light blue bars) and 15,000 (dark blue bars) cells/well in DMEM medium (Gibco A14430) containing 5mM glucose, 2mM glutamine and 10% dialyzed FBS (Gibco 26400036). Wells with medium only were included as controls. At indicated time points, 2.5µl of medium was removed and diluted in 97.5µl PBS. The samples were frozen and stored at –20°C. On the day of the assay, the samples were thawed and further diluted 2.5-fold in PBS (final medium dilution 100-fold). A portion of the sample (25µl) was transferred to a 384-well assay plate and 25µl of Glucose Detection Reagent was added. After 60 minutes at room temperature, luminescence was read using a Tecan instrument. The data represent the average of four replicates. The red line represents the luminescence values of the medium controls (average value 1,612,670 RLUs). The upper limit of the assay at 50µM glucose corresponded to 1,561,868 RLUs. All measured samples were within the linear range of the assay and the calculated glucose concentration in the media changed from 4.5mM to 0.5mM for 5,000 cells/well and from 4.4mM to 0mM for 15,000 cells/well.

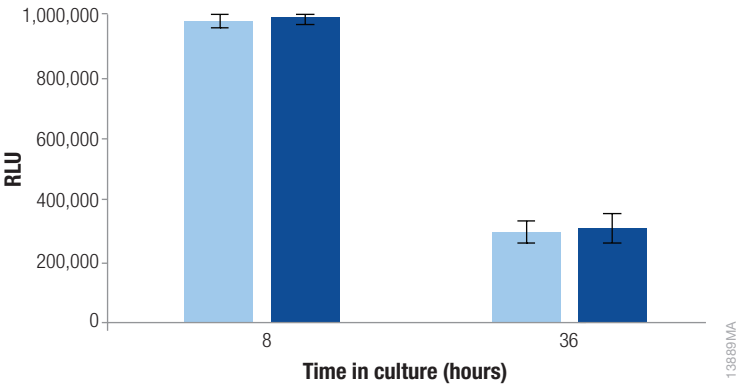

Figure 5. Glucose consumption by K562 cells. K562 cells were cultured in 75 cm^2 tissue culture flasks in RPMI medium (Sigma R1383) supplemented with 5mM glucose and 10% dialyzed FBS. After 8 and 36 hours, 1.5ml of cell culture was transferred into 1.5ml microcentrifuge tubes. A portion of each sample was diluted 10-fold in PBS directly (light blue bars) or after removing the cells by centrifugation (dark blue bars). All samples were frozen and stored at −20°C. For glucose detection, the samples were thawed, diluted with PBS to a 100-fold final dilution and 20µl was transferred into an assay plate. An equal volume of Glucose Detection Reagent was added to the samples. After 60 minutes at room temperature, luminescence was read using a Tecan instrument. The data represent the average from four separate flasks. Samples treated and not treated with Inactivation/ Neutralization solutions showed no significant difference in glucose concentrations (data not shown). The data show that a small volume of suspension cell culture can be removed, diluted and assayed directly without pelleting the cells or treating the samples for protein inactivation and NAD(P)H degradation.

4.B. Cell Lysates

The Glucose-Glo™Assay can be used for monitoring changes in intracellular glucose levels. The cell culture medium must be removed and the cells washed with PBS to avoid contamination from glucose in the medium. Upon uptake into cells, glucose is rapidly phosphorylated by hexokinase to glucose-6-phosphate, and this process has to be considered when determining the intracellular glucose concentration. We recommend using cold PBS for washes and working quickly to minimize changes in glucose metabolism.

After washing, the Inactivation Solution can be added directly to the cells in a 96-well plate, eliminating the need for sample centrifugation or deproteinization using 10K spin columns required by other methods. The Inactivation Solution rapidly stops metabolism, lyses the cells, inhibits activity of endogenous proteins and destroys reduced NAD(P)H dinucleotides. After neutralization, the samples can be assayed immediately or stored at −20°C. Instructions for preparing Inactivation and Neutralization Solutions are provided in Table 3.

Samples deproteinized using other methods might be acceptable but have to be tested for compatibility with the Glucose-Glo™ Assay. For example, perchloric acid/KOH treatment is not recommended with the Glucose-Glo™ Assay.

Table 3. Inactivation and Neutralization Solutions

Solution	Preparation
Inactivation Solution 0.6N HCl	Prepare 0.6N HCl from a concentrated stock solution, such as 1N HCl, by diluting with water. No pH adjustment is necessary.
Neutralization Solution 1M Tris base (Trizma®)	Dissolve 24.2g of Trizma® base powder (Sigma Cat. #T1503) in 200ml water. The final pH will be approximately 10.7. No pH adjustment is necessary.

Note: When homogenizing tissues or other hard-to-lyse samples, the addition of DTAB (dodecyltrimethylammonium bromide, Sigma Cat. #D8638) to the Inactivation Solution may increase the efficiency of homogenization. Prepare a 10% DTAB stock solution in water. If needed, warm the solution in a 37°C water bath to completely solubilize the DTAB. Add DTAB to the Inactivation Solution to a final concentration of 0.1–0.25% (v/v).

The following protocol measures changes in intracellular glucose.

1. Plate 20,000–50,000 cells in 96-well plates. Add compounds to the cells if treatment is part of the experimental design.
2. After the compound treatment, remove and discard the medium and wash the cells twice with 200µl of PBS per wash.
3. Add 25µl of PBS to the washed cells.
4. Add 12.5µl of Inactivation Solution. Mix by shaking the plate for 5 minutes.

   Note: PBS can be combined with Inactivation Solution and added together.
5. Add 12.5µl of Neutralization Solution. Mix by shaking the plate for 30–60 seconds.
6. Add 50µl of Glucose Detection Reagent prepared as described in Section 3.A.
7. Shake the plate for 30–60 seconds to mix.
8. Incubate for 60 minutes at room temperature.
9. Record luminescence.

4.C. Cell Cultures Using a Homogeneous Assay Format

The homogeneous Glucose-Glo™ Assay format was developed for measuring total glucose directly in the well containing cells and is well suited for high-throughput applications, such as measuring glucose production through gluconeogenesis. The protocol is applicable under experimental conditions when the total amount of glucose (extracellular glucose secreted into the medium plus the intracellular glucose concentration) is within the linear range of the assay (0.05–50µM).

1. Collect the cells, count, resuspend in glucose-free medium at 10,000–200,000 cells/ml and plate 25µl in 96-well plates. When working with cells plated in growth medium overnight, remove the medium, wash the cells with PBS and add 25µl of glucose-free medium.
2. Add 5µl of compounds diluted in the same glucose-free medium and pre-incubate for 5–15 minutes.
3. Add 10µl of glucose-free medium supplemented with 4X final concentration of glucose. For example, add 40mM glucose to give a final concentration of 10mM in the medium.
4. Incubate for the desired amount of time.

   Note: The optimal time has to be determined to make sure the glucose concentration is within the linear range of the assay.
5. Stop glucose production by adding 5µl of Inactivation Solution. Shake the plate for 3–5 minutes to mix.
6. Add 5µl of Neutralization Solution. Shake the plate for 30–60 seconds to mix.

   Note: At this point the samples can be stored at room temperature for up to 2 hours or at –20°C for longer storage.
7. Add 50µl of Glucose Detection Reagent prepared as described in Section 3.A.
8. Shake the plate for 30–60 seconds to mix.
9. Incubate for 60 minutes at room temperature.
10. Record luminescence.

An example of glucose production through gluconeogenesis using spheroid hepatocytes is shown in Figure 6. The cells were in transparent plates and required a transfer step to a luminometer assay plate. Transfer may not be required if the cells are cultured in plates compatible with luminescent measurements.

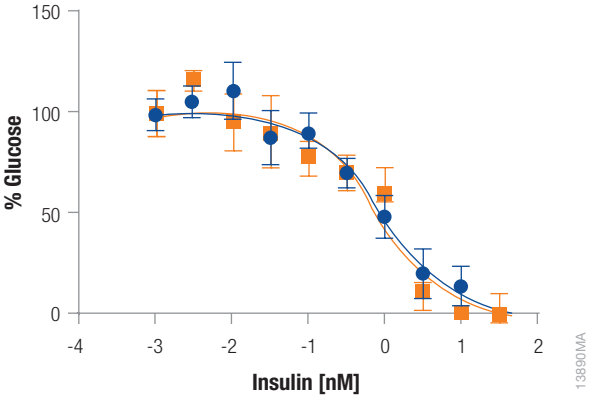

Figure 6. Insulin-mediated inhibition of gluconeogenesis in iPSC-derived human liver spheroids. Human liver spheroids were prepared from iCell® Hepatocytes 2.0 (Cellular Dynamics International). Cells were thawed and cultured for 5 days on collagen I prior to dissociating them, supplementing the cell resuspension with extracellular matrix, and then re-plating them at ~2,000 cells/well into a GravityTRAP™ Ultra-Low Attachment 96-well spheroid plate (InSphero). On the day of the assay, the spent medium was removed and the spheroids were washed twice to remove any residual glucose. The spheroids were incubated for 1.5 hours in gluconeogenesis medium: a modified, glucose-free Krebs-Ringer buffer supplemented with 10mM lactate and 2µM forskolin to inhibit glycolysis and promote hepatic glucose production. Next, the spheroids were washed twice and treated for 6 hours with gluconeogenesis medium (50µl) supplemented with increasing insulin concentrations to inhibit glucose production. After 6 hours, 25µl was removed from each well to a 96-well assay plate and an equal volume of Glucose Detection Reagent was added. To the 25µl remaining on the spheroids, 12.5µl Inactivation Solution was added and the plate was shaken for 5 minutes. An equal volume of Neutralization Solution was then added to each well. After mixing, Glucose Detection Reagent (50µl/well) was added and the plate was incubated at room temperature. After 60 minutes, a portion of each sample was transferred to a luminometer plate and luminescence was recorded using a GloMax® instrument. Similar results were obtained when glucose detection was performed using the medium (blue) or from cell lysates (orange).

4.D. Measuring Multiple Metabolites from One Sample

Four metabolites important to the energetic state of the cell—glucose, lactate, glutamate and glutamine—can be measured in parallel using the bioluminescent Glucose-Glo™ (Cat.# J6021), Lactate-Glo™ (Cat.# J5021), Glutamine/Glutamate-Glo™ (Cat.# J8021) and Glutamate-Glo™ (Cat.# J7021) Assays. Sample processing compatible with all of the bioluminescent metabolite assays allows the same sample to be used for detection of all four metabolites. This includes sample types such as culture media, sera, plasma and tissues.

When measuring metabolites in medium, only a small amount of sample is required for any assay. Therefore, cells can be grown in multiwell plates, and medium (2–5µl) can be collected at multiple time points from the same well. All four metabolites can then be assayed from the same collected medium samples (Figure 7).

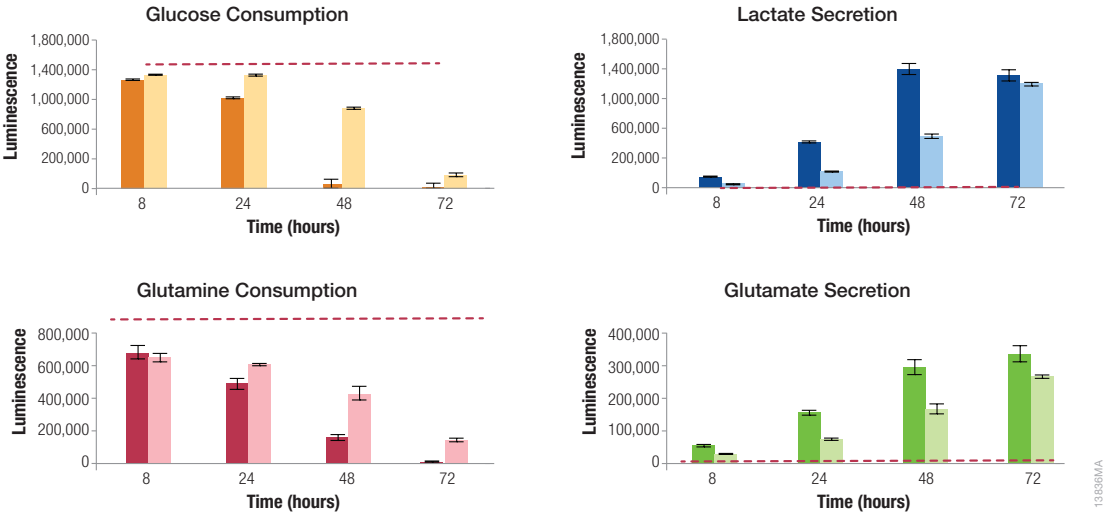

Figure 7. Measuring extracellular metabolites. A549 cells were plated at 15,000 (dark bars) or 5,000 (light bars) cells/well in 100µl DMEM with 5mM glucose, 2mM glutamine and 10% dialyzed serum. At the indicated time points, 2.5µl of medium was removed, diluted in 97.5µl PBS and stored frozen at –20°C. At the end of the experiment, samples were thawed and aliquots were transferred to a 384-well plate. Each sample was transferred into four wells, one for each metabolite. The following volumes were used from the thawed sample to detect each of the four metabolites: 25µl for lactate, 12.5µl plus an additional 12.5µl PBS for glucose, 12.5µl for glutamine and 12.5µl for glutamate. The metabolites were then detected using the Lactate-Glo™, Glucose-Glo™, and Glutamine/Glutamate-Glo™ Assays, respectively. Luminescence was recorded using a Tecan instrument. The red lines depict the signals from control wells containing medium but no cells.

4.E. Tissues

The Glucose-Glo™ Assay can be used to measure the glucose concentration in homogenized tissues (Table 4). We recommend homogenizing the tissues in 50mM Tris (pH 7.5) pre-mixed with Inactivation Solution (8:1 v/v) at 3–15 mg of tissue/ml. Other buffers, such as RIPA, can be used but should be tested for compatibility with the Glucose-Glo™ Assay. If other buffers are used, the Inactivation Solution should be added immediately after tissue homogenization. After homogenization, treat the samples with Neutralization Solution (the same volume as the Inactivation Solution) and, if necessary, diluted to the linear range of the assay. As a starting point, we recommend using use 0.05–0.3mg of tissue (0.005–0.03mg of protein) in a 50µl reaction volume.

See Table 3, Section 4.B for preparation of Inactivation and Neutralization Solutions.

1. Slice frozen tissue and place in a pre-weighed tube. Target 3–15mg tissue per slice. Weigh the sample and pre-weighed tube, then subtract the weight of the tube to get the tissue weight. Immediately place samples on dry ice.
2. Premix 50mM Tris (pH 7.5) buffer (Homogenization Buffer) with Inactivation Solution at a 8:1 ratio (e.g., 1ml buffer + 0.125ml of Inactivation Solution) and add 1.125ml for every 3–15mg of frozen tissue.
3. Homogenize for 20–30 seconds using a Tissue Tearor (BioSpec Cat.# 985370-07) or other mechanical homogenization.
4. Neutralize the tissue homogenate by adding 0.125ml of Neutralization Solution per 1.125ml of homogenate.

   Note: Samples of tissue homogenate can be used for protein determination. If detergents such as DTAB are included in the homogenization protocol, we recommend using the Pierce 660nm Protein Assay with Ionic Detergent Compatibility Reagent.
5. Make a sample dilution buffer by premixing Homogenization Buffer with Inactivation Solution and Neutralization Solution at a 8:1:1 ratio (1ml + 0.125ml + 0.125ml).
6. Dilute the tissue homogenate to fit the linear range of the assay.
7. Transfer 50µl of prepared samples into a 96-well assay plate.

   Note: The samples can be transferred directly. The centrifugation step commonly used by other methods is not required.
8. Add 50µl of Glucose Detection Reagent prepared as described in Section 3.A.
9. Shake the plate for 30–60 seconds to mix.
10. Incubate for 60 minutes at room temperature.
11. Record luminescence.

Table 4. Glucose in Tissues

Sample	RLUs
Assay background	1,297 ± 130
Control (25µM glucose)	1,428,224 ± 77,378
Glucose in liver tissue	1,074,110 ± 10,373

A sample of frozen mouse liver tissue (Bioreclamation IVT) was homogenized, neutralized, and diluted as described in Section 4.E to yield 3mg of tissue/ml (0.3mg/ml protein). Dilutions were prepared and aliquots were transferred to a 96-well assay plate. Wells containing 50µl of dilution buffer with or without 25µM glucose were included in the same plate as controls. The measurements were done in triplicate. The glucose concentration in the sample was approximately 75µM and the luminescent signal was 828-fold over background.

4.F. Plasma and Serum

Concentrations of glucose in plasma and serum (3–7mM) will be above the linear range of the glucose assay and, therefore, samples must be diluted to 0.05–50µM glucose. The sensitivity of the assay requires that only a small amount of plasma or serum be used, e.g., 10µl of sample diluted 150-fold or more.

1. Dilute the plasma or serum sample 150- to 500-fold in PBS. Multiple dilutions can be tested.
2. Transfer 50µl of diluted plasma or serum into the wells of a white 96-well assay plate.
3. Add 50µl of Glucose Detection Reagent prepared as described in Section 3.A.
4. Shake the plate for 30–60 seconds to mix.
5. Incubate for 60 minutes at room temperature.
6. Record luminescence.

Note: If plasma or serum is diluted less than 10-fold, the Inactivation and Neutralization Solutions may be required to inactivate endogenous enzymes. Add 25µl of the diluted sample to a well, followed by 12.5µl of Inactivation Solution. Mix and incubate for 5–10 minutes at room temperature. Then add 12.5µl of Neutralization Solution and proceed with Step 3 above.

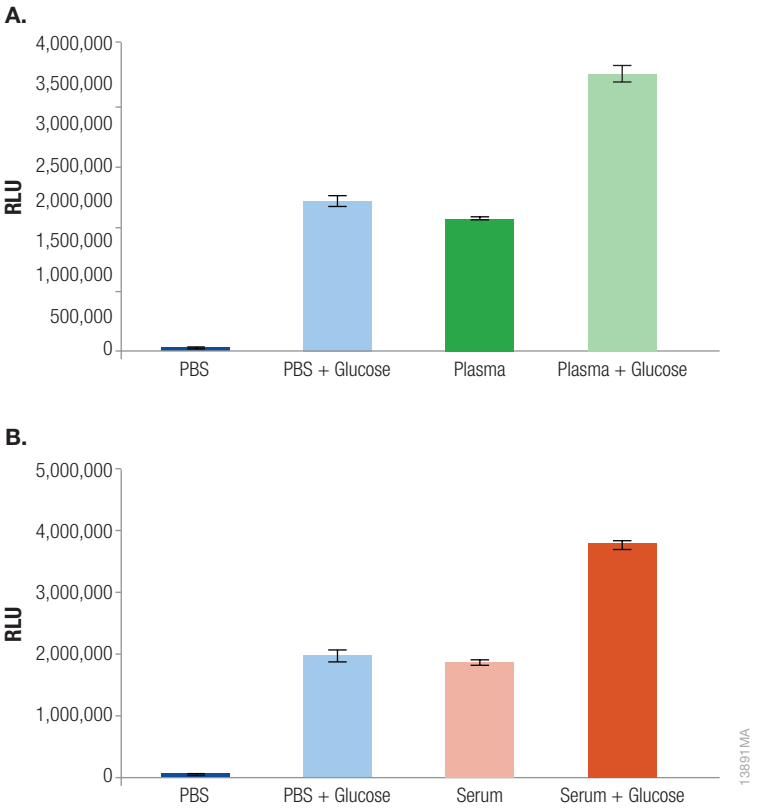

Figure 8. Glucose in human plasma and serum. Human plasma (Bioreclamation IVT Cat.# HMPLEDTA) and serum (Bioreclamation IVT Cat.# HMSRM) were diluted 500-fold in PBS. Half of each sample received a 10µM glucose spike. Aliquots (50µl) were combined with an equal volume of Glucose Detection Reagent. Luminescence signals were recorded with a GloMax® Luminometer after a 60 minute incubation at room temperature. The data represent average RLUs calculated from triplicate samples. Concentrations of glucose in the plasma and serum were determined from the glucose spike. The calculated concentration of glucose was 4.5mM for plasma samples and 5mM for serum samples.

5. Appendix

5.A. Assay Controls and Data Analysis

There is a linear relationship between luminescence signal and glucose concentration. Many luminescence measurements can be described simply in terms of RLUs. To calculate glucose concentrations, a standard curve using titrations of glucose can be used. Alternatively, a "spike" of glucose at a known concentration can be included in the experimental set up and assayed under the same experimental conditions, preferably on the same assay plate.

Different buffers can affect light output; therefore, controls should be prepared using the same buffers as the samples. The Glucose-Glo™ Assay includes 10mM Glucose as a **positive control**. Wells containing buffer only should be included as **negative controls**. These wells can be used to measure the background signal and calculate signal-to-background ratios.

5.B. Multiplexing and Normalization

The Glucose-Glo™ Assay can be multiplexed to normalize for changes in viability and to account for well-to-well variation. Changes in the glucose concentration in the medium can be measured by removing a small amount of medium (2–5µl) for glucose detection and using the remainder of the sample for RealTime-Glo™, CellTiter-Fluor™ and CellTiter-Glo® viability measurements, following the protocols provided with the respective assays.

Multiplexing intracellular glucose detection with viability assays starts by adding RealTime-Glo™ and/or CellTiter-Fluor™ reagents to the medium and measuring viability after incubation, followed by removal of the medium and lysing the cells (Section 4.B). An aliquot of the cell lysate can be removed for quantitation measurements using the CellTiter-Glo® Assay. An equal volume of Glucose Detection Reagent is added to the remainder of the cell lysate for measurement of intracellular glucose concentration.

5.C. Use of Medium and Serum

The formulations of commonly used cell culture media, such as DMEM and RPMI-1640, contain glucose, glutamine, amino acids and other components that may influence the metabolic rate of cells. Therefore, it is important to carefully define the culture medium used in assays measuring metabolic pathways. We recommend using medium lacking glucose, glutamine and pyruvate, and adding those components at the desired final concentration on the day of the experiment. We use DMEM (Gibco #14430) and add 5mM glucose and 2mM glutamine.

Supplementing the culture medium with 5–10% of fetal bovine serum (FBS) is a standard practice when culturing mammalian cells. We have found FBS may contain significant levels of metabolites that should be taken into account when planning experiments. For short-term experiments, media without serum can be used. Otherwise, we recommend using dialyzed serum (e.g., Gibco #26400036).

5.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 using.

Avoid the presence of DTT and other reducing agents in the samples to be tested. Reducing agents will react with the Reductase Substrate and increase background.

5.E. Assay Plates and Equipment

Most standard plate readers are designed for measuring luminescence and are suitable for this assay. Some instruments do not require gain adjustment, while others might require optimizing the gain settings to achieve sensitivity and dynamic range. An integration time of 0.25–1 second per well should serve as a guidance. For exact instrument settings, consult the instrument manual.

Use opaque, white multiwell plates that are compatible with your luminometer (e.g., Corning Costar® #3917 96-well or Costar® #3570 384-well plates). For cultured cell samples, white-walled, clear-bottom tissue culture plates (e.g., Corning Costar #3903 96-well plates) are acceptable. Light 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.

6. References

1. Zhou, W. *et al.* (2014) Self-Immolative Bioluminogenic Quinone Luciferins for NAD(P)H Assays and Reducing Capacity-Based Cell Viability Assays. *ChemBioChem*, **15**, 670-675.
2. Vidugiriene, J. *et al.* (2014) Bioluminescent Cell-based NAD(P)/NAD(P)H Assays for Rapid Dinucleotide Measurement and Inhibitor Screening. ASSAY and Drug Development Technologies, **12**, 514-526.
3. Leippe, D. *et al.* (2016) Bioluminescent Assays for Glucose and Glutamine Metabolism: High-Throughput Screening for Changes in Extracellular and Intracellular Metabolites. *SLAS Discovery* **22(4)**, 366–377.

7. Related Products

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

Cytotoxicity Assays

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

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 for viability, cytotoxicity and multiplex assays.

Energy Metabolism Assays

Product	Size	Cat.#
Glucose Uptake-Glo™ Assay	5ml	J1341
Glutamate-Glo™ Assay	5ml	J7021
Glutamine/Glutamate-Glo™ Assay	5ml	J8021
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
	100ml	G8001

Other sizes are available.

Oxidative Stress Assays

Product	Size	Cat.#
ROS-Glo™ H_2O_2 Assay	10ml	G8820
GSH-Glo™ Glutathione Assay	10ml	V6911
GSH/GSSG-Glo™ Assay	10ml	V6611

Other sizes are available.

Detection Instrumentation

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

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