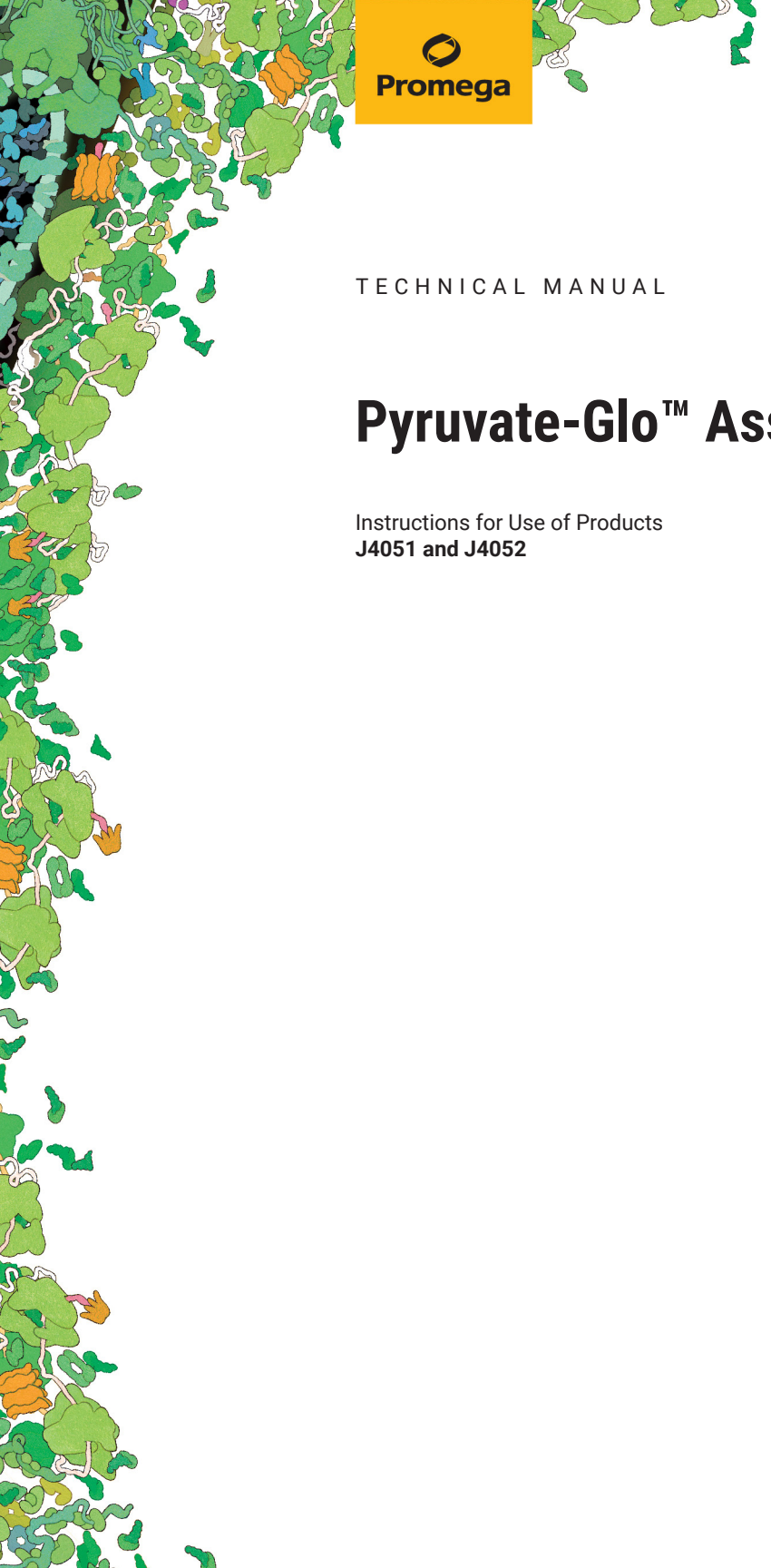

TECHNICAL MANUAL

Pyruvate-Glo™ Assay

Instructions for Use of Products
J4051 and J4052

Revised 1/24
TM709

Pyruvate-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

The Pyruvate-Glo™ Assay is a bioluminescent assay for rapid and sensitive detection of pyruvate. Pyruvate is a key energy metabolite that is an intermediate in various cell biochemical pathways, including the TCA cycle and glycolysis.

The Pyruvate-Glo™ Assay uses pyruvate oxidase enzyme to produce acetyl phosphate and H_2O_2 in the presence of pyruvate, followed by H_2O_2 measurement using a bioluminescent H_2O_2 detection technology. The result is a light signal proportional to the starting pyruvate concentration in the sample (Figure 1).

The Pyruvate-Glo™ Assay is a two-step assay that involves an initial incubation with pyruvate oxidase and H_2O_2 Substrate, followed by addition of Pyruvate -Glo™ detection reagent. Results are available in less than 90 minutes (Figure 2). The assay is linear to 50µM pyruvate and sensitive to <800nM (Figure 3, Table 1).

The Pyruvate-Glo™ Assay is versatile and compatible with many sample types. However, as with other enzyme coupled pyruvate detection methods, enzymes and H_2O_2 in the samples can interfere with the assay. Sample preparation such as deproteinization may be required for complex sample types, including serum, to limit assay interference. The workflows are compatible with 96- and 384-well plate formats and well suited for rapid screening of multiple samples.

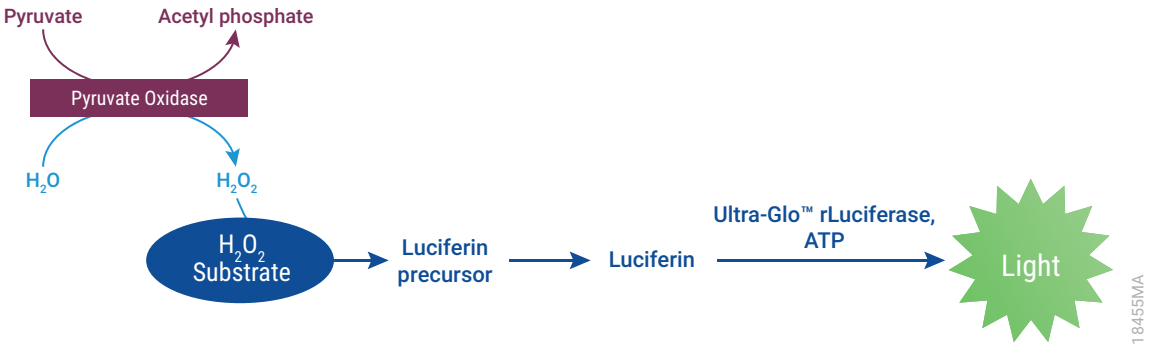

Figure 1. Schematic diagram of the Pyruvate-Glo™ Assay principle. Pyruvate Oxidase catalyzes the reaction of pyruvate to acetyl phosphate, producing H_2O_2. In the presence of H_2O_2, the H_2O_2 Substrate creates a luciferin precursor. Adding Pyruvate-Glo™ detection reagent converts a luciferin precursor to luciferin, which is detected using Ultra-Glo™ rLuciferase. The amount of light produced is proportional to the amount of pyruvate in the sample.

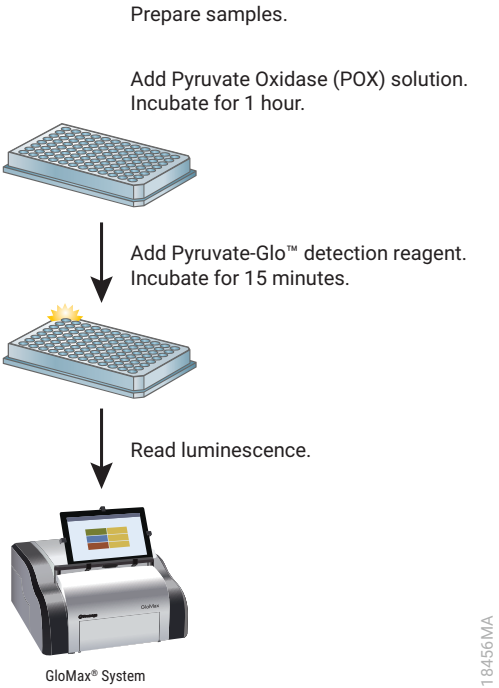

Figure 2. Pyruvate-Glo™ Assay protocol.

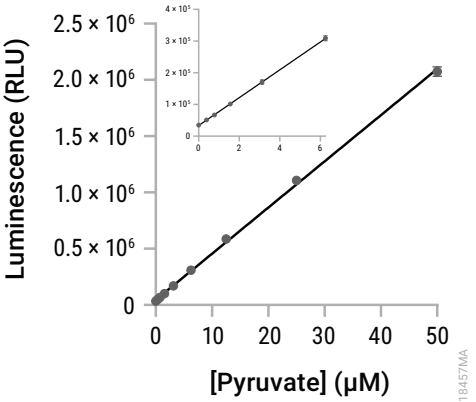

Figure 3. Pyruvate titration curve. Twofold serial dilutions of pyruvate were prepared in PBS starting with 50µM. Aliquots (30µl) of the prepared standards were transferred to a 96-well plate and the assay was performed following the protocol in Section 3.C. Data represent the average of four replicates from readings using a GloMax® Discover System.

1. Description (continued)

Table 1. Pyruvate Titration Data. Luminescence is shown in relative light units (RLU). The signal-to-background ratio (S/B) was calculated by dividing the mean sample signal by the mean negative control signal (no pyruvate). The signal-to-noise ratio (S/N) was calculated by dividing the net signal (mean signal minus mean negative control) by the standard deviation of the negative control.

Pyruvate (µM)	0	0.39	0.78	1.56	3.13	6.25	12.5	25	50
Average Luminescence (RLU × 10^3)	44	118	188	336	630	1,204	2,353	4,487	8,222
Standard Deviation (RLU × 10^3)	1.2	3.9	4.6	8.9	21.9	37.1	51.2	156.3	232.5
Coefficient of Variation (%)	3	3	2	3	3	3	3	2	3
S/B	1.0	2.7	4.3	7.7	14.4	27.5	53.8	102.5	187.9
S/N		61	120	243	488	967	1,924	3,702	6,815

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
Pyruvate-Glo™ Assay	**5ml**	**J4051**

The system contains sufficient reagents to perform 100 reactions in 96-well plates. Includes:

- 5ml Luciferin Detection Solution
- 2ml H_2O_2 Substrate Dilution Buffer
- 40µl H_2O_2 Substrate, 10mM
- 130µl Pyruvate Oxidase (POX)
- 100µl D-Cysteine, 100X
- 100µl Signal Enhancer Solution
- 50µl Pyruvate, 10mM
- 15ml 0.6N HCl
- 15ml Neutralization Buffer

PRODUCT	SIZE	CAT.#
Pyruvate-Glo™ Assay	**50ml**	**J4052**

The system contains sufficient reagents to perform 1,000 reactions in 96-well plates. Includes:

- 50ml Luciferin Detection Solution
- 10ml H_2O_2 Substrate Dilution Buffer
- 200µl H_2O_2 Substrate, 10mM
- 1.3ml Pyruvate Oxidase (POX)
- 500µl D-Cysteine, 100X
- 500µl Signal Enhancer Solution
- 50µl Pyruvate, 10mM
- 15ml 0.6N HCl
- 15ml Neutralization Buffer

Storage Conditions: Store complete kits at –30°C to –10°C. Store Neutralization Buffer and 0.6N HCl at +2°C to +10°C or at room temperature. **Do not** freeze-thaw the kit components more than three times. As needed, dispense kit components into single-use aliquots to minimize freeze-thaw cycles

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

3. Measuring Pyruvate

Materials to Be Supplied by the User

- phosphate-buffered saline (PBS; e.g., Sigma Cat.# D8537 or GIBCO™ Cat.# 14190)
- 96-well assay plates (opaque white-walled with white or clear bottom; e.g., Corning® Cat.# 3903 or 3912)
- luminometer (e.g., GloMax® Discover System, Cat.# GM3000)

3.A. Assay Considerations and Sample Preparation

The Pyruvate-Glo™ Assay is versatile and compatible with many sample types, including cell lysates, cell culture medium and blood-based samples. However, when working with complex biological samples such as cell lysates, plasma or serum, the presence of endogenous enzymes, H_2O_2 and other factors in the samples can impact the Pyruvate-Glo™ Assay signal and background. Therefore, additional sample preparation steps are needed for optimal assay performance.

Medium Considerations for Cell Culture Experiments

Metabolism is a dynamic process guided by fuel availability. Commonly used cell culture medium, such as DMEM and RPMI 1640, contain different amounts of small metabolites, including glucose, glutamine, pyruvate, amino acids and other components. In pyruvate-containing cell culture medium, the concentration of pyruvate is typically between 1–2mM. Supplementing the culture medium with 5–10% fetal bovine serum (FBS) is a standard practice when culturing mammalian cells. FBS also contains variable levels of different metabolites. Such supplements should be considered for experimental setup and data analysis. Using defined medium, for example DMEM (GIBCO™ Cat.# A1443001), lacking major fuel sources such as glucose, glutamine and pyruvate, and adding those components at the desired concentrations supplemented with dialyzed serum (e.g., GIBCO™ Cat.# A33820-01) enables better control when studying metabolic changes and should be considered.

Washes

For measuring pyruvate, wash cells at least three times with PBS and glucose. Glucose is added to avoid disrupting cellular metabolism during washes. Since the amount of glucose varies in different medium, for consistency use the glucose concentration present in the medium in which your cells are routinely cultured. We recommend three washes, but if medium contains high levels of pyruvate (>2mM), 4–5 washes may be necessary. The final wash can be collected and assayed to confirm pyruvate depletion.

Dilutions

If pyruvate concentration is above the linear range for this assay (>50µM), we recommend diluting the sample. Dilute samples in PBS or a compatible buffer (Section 5.A.), followed by 0.6N HCl treatment and neutralization. If diluting after 0.6N HCl/neutralization treatment, use dilution buffer (PBS and 0.6N HCl and Neutralization Buffer at a 6:1:1 ratio) to maintain optimal conditions for the assay. For example, when measuring pyruvate consumption in medium, initial pyruvate concentrations can range from 1–2mM. For accurate pyruvate consumption measurements, a 20-fold or higher sample dilution is required to remain within the linear range of the assay. Further, diluting the sample also helps diminish interference with some sample types, such as serum.

0.6N HCl Addition

To minimize interference and maintain proper pH, samples should be treated with the 0.6N HCl supplied with the Pyruvate-Glo™ Assay. Adding 0.6N HCl rapidly stops metabolism, inhibits activity of endogenous proteins and lyses cells, providing access to intracellular metabolites. For sample preparation, we recommend adding 0.6N HCl as soon as samples are washed, followed by a 1:1 addition of Neutralization Buffer (1M Tris base). At this point, samples can be assayed immediately or stored below –10°C until assayed. The use of 0.6N HCl and Neutralization Buffer also aid in maintaining the optimal pH for this assay. If 0.6N HCl treatment and neutralization steps are omitted, relative light units (RLU) may decrease, reducing the assay window. If high levels of pyruvate are present and the sample is diluted at least tenfold, 0.6N HCl and base treatment steps can be omitted if desired.

Table 2. General Recommendations for Sample Preparation.

Sample	Expected Sample Pyruvate Concentration	Recommendations
Cell culture medium (extracellular)	100µM–2mM	• Cells in DMEM (dialyzed FBS ± pyruvate) • Remove 5–10µl of medium at desired times. • Dilute medium samples 10- to 20-fold.
Cell lysates (intracellular)	0.5–2µM (25,000 cells lysed in 30µl) 0.5–2.0fmol/cell	• Remove medium and wash cells 3–5 times in PBS with glucose. • Add 0.6N HCl (1/6 sample volume). • Add Neutralization Buffer (1/6 sample volume). • If required, dilute cells in dilution buffer (PBS and 0.6N HCl and Neutralization Buffer, 6:1:1).
Cells in culture (extracellular and intracellular)	10—50µM (25,000 cells in 30µl)	• Cells in DMEM (pyruvate-free with dialyzed FBS) • For 30µl sample, use 96-well half-area plate. • Incubate for 1—2 hours at 37°C.
Filtered serum	40–160µM (human)	• Deproteinized serum (10kDa column) • Samples diluted 10- to 40-fold in PBS or dilution buffer (above).

Note: For detailed example protocols, see Section 4. Samples may contain higher or lower concentrations of pyruvate, depending on sample type and volumes. Recommendations are based on experiments provided in this technical manual.

3.B. Reagent Preparation

This protocol is for a 100µl reaction (30µl of sample, 5µl of 0.6N HCl, 5µl of Neutralization Buffer, 10µl of Pyruvate Oxidase (POX) solution and 50µl of Pyruvate-Glo™ detection reagent) in a 96-well plate. The assay can be adapted to other volumes provided the ratio of Pyruvate-Glo™ detection reagent volume to sample volume and POX solution is maintained (Section 3.C.).

1. Thaw all components on ice or at room temperature. Once thawed, equilibrate the Luciferin Detection Solution to room temperature; place all other components on ice. Thaw H_2O_2 Substrate at room temperature immediately before use, and then place on ice after use. Mix thawed components to ensure homogeneous solutions prior to use.
2. Immediately before use, prepare 10µl of Pyruvate Oxidase (POX) solution per assay well.

   Note: Amount per reaction is for a 96-well plate format. Prepare the amount of reagent needed for your experiment, factoring in some volume that may be lost during pipetting. **Do not** store unused POX solution.

Component	Volume Per Reaction	Volume Per 100 Reactions
H_2O_2 Substrate Dilution Buffer	10µl	1ml
H_2O_2 Substrate	0.125µl	12.5µl
Pyruvate Oxidase (POX)	1.3µl	130µl

3. Prepare 50µl of Pyruvate-Glo™ detection reagent per plate well. Mix well before addition to assay plate.

   Note: The amount per reaction is for a 96-well plate format. Prepare the amount of reagent needed for your experiment, factoring in volume that may be lost during pipetting. **Do not** store unused Pyruvate-Glo™ detection reagent.

Component	Volume Per Reaction	Volume Per 100 Reactions
Luciferin Detection Solution	50µl	5ml
D-Cysteine	0.5µl	50µl
Signal Enhancer Solution	0.5µl	50µl

3.C. Protocol

When performing the Pyruvate-Glo™ Assay, be sure to use assay plates that are compatible with your luminometer. See Section 5.B for more information. Information on preparing and using appropriate positive and negative controls for the Pyruvate-Glo™ Assay can be found in Section 3.D.

Recommended Reaction Volumes

Component	96-Well Plate	384-Well Plate
Sample/Pyruvate Standard	30µl	7.5µl
0.6N HCl	5µl	1.25µl
Neutralization Buffer	5µl	1.25µl
POX Solution	10µl	2.5µl
Pyruvate-Glo™ detection reagent	50µl	12.5µl
Total Volume	**100µl**	**25µl**

1. Prepare samples using the appropriate method for your sample type. See Sections 3.A and 4 for more information.
2. Transfer 30µl of sample or pyruvate standard (positive control) into a 96-well plate. Include negative controls (buffer only and no-POX control) for determining assay background (Section 3.D).
3. Add 5µl of 0.6N HCl and mix by shaking the plate for 5 minutes.
4. Add 5µl of Neutralization Buffer, mix well.
   Note: For standards, 0.6N HCl and Neutralization Buffer can be premixed 1:1 and 10µl added per well.
5. Add 10µl of POX solution, prepared as described in Section 3.B, to each assay well.
6. Mix by shaking the plate for 30–60 seconds.
7. Incubate for 60 minutes at room temperature.
8. Add 50µl of Pyruvate-Glo™ detection reagent as prepared in Section 3.B.
9. Mix by shaking the plate for 30–60 seconds.
10. Incubate at room temperature for 10–15 minutes.
11. Record luminescence using a plate-reading luminometer, as directed by the luminometer manufacturer.

3.D. Assay Controls and Data Analysis

With the Pyruvate-Glo™ Assay, there is a linear relationship between luminescence signal and pyruvate concentration. Many luminescent measurements can be described in terms of relative light units (RLU). The data can be analyzed as the change in RLU between the experimental controls and test conditions.

To calculate pyruvate concentration and determine if your samples are within the linear range of the assay, create a standard curve using a titration of 10mM Pyruvate, included in the kit. If RLU of the sample are higher than RLU of the 50µM pyruvate standard, dilute samples and reassay. Quantify pyruvate using the standard curve, after subtracting background.

As an alternative to running a full standard curve, quantitate pyruvate in unknown samples by preparing 2–4 pyruvate standard concentrations. We recommend using a high (50µM or 25µM) and a low pyruvate concentration (1–2µM). Adjust these amounts based on the expected sample pyruvate concentrations.

Wells containing buffer only should be included as negative controls. Different buffers can affect light output; therefore, controls should be prepared using the same buffers as the samples.

To test for endogenous interference in samples, you should also run no-POX controls by omitting the enzyme from POX solution to measure the luminescent signal from H_2O_2 or other assay interference. Sample preparation such as deproteinization is recommended for samples that exhibit assay interference (i.e., no-POX values are high or identical to RLU of the sample with enzyme).

Calculate pyruvate concentration in the sample with the following formula using one pyruvate standard concentration:

$$[\text{Pyruvate}] = \frac{[\text{Pyruvate standard}]\ (\mu M) \times (RLU_{sample} - RLU_{background})}{(RLU_{\text{Pyruvate standard}} - RLU_{background})}$$

For background, use either buffer or no-POX control, whichever is larger.

4. Example Protocols and Data for Various Sample Types

4.A. Pyruvate Secretion in Cell Culture Medium

The Pyruvate-Glo™ Assay can be used for monitoring secretion and consumption of pyruvate over time. For measuring either pyruvate consumption or secretion, medium and serum used for these experiments should be considered (Section 3.A). To measure pyruvate secretion, use medium without pyruvate. Some common medium contain 1–2mM of pyruvate, making it difficult to measure low levels of pyruvate secretion. For measuring pyruvate consumption, use medium containing a known concentration of pyruvate. Samples can be taken and assayed immediately or frozen for later analysis. Depending on pyruvate concentration, dilute medium to fit the linear window of the assay (Section 3.A).

1. Plate cells at desired densities and allow to adhere overnight.
2. Remove medium from wells and wash 3X with 100µl of PBS and 10mM glucose (or PBS only), then add 100µl of pyruvate-free medium.
3. Remove 5–10µl medium samples at desired timepoints.

   Note: Samples can be assayed immediately or frozen at or below –10°C.
4. Dispense 30µl of sample per assay well. Include a negative control (medium only, no cells) for determining assay background and a positive pyruvate control (1µM–10µM).

   Note: Samples may need to be diluted depending on incubation time and secretion rates. Dilute samples in PBS.
5. Add 5µl of 0.6N HCl to each assay well and mix by shaking the plate for 5 minutes.
6. Add 5µl of Neutralization Buffer to each assay well. Mix by shaking the plate for 30–60 seconds.
7. Add 10µl of POX solution, prepared as described in Section 3.B, to each assay well.

   Note: Include a no-POX control to determine any H_2O_2 interference in spent medium.
8. Mix by shaking the plate for 30–60 seconds.
9. Incubate for 60 minutes at room temperature.
10. Add 50µl of Pyruvate-Glo™ detection reagent, prepared as described in Section 3.B.
11. Mix by shaking the plate for 30–60 seconds.
12. Incubate for 10–15 minutes at room temperature.
13. Record luminescence.

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4.B. Pyruvate Consumption in Cell Culture Medium

1. Plate cells at desired densities and allow to adhere overnight.
2. Remove medium from wells and add 100µl of medium containing a known concentration of pyruvate.

   Note: Some common cell culture media have 1–2mM pyruvate added. A no-cell control with the same medium will be essential for determining the starting concentration of pyruvate.
3. Remove medium samples at desired timepoints.

   Note: Samples may be assayed immediately or frozen at –10°C or below.
4. Dilute medium sample (20-fold or more) and dispense 30µl of sample per assay well. Include a negative control (medium only, no cells) for determining starting pyruvate concentration, and a positive pyruvate control (1µM–10µM).

   Note: Dilutions may need to be optimized depending on cell type or conditions. Dilute samples in PBS.
5. Add 5µl of 0.6N HCl to each assay well and mix by shaking the plate for 5 minutes.
6. Add 5µl of Neutralization Buffer to each assay well. Mix by shaking the plate for 30–60 seconds.
7. Add 10µl of POX solution, prepared as described in Section 3.B, to each assay well.

   Note: Include a no-POX control to determine any H_2O_2 interference in spent medium.
8. Mix by shaking the plate for 30–60 seconds.
9. Incubate for 60 minutes at room temperature.
10. Add 50µl of Pyruvate-Glo™ detection reagent, prepared as described in Section 3.B.
11. Mix by shaking the plate for 30–60 seconds.
12. Incubate for 10–15 minutes at room temperature.
13. Record luminescence.

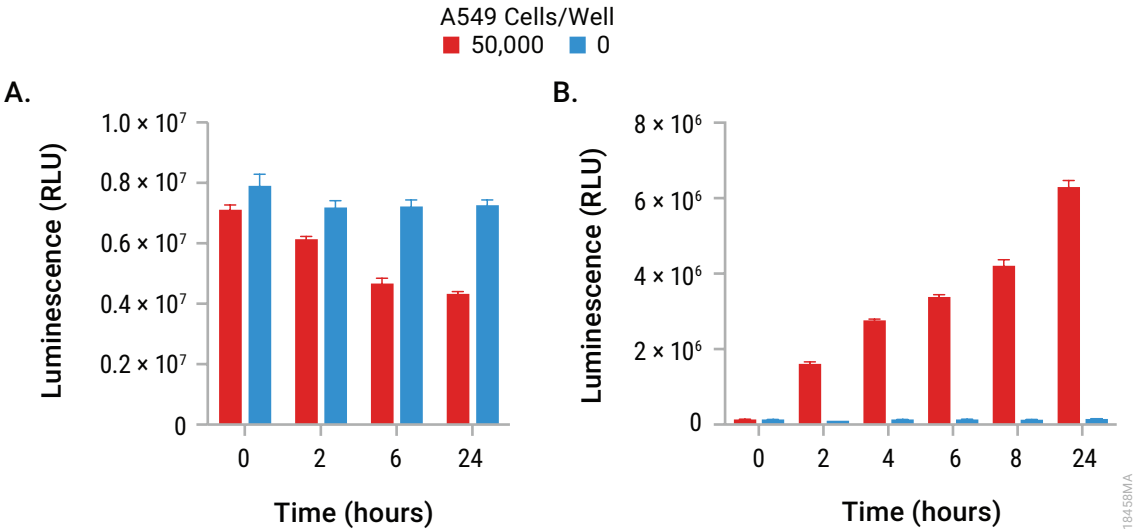

Figure 4. Pyruvate in cell medium. A549 cells (50,000 cells/well) were plated overnight, washed 3X with PBS and supplemented with DMEM (GIBCO™ Cat.# A1443001), dialyzed FBS (GIBCO™ Cat.# A33820-01), 10mM glucose and 1mM glutamine, either with 1mM pyruvate (**Panel A**) or without pyruvate (**Panel B**). Medium samples were taken between 0 and 24 hours post addition and stored at –20°C. Samples were then diluted 20-fold (consumption, **Panel A**) or 10-fold (secretion, **Panel B**). Both experiments were compared to no-cell controls, which were also removed at each time point for baseline measurements. Data represent the average of three replicates.

4.C. Collected Mammalian Cells

The Pyruvate-Glo™ Assay can be used for monitoring changes in intracellular pyruvate levels in suspension cell lines or trypsinized cells. After removing cell culture medium and washing three times with PBS and glucose (Section 3.A), cells can be collected, lysed and transferred to 96- or 384-well plates for pyruvate measurement.

Plated adherent cells are not recommended for this assay due to high assay interference. If performing this assay with adherent cells, luminescence values (RLU) of the no-POX control should be subtracted before calculating pyruvate concentrations.

1. Collect cells, wash 3X with cold PBS and 10mM glucose and resuspend in PBS at 0.2×10^5 – 1.67×10^6 cells/ml.
2. Add a 1/6 volume of 0.6N HCl. For example, add 125µl of 0.6N HCl to 750µl of cells in PBS, and mix.
3. Add the same volume of Neutralization Buffer as 0.6N HCl in Step 2 and mix.

   Note: If cells need to be diluted after neutralization, use dilution buffer (premixed PBS, 0.6N HCl and Neutralization buffer in a 6:1:1 ratio).

4.C. Collected Mammalian Cells (continued)

4. Transfer 40µl of the sample to an assay plate. Include negative (buffer) and positive (1µM pyruvate) controls.
   Note: Prepare controls in dilution buffer as described in Step 3.
5. Add 10µl of POX solution prepared as described in Section 3.B, to each assay well.
   Note: Include a no-POX control to determine any H_2O_2 or assay interference in cell lysates.
6. Mix by shaking the plate for 30–60 seconds.
7. Incubate for 60 minutes at room temperature.
8. Add 50µl of Pyruvate-Glo™ detection reagent, prepared as described in Section 3.B.
9. Mix by shaking the plate for 30–60 seconds.
10. Incubate for 10–15 minutes at room temperature.
11. Record luminescence.

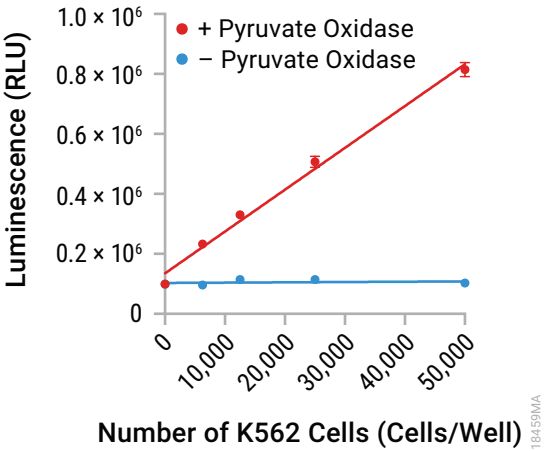

Figure 5. K562 cell titration. K562 cells were collected, washed with cold PBS and 10mM glucose and resuspended in 600µl of PBS at 1.7 × 10^6 cells/ml. 0.6N HCl (100µl) was added to the cells. The samples were neutralized with 100µl of Neutralization Buffer. Samples (40µl) were transferred to a 96-well assay plate and pyruvate was detected as described in Section 4.C. Data shows a linear relationship between light output and cell density, indicating that pyruvate is detected within the linear range of the assay. A 2.3-fold signal above assay background was measured for 6,250 K562 cells in 30µl. The calculated pyruvate concentration in K562 cells corresponded to approximately 1.5fmol/cell. Data represent the average of three replicates.

4.D. Homogeneous Assay Format

The Pyruvate-Glo™ Assay can be used in a homogeneous assay format, to measure total pyruvate in a sample (both intracellular and extracellular pyruvate). This format is well suited for compound screening, as shown in Figure 6. Cells should be collected and washed 3X with PBS and 10mM glucose and resuspended in medium containing no pyruvate.

For compound addition, 30–60µl of cells plus compounds can be incubated for 0–2 hours before addition of 0.6N HCl. We recommend using a half-area 96-well plate (e.g., Corning® Cat.# 3696) due to the low volume (30µl) of cells used. Alternatively, 60µl of cells can be plated, and half of sample can be removed from the assay for use with cell viability assays (e.g., CellTiter-Glo™ Luminescent Cell Viability Assay, Cat.# G7570; Figure 6, Panel B).

1. Collect cells, wash 3X with PBS and 10mM glucose, and resuspend in medium containing no pyruvate at 1.67×10^6 cells/ml.
2. Add 30µl of cells to a half-area 96-well plate. Include negative (medium only, no cells) and positive (1–10µM pyruvate) controls.

   Notes:

   a. Compounds can be added and incubated at this step for 0–2 hours.

   b. For cell viability measurement shown in Figure 6, 60µl of cells is plated, and post-incubation, 30µl of sample is removed and used with CellTiter-Glo® Luminescent Cell Viability Assay (Cat.# G7570; 1:1 ratio).
3. Add 5µl of 0.6N HCl to each well. Mix by shaking the plate for 5 minutes.
4. Add 5µl of Neutralization Buffer to each well. Mix by shaking the plate for 30–60 seconds.
5. Add 10µl of POX solution to each well, prepared as described in Section 3.B.
6. Mix by shaking the plate for 30–60 seconds.
7. Incubate for 60 minutes at room temperature.
8. Add 50µl of Pyruvate-Glo™ detection reagent to each well, prepared as described in Section 3.B.
9. Mix by shaking the plate for 30–60 seconds.
10. Incubate for 10–15 minutes at room temperature.
11. Record luminescence.

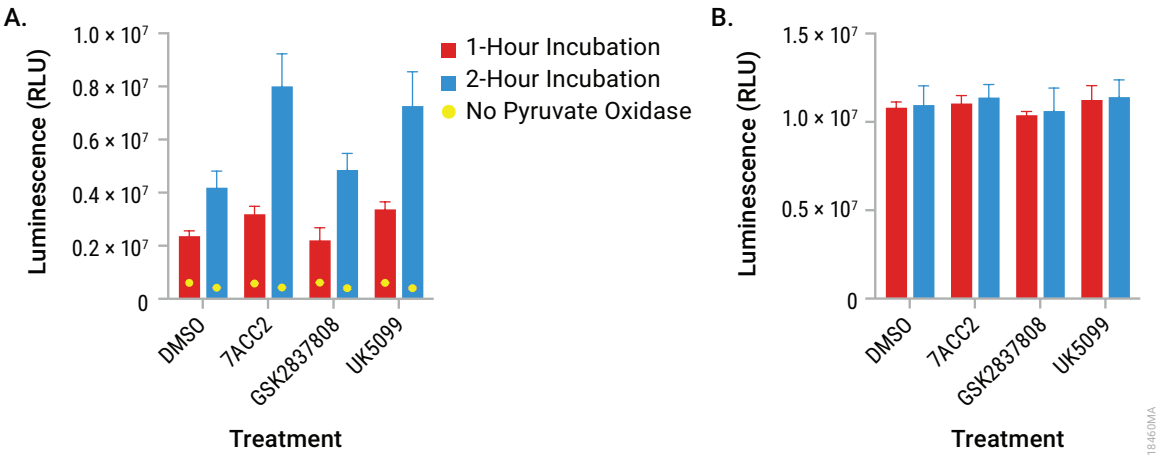

Figure 6. Homogeneous assay format for compound treatment. K562 cells were collected, washed with cold PBS and 10mM glucose and resuspended at 1.7 × 10^6 cells/ml (100,000 cells/well) in DMEM (GIBCO™, Cat.# A1443001) with dialyzed FBS (GIBCO™, Cat.# A33820-01), 10mM glucose and 1mM glutamine in a 96-well half-area plate. Cells (60µl) were then incubated with DMSO (negative control), 10µM GSK2837808 (lactate dehydrogenase A inhibitor), 10µM 7ACC2 (monocarboxylate transporter 1-4 [MCT1-4] inhibitor) or 10µM UK5099 (mitochondrial pyruvate carrier inhibitor) at 37°C for 60 or 120 minutes. After incubation, 30µl (half of volume) was removed for cell viability analysis, using the CellTiter-Glo® Luminescent Cell Viability Assay (Cat.# G7570; **Panel B**). There was no decrease in viability during treatment, when compared to DMSO control. Data represent the average of three replicates. Samples containing no pyruvate oxidase are displayed (**Panel A**). Pyruvate levels increased approximately twofold after a 2-hour incubation with UK5099 (23.4µM) and 7ACC2 (21.5µM), compared to DMSO (11.4µM control). Increase of pyruvate levels with these inhibitors is concurrent with other studies (1,2).

4.E. Serum

The Pyruvate-Glo™ Assay can be used to measure amount of pyruvate in deproteinized serum or plasma samples. Serum that has not been deproteinized will interfere with the Pyruvate-Glo™ Assay. To determine sample interference we recommend running a no-POX control. Dilute samples to minimize interference (see Section 3.A).

1. Dilute 10kDa filtered serum (or plasma, following recommendations in Table 2). Add 30µl to each well. Include a positive pyruvate control (1µM–10µM) for quantification.

   Note: Serum that has not been deproteinized will interfere with this assay.

2. Add 5µl of 0.6N HCl per well.
3. Add 5µl of Neutralization Buffer per well. Mix by shaking the plate for 30–60 seconds.
4. Add 10µl of POX solution, prepared as described in Section 3.B.
5. Mix by shaking the plate for 30–60 seconds.

6. Incubate for 60 minutes at room temperature.
7. Add 50µl of Pyruvate-Glo™ detection reagent, prepared as described in Section 3.B.
8. Mix by shaking the plate for 30–60 seconds.
9. Incubate for 10–15 minutes at room temperature.
10. Record luminescence.

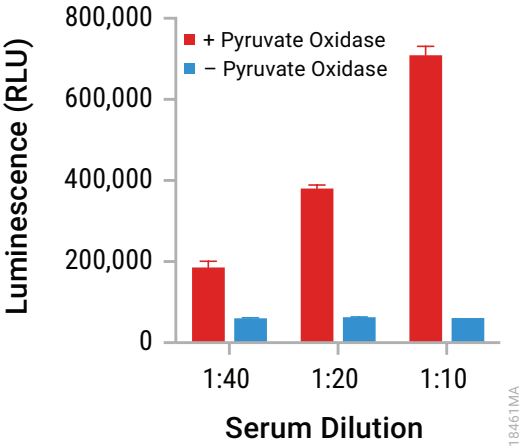

Figure 7. Pyruvate detection in serum. Serial dilutions of human serum (BioIVT Cat.# HMSRM, 10kDa filtered) were prepared in PBS at dilutions of 1:10 to 1:40. Dilutions were transferred to a white 96-well plate (30µl per well) and the Pyruvate-Glo™ Assay performed as described in Section 4.E. A no-POX control was included to determine assay background. Data are the average of three replicates. The amount of pyruvate measured in filtered serum samples was approximately 52µM.

5. Appendix

5.A. Temperature and Reagent Compatibility

The intensity and stability of the luminescent signal is temperature sensitive. For consistent results, equilibrate reagents to room temperature before use.

The compatibility of buffers with this assay can vary. For optimal assay results, test buffers in the assay with the provided standards, comparing results to those obtained with PBS. Background values can increase, depending on the buffer, especially for buffers containing detergents, which can decrease sensitivity and assay window. pH can also influence performance of this assay, resulting in luminescence signal variations.

5.B. Plates and Equipment

Most standard plate readers are designed to measure 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 seconds per well should serve as a guide. For exact instrument settings, consult the instrument manual.

Use opaque, white multiwell plates that are compatible with your luminometer (e.g., Corning® Costar® Cat.# 3917, 96-well or Costar® Cat.# 3570, 384-well plates). For cultured cell samples, white-walled clear-bottom tissue culture plates (e.g., Corning® Costar® Cat.# 3903, 96-well plates) are acceptable. Luminescent metabolite assays are well suited for miniaturization. When samples are limited, consider using 96-well half-area (Corning® Cat.# 3696), 384-well (Costar® Cat.# 3570) or 384-low volume (Corning® Cat.# 4512) plates. Light signal is diminished in black plates and well-to-well crosstalk is increased in clear plates.

The RLU values shown in the figures in this technical manual vary, depending on the assay plate and luminometer used to generate data. Relative luminescence may vary, depending on the instrument used, but this variation does not affect assay performance.

5.C. References

1. Corbet, C. *et al.* (2018) Interruption of lactate uptake by inhibiting mitochondrial pyruvate transport unravels direct antitumor and radiosensitizing effects. *Nat. Commun.* **9**, 1208.
2. Vacanti, N.M. *et al.* (2014) Regulation of substrate utilization by the mitochondrial pyruvate carrier. *Mol. Cell.* **56**, 425–35.

5.D. Related Products

Energy Metabolism Assays

Product	Size	Cat.#
BCAA-Glo™ Assay	5ml	JE9300
BHB-Glo™ (Ketone Body) Assay	5ml	JE9500
Cholesterol/Cholesterol Ester-Glo™ Assay	5ml	J3190
Dehydrogenase-Glo™ Detection System	5ml	J9010
Glucose-Glo™ Assay	5ml	J6021
Glucose Uptake-Glo™ Assay	5ml	J1341
Glutamine/Glutamate-Glo™ Assay	5ml	J8021
Glycerol-Glo™ Assay	5ml	J3150
Glycogen-Glo™ Assay	5ml	J5051
Lactate-Glo™ Assay	5ml	J5021
Malate-Glo™ Assay	5ml	JE9100
Metabolite-Glo™ Detection System	5ml	J9030
Triglyceride-Glo™ Assay	5ml	J3160

Additional sizes available.

Oxidative Stress Assays

Product	Size	Cat.#
GSH/GSSG-Glo™ Assay	10ml	V6611
NAD/NADH-Glo™ Assay	10ml	G9071
NADP/NADPH-Glo™ Assay	10ml	G9081
ROS-Glo™ H_2O_2 Assay	10ml	G8820

Additional sizes available.

Promega

5.D. Related Products (continued)

Cell Viability, Cytotoxicity and Apoptosis Assays

Product	Size	Cat.#
Caspase-Glo® 3/7 Assay System	2.5ml	G8090
CellTiter-Glo® 2.0 Cell Viability Assay	10ml	G9241
CellTiter-Glo® 3D Cell Viability Assay	10ml	G9681
CellTiter-Fluor™ Cell Viability Assay	10ml	G6080
LDH-Glo™ Cytotoxicity Assay	10ml	J2380
RealTime-Glo™ Annexin V Apoptosis and Necrosis Assay	100 assays	JA1011
RealTime-Glo™ MT Cell Viability Assay	100 assays	G9711

Additional sizes available.

6. Summary of Changes

The following change was made to the 1/24 revision of this document:

1. The volume of cells/well used in Figure 6 was changed to 100,000.

[a]Patents pending.