TECHNICAL MANUAL

Lactate-Glo™ Assay

Instructions for Use of Products
J5021 and J5022

3/17
TM493

Promega

Lactate-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

1. Description 2
2. Product Components and Storage Conditions 5
3. Measuring Lactate 6
   3.A. Reagent Preparation 6
   3.B. Protocol 7
   3.C. Sample Processing 7
4. Example Protocols and Data for Various Sample Types 9
   4.A. Cell Culture Medium 9
   4.B. Cell Lysates 12
   4.C. Cell Cultures Using a Homogeneous Assay Format 14
   4.D. Measuring Multiple Metabolites From One Sample 17
   4.E. Tissues 18
   4.F. Plasma and Serum 19
5. Appendix 21
   5.A. Assay Specificity 21
   5.B. Assay Controls and Data Analysis 22
   5.C. Multiplexing and Normalization 22
   5.D. Use of Medium and Serum 24
   5.E. Temperature and Reagent Compatibility 24
   5.F. Assay Plates and Equipment 24
6. References 25
7. Related Products 25

Promega

1. Description

The Lactate-Glo™ Assay is a bioluminescent assay for rapid, selective and sensitive detection of L-Lactate in biological samples. Lactate is produced by glycolysis, a major metabolic pathway responsible for glucose homeostasis and energy production (1, 2). Once considered merely a byproduct of glycolysis, lactate is now considered an important regulatory molecule of intermediate metabolism involved in cancer development, diabetes and other diseases (3, 4).

The Lactate-Glo™ Assay couples lactate oxidation and NADH production with a bioluminescent NADH detection system (5) (Figure 1). Lactate dehydrogenase uses lactate and NAD^+ to produce pyruvate and NADH. In the presence of NADH a pro-luciferin Reductase Substrate is converted by Reductase to luciferin, which is then used in a luciferase reaction to produce light. The Lactate-Glo™ Assay contains an L-lactate selective lactate dehydrogenase to confer specificity for L-lactate, the major stereoisomer found in mammalian cells (Section 5.A).

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

The Lactate-Glo™ Assay is a versatile system that is amenable to higher-throughput formats (6) 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, or 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 plate formats and do not require sample centrifugation or spin columns.

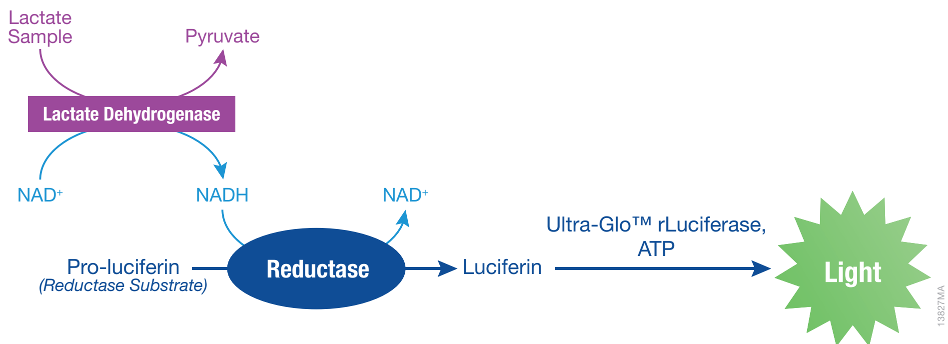

Figure 1. Schematic diagram of the Lactate-Glo™ Assay principle. Lactate dehydrogenase catalyzes the oxidation of lactate with concomitant reduction of NAD^+ to NADH. In the presence of NADH, Reductase enzymatically reduces a pro-luciferin Reductase Substrate to luciferin. Luciferin is detected in a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

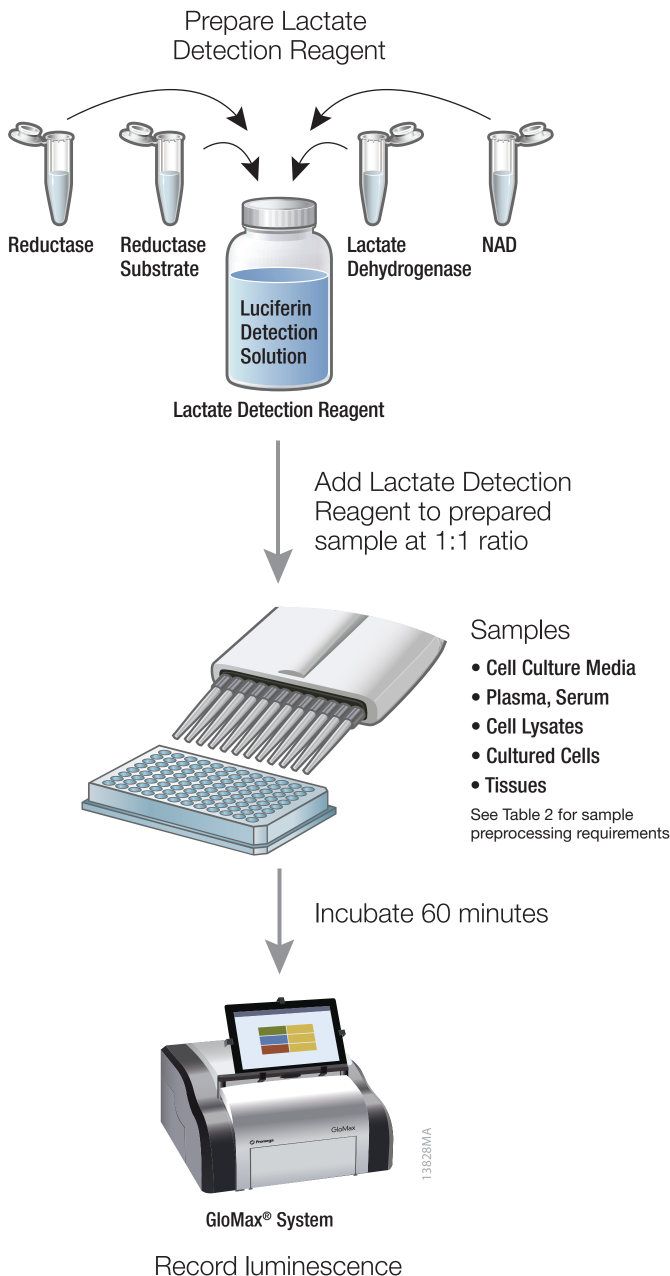

Figure 2. Lactate-Glo™ Assay reagent preparation and protocol.

1. Description (continued)

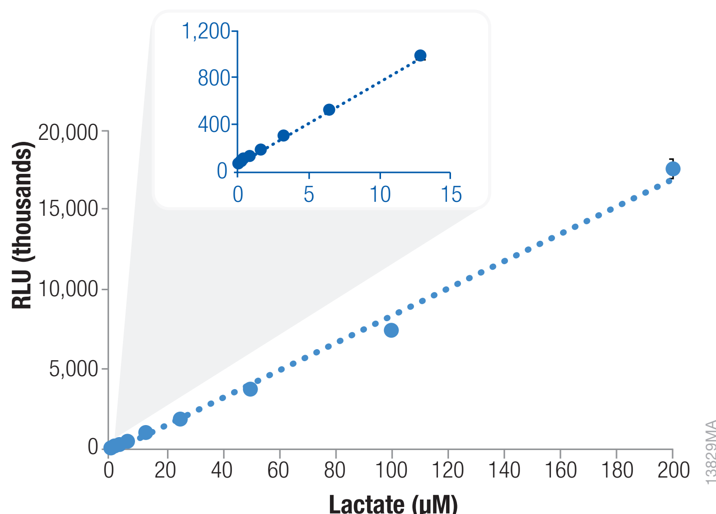

Figure 3. Lactate titration curve. Twofold serial dilutions of lactate were prepared in PBS starting from 200µM. Aliquots of the prepared standards (50µl) were transferred to a 96-well plate and the assay was performed following the protocol in Section 3.B. Data represent the average of four replicates from readings using a GloMax® Luminometer.

Table 1. Lactate Titration Data

Lactate, µM	0	0.20	0.39	0.78	1.56	3.13	6.25	12.5	25	50	100	200
Ave. RLU (thousands)	72	88	104	134	194	297	524	980	1,883	3,723	7,427	17,466
St. Dev. (thousands)	1.6	1.2	1.3	1.4	2.2	4.9	8.1	17.8	27.9	61.1	110	592
CV	2%	1%	1%	1%	1%	2%	2%	2%	1%	2%	1%	3%
S/B	1.0	1.2	1.5	1.9	2.6	4.1	7.3	13.7	26.3	52.0	103.7	224.0
S/N	-	10	20	39	70	140	281	564	1,124	2,265	4,563	10,791

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.

Promega

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
Lactate-Glo™ Assay	**5ml**	**J5021**

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

- 5ml Luciferin Detection Solution
- 55µl Reductase
- 55µl Reductase Substrate
- 1 vial Lactate Dehydrogenase
- 30µl NAD
- 50µl Lactate (10mM)

PRODUCT	SIZE	CAT.#
Lactate-Glo™ Assay	**50ml**	**J5022**

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

- 50ml Luciferin Detection Solution
- 275µl Reductase
- 275µl Reductase Substrate
- 1 vial Lactate Dehydrogenase
- 275µl NAD
- 50µl Lactate (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 Lactate

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 Lactate Detection Reagent in a 96-well plate. The assay can be adapted to other volumes provided the 1:1 ratio of Lactate Detection Reagent volume to sample volume is maintained (e.g., 12.5µl of sample and 12.5µl Lactate 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. Reconstitute Lactate Dehydrogenase with 275µl of water. Gently mix to dissolve and put on ice.
3. Immediately before use, prepare Lactate Detection Reagent as shown in the table below. The amount of Lactate 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
Lactate Dehydrogenase	0.25µl	25µl
NAD	0.25µl	25µl

Note: For best results, we recommend preparing the Lactate Detection Reagent immediately before use. The background will increase with time at room temperature; for example, a fourfold increase in background was measured after 8 hours at room temperature.

4. Mix by gently inverting five times.

Note: Return unused Luciferin Detection Solution, Reductase, NAD and Lactate 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 Lactate 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 lactate control into a 96-well plate. Include a negative control (buffer only) for determining assay background.
2. Add 50µl of Lactate 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 lactate is consumed and the signal plateaus. At any time point the signal is directly proportional to the lactate concentration.
5. Record luminescence using a plate-reading luminometer as directed by the luminometer manufacturer.

3.C. Sample Processing

The lactate concentration in samples can vary significantly. For example, with cultured mammalian cells, the lactate concentration can range from low micromolar values in freshly added medium to millimolar levels as cells grow and secrete lactate. The presence of dehydrogenases, reduced NAD(P)H dinucleotides and other factors in the samples can affect the Lactate-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 lactate concentration ranges in samples and suggestions for sample preparation. Section 4 provides example protocols for processing various sample types.

Table 2. Recommendations for Sample Processing

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

Sample Type	Lactate Concentration in Sample	Processing Recommendations
Cell culture medium (extracellular)	200µM to 10mM	• Dilute medium sample 40- to 100-fold in PBS
Cell lysates (intracellular)	5–10µM for 20,000 cells lysed in 50µl	• 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 200µM[1]	• Cells in medium or PBS • Add Inactivation Solution (1/8 of sample volume) • Add Neutralization Solution (the same volume as Inactivation Solution)
Tissues	50µM if 3mg of liver tissue is homogenized in 1ml	• Tissues in homogenization buffer • Add Inactivation Solution (1/8 of sample volume)[2] • Add Neutralization Solution (the same volume as Inactivation Solution)
Plasma and serum	500µM to 2mM	• Dilute plasma or serum samples 10- to 100-fold in PBS

[1] Applicable for short incubation times (1–2 hours) when total lactate production is within the linear range of the assay.
[2] 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 Lactate-Glo™Assay can be used to measure changes in lactate concentration in mammalian cell culture medium. Mammalian culture media typically do not contain lactate. However, the lactate concentration in fetal bovine serum is in the millimolar range and must be taken into account when calculating the rate of lactate secretion. We recommend using dialyzed serum (e.g., Gibco 26400-036) that contains significantly lower lactate and will allow detection of small changes in lactate secretion with a larger assay window.

As growing cells continuously secrete lactate, the concentration of lactate in the medium will increase depending on cell type, cell density and time. Therefore, samples of the medium typically require dilution into the linear range of the Lactate-Glo™ Assay. As a starting point, if the cells are plated in fresh medium with dialyzed FBS and the secretion of lactate is measured within 1–2 hours of incubation, no dilution is required. For longer incubation times we recommend a 40–100 fold dilution in PBS.

An example showing measurement of lactate secretion 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 5,000–20,000 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 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 adhesive plate sealer and a plastic plate lid.
4. On the day of the assay, thaw the samples and transfer 50µl to a 96-well assay plate.
5. Add 50µl of Lactate Detection Reagent prepared as described in Section 3.A.
6. Shake the plate for 30–60 seconds to mix.
7. Incubate 60 minutes at room temperature.
8. Record luminescence.

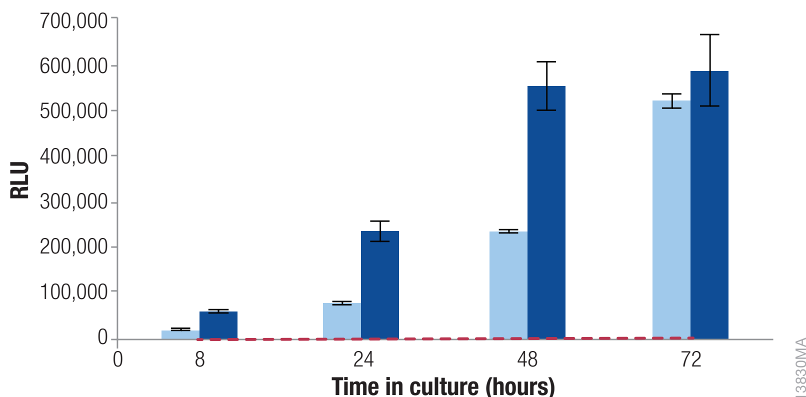

Figure 4. Lactate secretion by A549 cells. A549 cells were plated at 5,000 (light blue bars) and 15,000 (dark blue bars) cells/well in DMEM (Gibco A14430) containing 5mM glucose, 2mM glutamine and 10% dialyzed FBS (Gibco 26400036). Wells with medium only were included as controls. At indicated time points, 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 was 100-fold). A portion of the sample (25µl) was transferred to a 384-well assay plate and 25µl of Lactate Detection Reagent was added. After 60 minutes at room temperature, luminescence was read using a Tecan instrument. Data represent the average of four replicates. The red line represents the luminescence values of the medium controls (average value is 4,157 RLUs). The upper limit of the assay at 200µM lactate corresponded to 1,342,969 RLUs and is not shown on the graph. All measured samples were within the linear range of the assay and the calculated lactate concentration in the medium changed from 0.4mM to 8.3mM for 5,000 cells/well and from 1mM to 9.3mM for 15,000 cells/well.

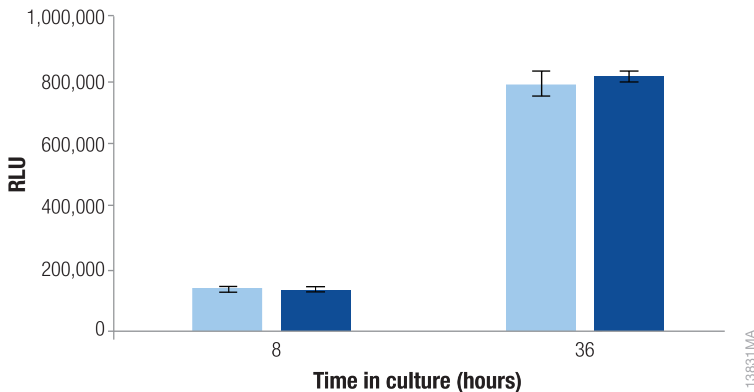

Figure 5. Lactate secretion by K562 cells. K562 cells were cultured in 75 cm^2 tissue culture flasks in RPMI (Sigma R1383) supplemented with 5mM glucose and 10% dialyzed FBS. After 8 and 36 hours, 1.5ml of cell culture was transferred into 1.5ml microcentrifuge tubes. A portion of the samples was diluted 10-fold in PBS directly (light blue bars) or after removing the cells by centrifugation (dark blue bars). The diluted samples were frozen and stored at –20°C. For lactate detection, the samples were thawed, diluted with PBS to a 40-fold final dilution and 20µl was transferred into an assay plate. An equal volume of Lactate Detection Reagent was added to the samples, and 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 lactate 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 Lactate-Glo™Assay can be used for monitoring changes in intracellular lactate levels. To measure intracellular lactate concentration, the cell culture medium must be removed and cells washed with PBS to avoid contamination from lactate in the media. Work fast and use cold PBS to minimize the changes in lactate metabolism.

After washing, the Inactivation Solution can be added directly to the cells in the 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 Lactate-Glo™ Assay. For example, perchloric acid/KOH treatment is not recommended for use with the Lactate-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 required.

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 and the release of lactate. 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 is a protocol for measuring changes in intracellular lactate.

1. Plate 5,000–50,000 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 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 Lactate Detection Reagent prepared as described in Section 3.A.
7. Shake the plate for 30–60 seconds to mix.
8. Incubate 60 minutes at room temperature.
9. Record luminescence.

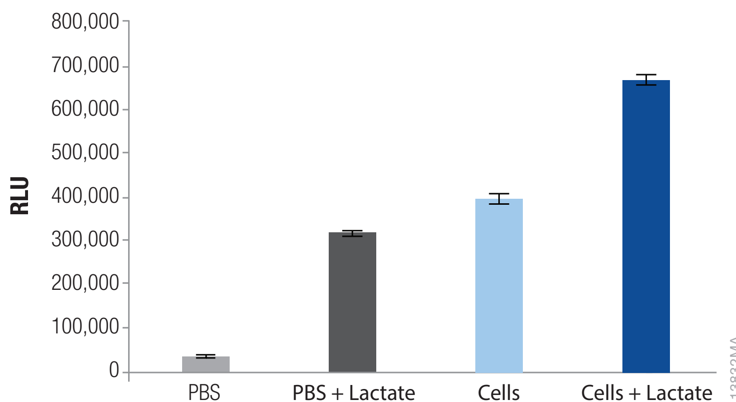

Figure 6. Intracellular lactate in A549 cells. A549 cells in DMEM (Gibco A14430) supplemented with 10% dialyzed serum, 5mM glucose and 2mM glutamine were plated at 30,000 cells/well in 96-well plates. After 24 hours incubation, the medium was removed, cells were washed twice with 200µl PBS, and then 25µl PBS or PBS containing 20µM lactate was added to the cells. PBS, and PBS containing 20µM lactate were included as controls. The cells with lactate spike were used to determine the efficiency of lactate recovery and to calculate the lactate concentration in the cell lysate. Samples were processed following the protocol in Section 4.B. Date represent the average of 6 replicates. The calculated lactate concentration in the A549 cell lysate corresponded to 24µM (0.6nmol/25µl).

4.C. Cell Cultures Using a Homogeneous Assay Format

The homogeneous Lactate-Glo™ Assay format was developed for measuring total lactate directly in the well containing cells and is well suited for high-throughput applications. The protocol is only applicable under experimental conditions when the total amount of lactate (extracellular lactate secreted into the medium plus the intracellular concentration) is within the linear range of the assay (0.2–200µM).

We recommend adding compounds to cells plated in glucose-free media. In the presence of glucose, the high rate of glycolysis can generate significant amounts of lactate that will decrease the sensitivity of detecting compound-induced changes in lactate production.

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 media, remove media, wash the cells with PBS and add 25µl glucose-free media.
2. Add 5µl compounds diluted in the same glucose-free medium and pre-incubate for 5–15 minutes.
3. Add 10µl glucose-free medium supplemented with 4X final concentration of glucose. For example add 20mM glucose to give a final concentration of 5mM in the media.
4. Incubate for the desired amount of time.

   Note: The optimal time has to be determined to make sure the lactate concentration is within the linear range of the assay. Typically it is less than 2 hours after addition of glucose-containing medium.
5. Stop lactate production by adding 5µl Inactivation Solution. Shake the plate for 3–5 minutes to mix.
6. Add 5µl Neutralization Solution. Shake the plate for 30–60 seconds to mix.

   Note: At this point the samples can be stored at room temperature for short times (up to 2 hours) or at –20°C for longer storage.
7. Add 50µl of Lactate 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.

Promega

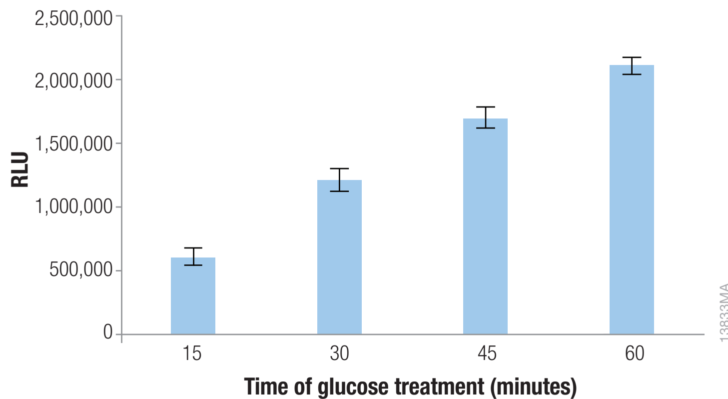

Figure 7. Lactate production over time in A549 cells. A549 cells in DMEM (Gibco A14430) with 10% dialyzed serum, 5mM glucose and 2mM glutamine were plated at 15,000 cells/well in 96-well plates. After 24 hours, medium was removed and cells were washed twice with 200µl PBS. To start lactate production, 40µl of PBS containing 5mM glucose was added to the cells. The plate was incubated at room temperature, and at the indicated time points, lactate production was stopped by adding 5µl Inactivation Solution followed by the addition of 5µl Neutralization Solution. At the end of incubation, 50µl of Lactate Detection Reagent was added to all samples and luminescence was read after 60 minutes at room temperature using a Tecan instrument. Lactate production was measured as early as 15 minutes with an approximately 17-fold increase in signal (610,185 RLU) above background (34,492 RLU). The light continued to increase over time and reached 60-fold above background after 60 minutes, with the signal remaining within the linear range of the lactate detection assay.

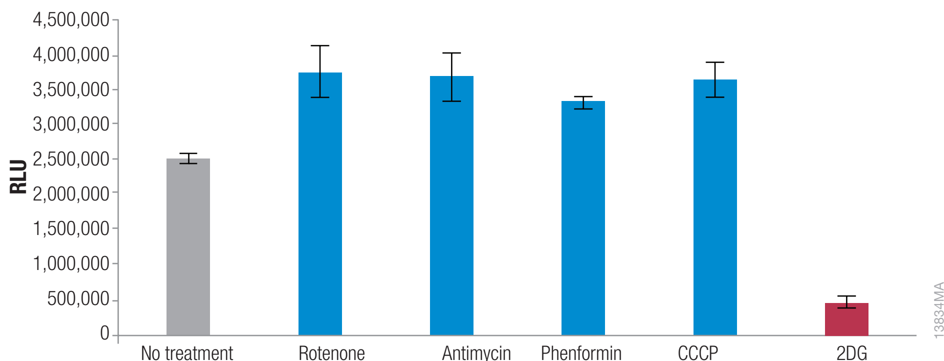

Figure 8. Changes in glycolytic rate. A549 cells in DMEM (Gibco A14430) supplemented with 10% dialyzed serum, 5mM glucose and 2mM glutamine were plated at 15,000 cells/well in 96-well plate. After 24 hours, medium was removed and cells were washed twice with 200µl PBS. Thirty microliters of glucose-free medium (Gibco A14430) containing mitochondrial inhibitors (5µM rotenone, 5µM antimycin, 2.5mM phenformin, 50µM CCCP), glycolysis inhibitor (10mM 2-deoxyglucose) or no compound was added to the samples. After 5 minutes pre-treatment at room temperature, 10µl of medium containing 4mM glucose (1mM final concentration) was added to the samples. The plate was incubated at 37°C, 5% CO_2 and after 1 hour the samples were processed following the protocol described in Section 3.B. The data shows an increase in glycolysis in response to compounds impacting mitochondrial function as determined by increased lactate production. Inhibition of glycolysis by 2DG showed 80% decrease in lactate production compared to control samples. The compound effect was greater when the experiments were set up at 1mM glucose concentration (7, 8).

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 9).

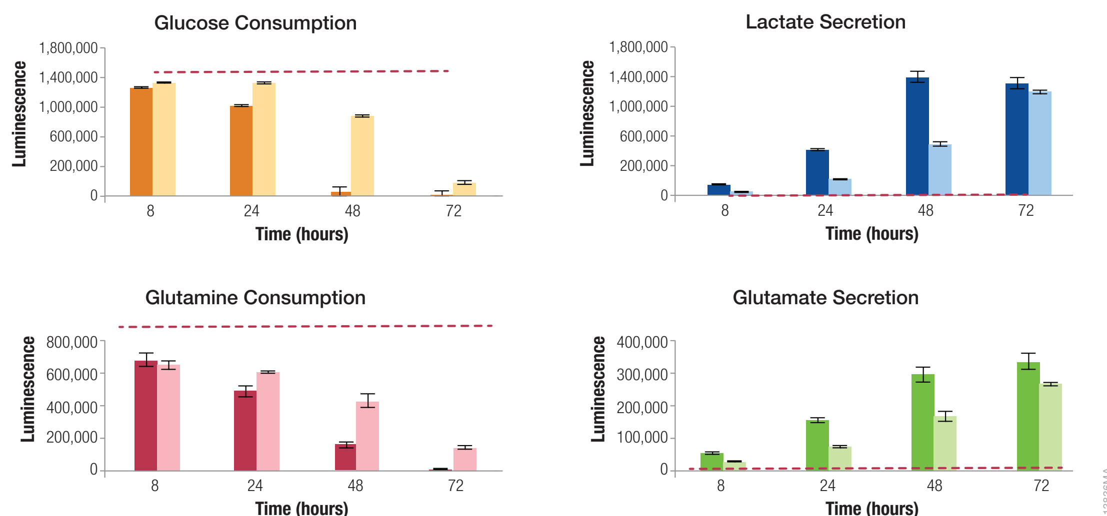

Figure 9. Measuring Extracellular Metabolites. A549 cells were plated at 15,000 (dark bars) or 5,000 (light bars) cells/well in 100µl DMEM with 5mM glucose, 2mM glutamine and 10% dialyzed serum. At the indicated time points, 2.5µl of medium was removed, diluted in 97.5µl PBS and stored frozen at –20°C. At the end of the experiment, samples were thawed and aliquots were transferred to a 384-well plate. Each sample was transferred into 4 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 Lactate-Glo™ Assay can be used to measure the lactate concentration in homogenized tissues. 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 Lactate-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 dilute 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 50µl reaction volume.

Note: Recipes for Inactivation and Neutralization Solutions are provided in Table 3 (Section 3.B).

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 an 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 or other mechanical homogenization.
4. Neutralize the tissue homogenate by adding 0.125ml 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 an 8:1:1 ratio.
6. Dilute the tissue homogenate to fit the linear range.
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 Lactate Detection Reagent prepared as described in Section 3.A.
9. Shake the plate for 30–60 seconds to mix.
10. Incubate at room temperature for 60 minutes.
11. Record luminescence.

Table 4. Lactate in Tissues

Sample	RLUs
Assay background	104,092 ± 7,209
Control (50µM lactate)	6,879,187 ± 52,068
Lactate in liver tissue	1,834,679 ± 34,482

A sample of frozen mouse liver tissue (BioreclamationIVT) was homogenized, neutralized and diluted in dilution buffer to yield 3mg of tissue/ml (0.3mg protein/ml). Dilutions were prepared and aliquots (50µl) transferred to a 96-well assay plate. Wells containing 50µl of dilution buffer with or without 50µM lactate were included in the same plate as controls. The measurements were done in triplicate. The lactate concentration in the sample was about 13.3µM and the luminescent signal was 17-fold above background.

4.F. Plasma and Serum

Concentrations of lactate in plasma and serum (0.5–2mM) will typically be above the linear range of the Lactate-Glo™ Assay and, therefore, plasma and serum samples must be diluted to 10–200µM lactate. The sensitivity of the assay requires that only a small amount of plasma or serum be used, e.g., 10µl diluted 10-fold or more.

1. Dilute the plasma or serum sample 10- to 100-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 Lactate Detection Reagent prepared as described in Section 3.A.
4. Shake the plate for 30–60 seconds to mix.
5. Incubate at room temp for 60 minutes.
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 Inactivation Solution. Mix and let sit for 5–10 minutes at room temperature. Then add 12.5µl Neutralization Solution and proceed with Step 3 above.

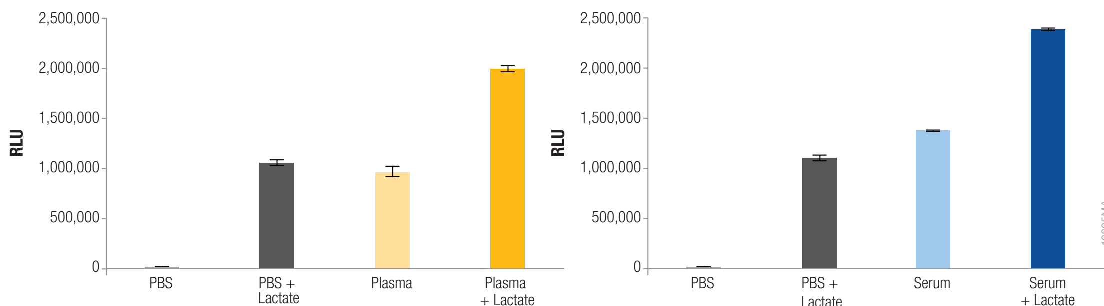

Figure 10. Lactate in human plasma and serum. Human plasma (BioreclamationIVT Cat. # HMPLEDTA) and serum (BioreclamationIVT Cat. # HMSRM) were diluted 40-fold in PBS. Half of each dilution received a 25µM lactate spike. Aliquots (50µl) were transferred to triplicate wells of a 96-well assay plate and 50µl Lactate Detection Reagent was added. Luminescence signals were recorded after a 60 minute incubation at room temperature with a GloMax® Luminometer. The data represent average RLUs calculated from triplicate samples. Concentrations of lactate in the plasma and serum were determined from the lactate spike. The calculated concentration of lactate was 1mM for plasma samples and 1.35mM for serum samples.

5. Appendix

5.A. Assay Specificity

The Lactate-Glo™ Assay uses L-lactate specific lactate dehydrogenase with >400-fold higher specificity for L-lactate compared to D-lactate. However, the core assay chemistry can be used for measuring D-lactate by using D-Lactate specific dehydrogenase as shown in Figure 11. When L-lactate specific dehydrogenase was replaced with D-lactate specific dehydrogenase in the Lactate Detection Reagent no significant signal was observed with L-lactate while a robust signal was obtained with D-lactate.

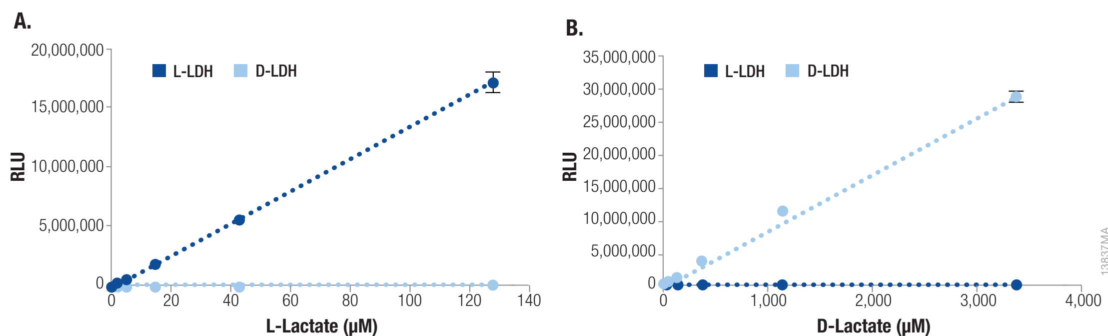

Figure 11. Specificity for L-lactate. L- or D-lactate were serially diluted 3-fold in PBS. Lactate Detection Reagents were prepared as described in Section 3.A using L-specific lactate dehydrogenase (L-LDH, provided in the kit) or D-specific lactate dehydrogenase (D-LDH, from Lactobacillus leichmanii, Sigma Cat. # L3888, prepared as 1000U/ml stock). To start the reaction 50µl of L- or D-lactate controls were transferred into 96-well plates and 50µl of Lactate Detection Reagent containing L-LDH (dark blue symbols) or D-LDH (light blue symbols) was added. Luminescence was recorded after a 60 minute incubation at room temperature.

5.B. Assay Controls and Data Analysis

There is a linear relationship between luminescence signal and lactate concentration, and many luminescence measurements can be described simply in terms of RLUs. The most important information is the change in RLUs that occurs between the experimental controls and test conditions. To calculate lactate concentration, a standard curve using a titration of lactate can be used. Alternatively, a "spike" of lactate 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 Lactate-Glo™ Assay includes 10mM Lactate 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.C. Multiplexing and Normalization

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

Multiplexing intracellular lactate 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 CellTiter-Glo® and protein assays. An equal volume of Lactate Detection Reagent is added to the remainder of the cell lysate for measurement of intracellular lactate concentration.

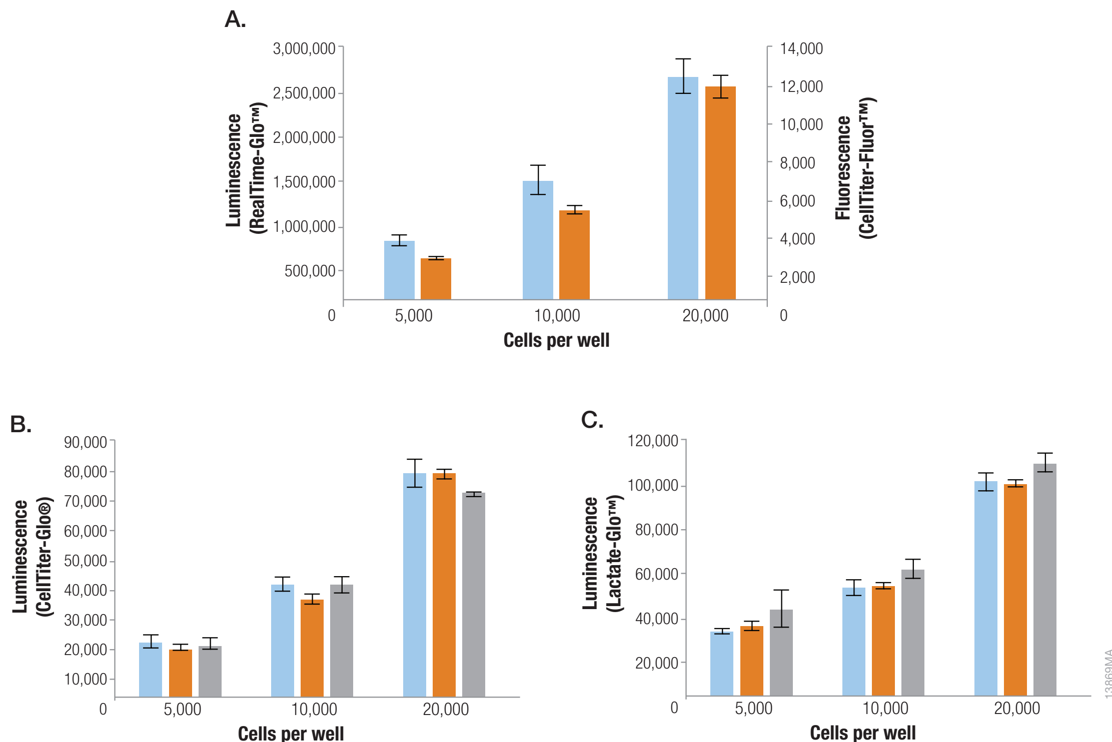

Figure 12. Multiplexing with viability assays. A549 cells were diluted in DMEM with 5mM glucose, 2mM glutamine, and 10% dialyzed serum at three cell densities (100µl per well). After overnight incubation, 25µl of 5X RealTime-Glo™ (blue bars) or CellTiter-Fluor™ (orange bars) was added to the wells. Medium without viability reagent was added to a third set of wells (grey bars). After 30 minutes at 37°C, luminescence and fluorescence was recorded. All medium was removed and the cells were washed twice with cold PBS. After the second wash, 25µl of PBS was added to each well of cells followed by 12.5µl of Inactivation Solution. After 5 minutes mixing on a plate shaker, 12.5µl of Neutralization Solution was added to the wells. An aliquot (2µl) of the cell lysate was removed to a 384-well luminometer plate and mixed with an equal volume of CellTiter-Glo™ reagent. After 10 minutes at room temperature, luminescence was recorded. The remaining cell lysate was combined with an equal volume of Lactate Detection Reagent. After 60 minutes at room temperature, the luminescence was recorded using a GloMax® luminometer. A linear increase in signal with increasing amounts of cells was detected with the viability assays (Panels A and B). The addition of viability reagents to the medium had no effect on lactate measurements (Panel C).

Promega

5.D. 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 glycolytic rate of cells. Therefore, it is important to carefully define the culture medium used in assays measuring glycolysis or other 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. FBS often contains high levels (1.3–3.5mM) of lactate. For short term experiments, medium without serum can be used. Otherwise, we recommend using dialyzed serum (e.g., Gibco 26400-036), which contains substantially lower amounts of lactate (0.01–0.02mM).

5.E. 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.F. 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 in this technical manual vary depending on the plates and luminometers used to generate the data.

6. References

1. Philp, A., Macdonald, A.L. and Watt, P.W. (2005) Lactate–a signal coordinating cell and systemic function. *Journal of Experimental Biology,* **208**, 4561-4575.
2. Goodwin, M.L. *et al.* (2015) Lactate and cancer: revisiting the Warburg effect in an era of lactate shuttling. *Frontiers in Nutrition*, **1**, Article 27, 1-3.
3. Doherty, J.R. and Cleveland, J.L. (2013) Targeting lactate metabolism for cancer therapeutics. *Journal of Clinical Investigation*, **123(9)**, 3685-3692.
4. Arbelaez, A.M. and Cryer, P.E. (2013) Lactate and the mechanism of hypoglycemia-associated autonomic failure in diabetes. *Diabetes*, **62**, 3999-4001.
5. 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.
6. 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. Birsoy, K. *et.al.* (2014) Metabolic determinants of cancer cell sensitivity to glucose limitation and biguanides. *Nature*, **508**, 108–112.
8. Gui, D. Y. *et al.* (2016) Environment Dictates Dependence on Mitochondrial Complex I for NAD+ and Aspartate Production and Determines Cancer Cell Sensitivity to Metformin. *Cell Metabolism*, **24**, 1–12.

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.

Metabolism Assays

Product	Size	Cat.#
Glucose Uptake-Glo™ Assay	5ml	J1341
Glucose-Glo™ Assay	5ml	J6021
Glutamate-Glo™ Assay	5ml	J7021
Glutamine/Glutamate-Glo™ Assay	5ml	J8021
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 Instruments

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

Promega

U.S. Pat. No. 9,273,343 and other patents pending.

U.S. Pat. No. 6,602,677, 7,241,584, 8,030,017 and 8,822,170 and other patents pending.

© 2017 Promega Corporation. All Rights Reserved.

CellTiter-Glo and GloMax are registered trademarks of Promega Corporation. Lactate-Glo, CellTiter-Fluor, CellTox, CytoTox-Fluor, CytoTox-Glo, Glucose-Glo, Glucose Uptake-Glo, Glutamate-Glo, Glutamine-Glo, Glutamine/Glutamate-Glo, GSH-Glo, GSH/GSSG-Glo, NAD/NADH-Glo, NADP/NADPH-Glo, NAD(P)H-Glo, RealTime-Glo, ROS-Glo and Ultra-Glo are trademarks of Promega Corporation.

Products may be covered by pending or issued patents or may have certain limitations. Please visit our Web site for more information.

All prices and specifications are subject to change without prior notice.

Product claims are subject to change. Please contact Promega Technical Services or access the Promega online catalog for the most up-to-date information on Promega products.