Promega

Energy Metabolism Assays

Glucose-Uptake | Glucose | Lactate | Glutamate
Glutamine | NAD(P)H | Oxidative Stress

Highly sensitive, plate-based bioluminescent methods
Simple “Add-mix-measure” protocols

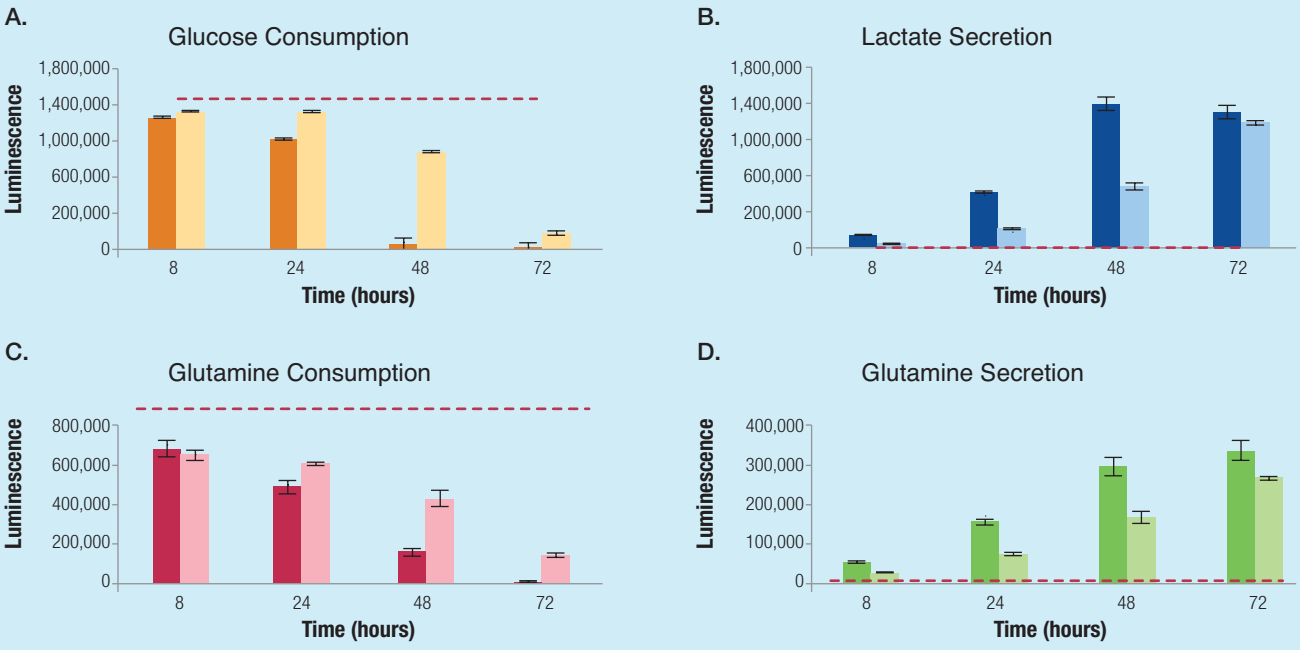
Measurement of individual intracellular metabolites or multiple secreted metabolites at different time points

Bioluminescent Energy Metabolite Assays for Glucose, Lactate, Glutamate and Glutamine Detection

Cellular metabolism is of growing interest in many areas of research including cancer, diabetes, immunology, stem cell differentiation, and neuroscience. This has increased the need for new technologies that can be used to measure key metabolites, especially plate-based assays that require minimal and rapid sample preparation and are amenable to higher throughput formats. Promega has developed four bioluminescent assays for robust detection of key metabolites: glucose, lactate, glutamate, and glutamine, in a plate-based format. The assays that involve simple and rapid sample preparation which is compatible with various sample types, are sensitive enough for intracellular metabolite detection, and facilitate measurement of multiple metabolites from a single sample.

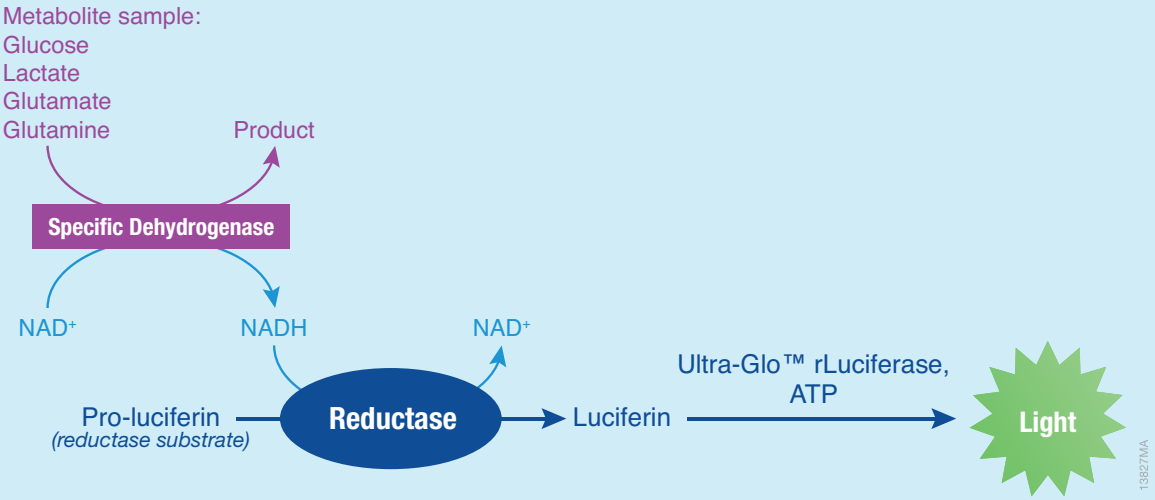
Measuring multiple metabolites from one sample

Four metabolites glucose, lactate, glutamate and glutamine can be measured in parallel using the bioluminescent **Glucose-Glo™, Lactate-Glo™, Glutamate-Glo™** and **Glutamine/ Glutamate-Glo™** Assays. Compatible sample processing 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, serum, plasma and tissues.

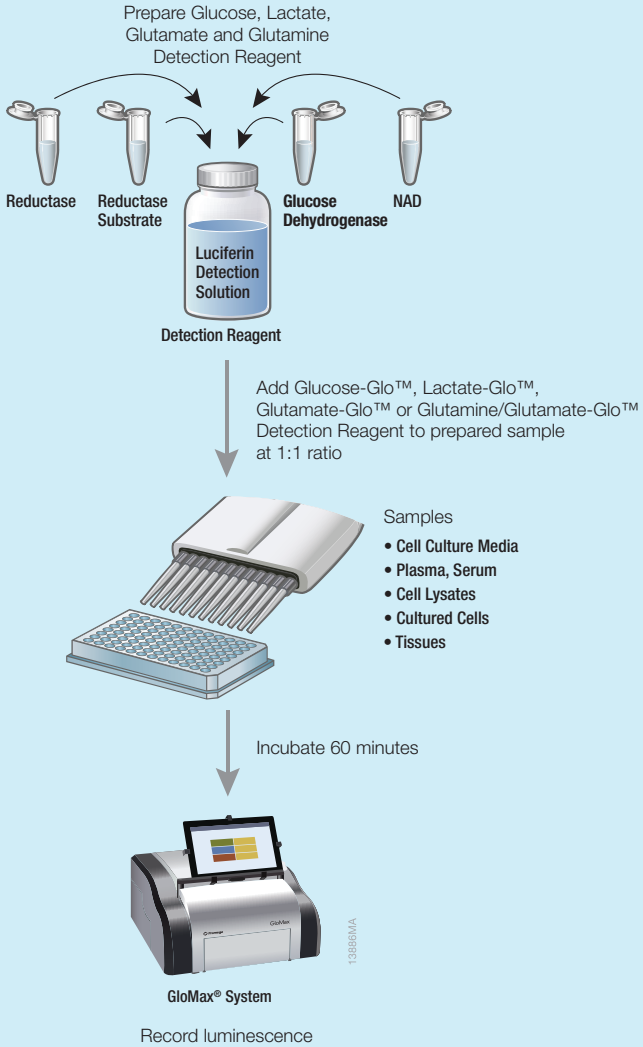


Red line represents luminescence values of medium-only controls.

Assay principles of Glucose-Glo™, Lactate-Glo™, Glutamate-Glo™ or Glutamine/Glutamate-Glo™



Simple “Add-mix-measure” protocols



Glucose-Glo™ Assay

Cell-based

Applications

Monitoring of glucose levels in cells, lysates, tissue, plasma and serum due to changes in glycolysis or glucose production during gluconeogenesis.

Assay description

The **Glucose-Glo™ Assay** is a bioluminescent assay for rapid, selective and sensitive detection of glucose in biological samples. The Glucose-Glo™ Assay couples glucose oxidation and NADH production with a bioluminescent NADH detection system. The Glucose-Glo™ Assay is a versatile system that is amenable to high-throughput formats and compatible with many sample types and does not require sample centrifugation or spin columns.

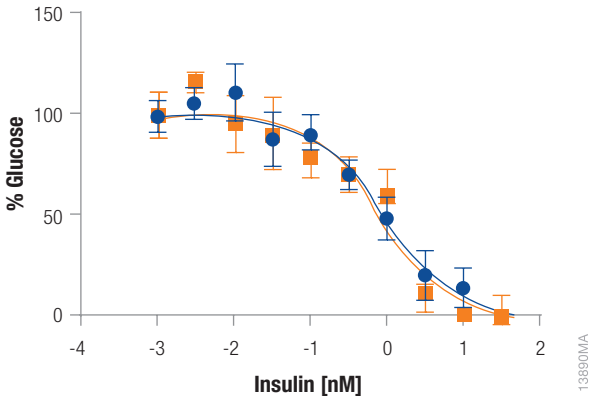
Assay principle

When Glucose Detection Reagent, which contains glucose dehydrogenase (GDH), 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. Glucose dehydrogenase catalyses the oxidation of glucose with concomitant reduction of NAD^+ to 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. The luminescent signal is proportional to the amount of glucose in the sample and increases until all glucose is consumed, at which time a stable luminescent signal is achieved.

Assay features

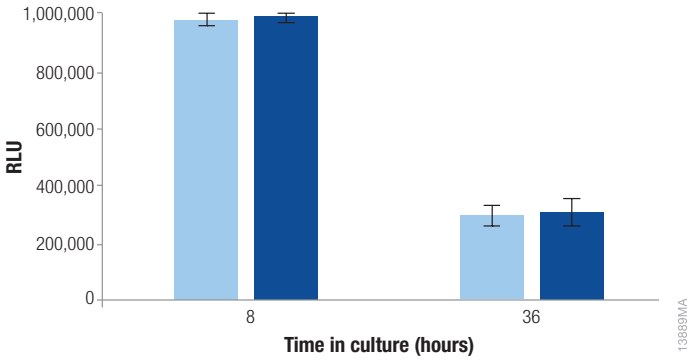
Assay type	Luminescent (glow-type), one step assay
Markers	Glucose
Applications	Monitoring of glucose levels
Sample	Cells, lysates, tissue, plasma and serum
Time required	60 minutes
Sensitivity	5 nM with linear range up to 50 µM, S/B max > 1000
Robustness	Easily scalable from 96- to 384-well plates

Insulin-mediated inhibition of gluconeogenesis in iPSC-derived human liver spheroids



Human liver spheroids were prepared from iCell® Hepatocytes 2.0 (Cellular Dynamics). Cells were thawed and cultured for 5 days on collagen and then plated at ~2,000 cells/well into a GravityTRAP™ 96-well spheroid plate (InSphero). On the day of the assay, the spent medium was removed and cells were incubated for 1.5 hours in gluconeogenesis medium to inhibit glycolysis and promote hepatic glucose production. Next, the spheroids were treated for 6 hours with gluconeogenesis medium supplemented with increasing insulin concentrations to inhibit glucose production. After 6 hours, 25 µl medium (blue) was removed from each well to a 96-well assay plate and an equal volume of Glucose Detection Reagent was added, incubated at room temperature for 60 minutes and luminescence was recorded using a GloMax® instrument. Similar results were obtained when glucose detection was performed using cell lysates (orange).

Glucose consumption by K562 cells



K562 cells were cultured in 75 cm^2 tissue culture flasks in RPMI medium (Sigma R1383) supplemented with 5 mM glucose and 10 % dialyzed FBS. After 8 and 36 hours, 1.5 ml of cell culture was transferred into 1.5 ml 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 and 20 µl was transferred into an assay plate and equal volume of Glucose Detection Reagent was added to the samples.

Lactate-Glo™ Assay

Cell-based

Applications

Monitoring of lactate levels in cells, lysates, tissue, plasma and serum.

Assay description

The **Lactate-Glo™ Assay** is a bioluminescent assay for rapid, selective and sensitive detection of L-lactate in biological samples. The assay couples lactate oxidation and NADH production with a bioluminescent NADH detection system. The Lactate-Glo™ Assay contains an L-lactate-selective lactate dehydrogenase to confer specificity for L-lactate, the major stereoisomer found in mammalian cells. The Lactate-Glo™ Assay is a versatile system that is amenable to high-throughput formats and compatible with many sample types and does not require sample centrifugation or spin columns.

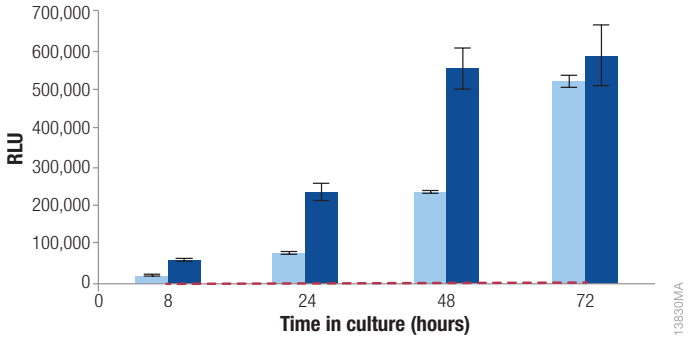
Assay principle

When Lactate Detection Reagent, which contains lactate dehydrogenase (LDH), 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. 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 luminescent signal is proportional to the amount of lactate in the sample and increases until all lactate is consumed at which time a stable luminescent signal is achieved.

Assay features

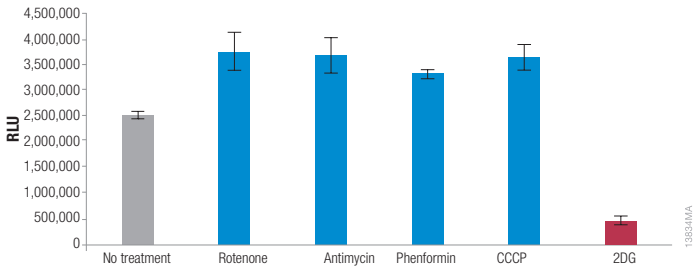
Assay type	Luminescent (glow-type), one step assay
Markers	Lactate
Applications	Monitoring of lactate levels
Sample	Cells, lysates, tissue, plasma and serum
Time required	60 minutes
Sensitivity	100 nM with linear range up to 200 µM, S/B max > 200
Robustness	Easily scalable from 96- to 384-well plates

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 containing 5 mM glucose, 2 mM glutamine and 10 % dialyzed FBS. At indicated time points, 2.5 µl of medium was removed and diluted in 97.5 µl PBS. The samples were frozen and stored at –20°C. On the day of the assay, the samples were thawed, 25 µl of each sample 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 GloMax® instrument. Data represent the average of four replicates.

Changes in glycolytic rate



A549 cells in DMEM supplemented with 10 % dialyzed serum, 5 mM glucose and 2 mM glutamine were plated at 15,000 cells/well in 96-well plate. After 24 hours, medium was removed. Thirty microliters of glucose-free medium containing mitochondrial inhibitors (5 µM rotenone, 5 µM antimycin, 2.5 mM phenformin, 50 µM CCCP), glycolysis inhibitor (10 mM 2-deoxyglucose) or no compound was added to the samples. After 5 minutes pre-treatment at room temperature, 10 µl of medium containing 4 mM glucose was added to the samples, incubated for 1 hour and then Lactate Detection Reagent was added. 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.

Glutamate-Glo™ Assay

Cell-based

Applications

Monitoring of glutamate levels in cells, lysates, tissue, plasma and serum.

Assay description

The **Glutamate-Glo™ Assay** is a bioluminescent assay for rapid, selective and sensitive detection of glutamate in biological samples. The assay couples glutamate oxidation and NADH production with a bioluminescent NADH detection system. The Glutamate-Glo™ Assay is a versatile system that is amenable to high-throughput formats and compatible with many sample types and does not require sample centrifugation or spin columns.

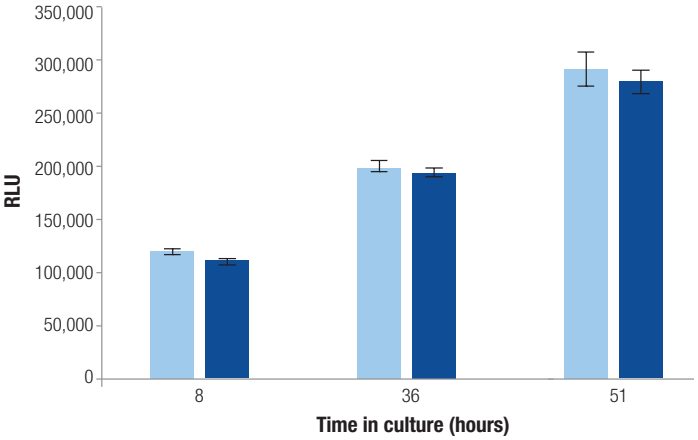
Assay principle

When Glutamate Detection Reagent, which contains glutamate dehydrogenase (GlutDH), NAD^+, Reductase, Reductase Substrate and Luciferase, is added to a sample containing glutamate at a 1:1 ratio, the enzyme-coupled reactions start and run simultaneously. Glutamate dehydrogenase uses glutamate and NAD^+ to produce α-ketoglutarate and 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. The luminescent signal is proportional to the amount of glutamate in the sample and increases until all glutamate is consumed, at which time a stable luminescent signal is achieved.

Assay features

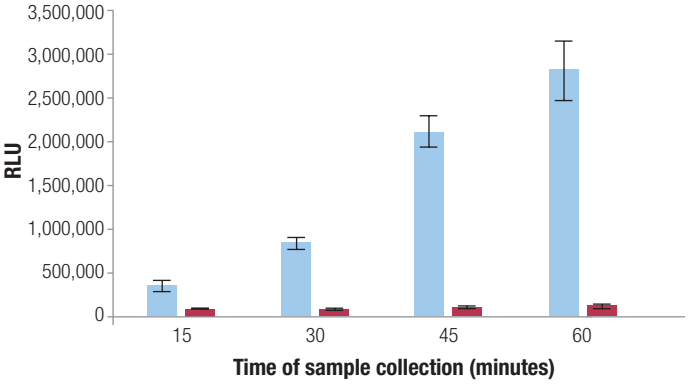
Assay type	Luminescent (glow-type), one step assay
Markers	Glutamate
Applications	Monitoring of glutamate levels
Sample	Cells, lysates, tissue, plasma and serum
Time required	60 minutes
Sensitivity	5 nM with linear range up to 50 µM, S/B max > 100
Robustness	Easily scalable from 96- to 384-well plates

Glutamate secretion by K562 cells



K562 cells were cultured in RPMI medium supplemented with 5 mM glucose and 10% dialyzed FBS. After 8, 36 and 51 hours, 1.5 ml of cell culture was transferred into 1.5 ml 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). The diluted samples were frozen and stored at –20°C. For glutamate detection, the samples were thawed and 20 µl was transferred into an assay plate. An equal volume of Glutamate Detection Reagent was added to the samples and luminescence was read. 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.

Homogeneous assay with glutaminase inhibitor BPTES



A549 cells were plated at 5,000 cells/ well in 100 µl culture medium. After an overnight incubation, thirty microliters of DMEM with 27 µM BPTES (red bars) or without BPTES (blue bars) was added to the cells. 10 µl of DMEM with glucose (20 mM) and glutamine (8 mM) was added to the cells. At indicated time points, 5 µl of Inactivation Solution was added to stop cell metabolism. After incubation for 2–5 minutes, 5 µl of Neutralization Solution was added and glutamate was measured by adding 50 µl of Glutamate Detection Reagent. Cells without BPTES produced increasing amounts of glutamate with time. However, in cells exposed to BPTES, glutaminase was inhibited, preventing the conversion of glutamine to glutamate.

Glutamine/Glutamate-Glo™ Assay

Cell-based

Applications

Monitoring of glutamine and glutamate levels in cells, lysates, tissue, plasma and serum.

Assay description

The **Glutamine/Glutamate-Glo™ Assay** is a bioluminescent assay for rapid, selective and sensitive detection of glutamine and glutamate in biological samples. The Glutamine/Glutamate-Glo™ Assay is based on the conversion of glutamine to glutamate by Glutaminase enzyme. Next, glutamate oxidation and NADH production are coupled with a bioluminescent NADH detection system. The assay is a versatile system that is amenable to high-throughput formats and compatible with many sample types and does not require sample centrifugation or spin columns.

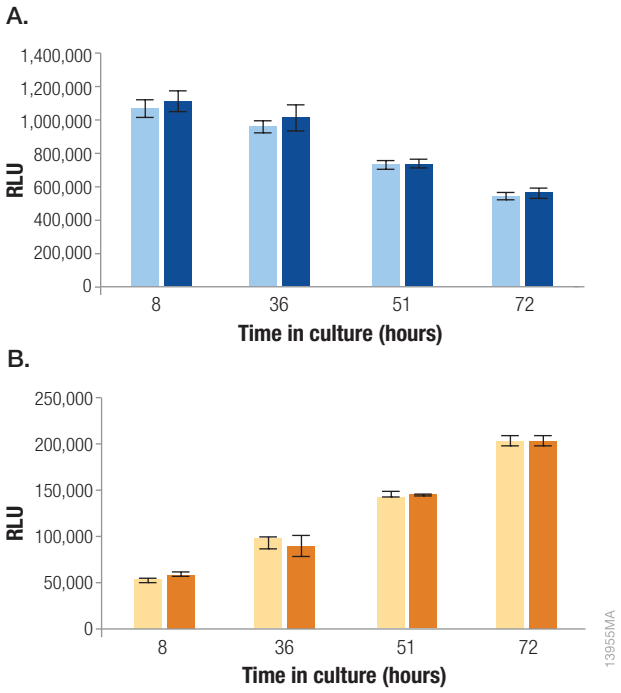
Assay principle

This assay requires two steps: i) glutamine conversion to glutamate by Glutaminase; and ii) glutamate detection with the Glutamate Detection Reagent. When Glutamate Detection Reagent, which contains glutamate dehydrogenase (GlutDH), NAD^+, Reductase, Reductase Substrate and Luciferase, is added to a sample containing glutamate at a 1:1 ratio, the enzyme-coupled reactions start and run simultaneously. The luminescent signal is proportional to the amount of glutamate and increases until all glutamate is consumed, at which time a stable luminescent signal is achieved.

Assay features

Assay type	Luminescent (glow-type), two step assay
Markers	Glutamine
Applications	Monitoring of glutamine levels
Sample	Cells, lysates, tissue, plasma and serum
Time required	60 minutes
Sensitivity	5 nM with linear range up to 50 µM, S/B max > 300
Robustness	Easily scalable from 96- to 384-well plates

Glutamine consumption and glutamate secretion by K562 cells



Glutamine consumption (Panel A) and glutamate secretion (Panel B) by K562 cells were monitored over time. K562 cells were cultured in RPMI medium supplemented with 5 mM glucose and 10 % dialyzed FBS. After 8, 36, 51 and 72 hours, 1.5 ml of cell culture was transferred into 1.5 ml microcentrifuge tubes. A portion of each sample was diluted 10-fold in PBS directly (light bars) or after removing the cells by centrifugation (dark bars). The diluted samples were frozen and stored at –20°C. The samples were thawed, diluted with PBS to a 40-fold final dilution and two 10 µl aliquots were transferred into a 384-well assay plate. Ten microliters of Glutaminase Buffer or Glutaminase Enzyme Solution was added, and the reactions were incubated for 30 minutes at room temperature. Twenty microliters of Glutamate Detection Reagent was added to each sample. After 60 minutes at room temperature, luminescence was read using a GloMax® instrument. 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.

Glucose Uptake-Glo™ Assay

Cell-based

Applications

Monitoring glucose uptake in mammalian cells, including insulin-sensitive cell types and cancer cells.

Assay description

The **Glucose Uptake-Glo™ Assay** is a plate-based, homogeneous bioluminescent method for measuring glucose uptake in cells, based on the detection of 2-deoxyglucose-6-phosphate (2DG6P).

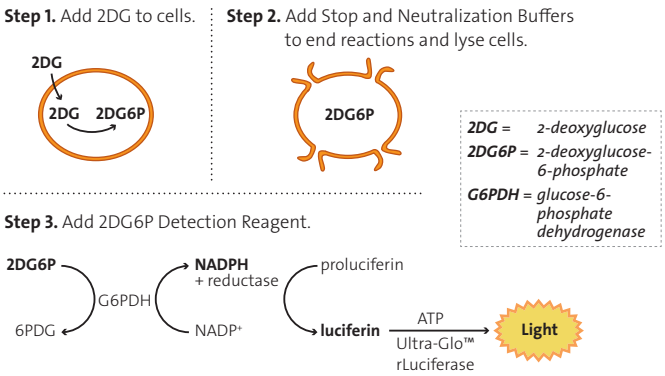
Assay principle

When 2-deoxyglucose (2DG) is added to cells, it is transported across the membrane and rapidly phosphorylated in the same manner as glucose. Enzymes that further modify glucose-6-phosphate (G6P) cannot modify 2DG6P, and thus this membrane-impermeable analyte accumulates in the cell. After a brief period of incubation, a Stop Buffer is added to lyse cells, terminate uptake and destroy any NADPH within the cells. A Detection Reagent containing glucose-6-phosphate dehydrogenase (G6PDH), $NADP^+$, Reductase, Ultra-Glo™ Recombinant Luciferase and proluciferin substrate is added to the sample wells. G6PDH oxidizes 2DG6P to 6-phosphodeoxygluconate and simultaneously reduces $NADP^+$ to NADPH. The Reductase uses NADPH to convert the proluciferin to luciferin, which is then used by Ultra-Glo™ Recombinant Luciferase to produce a luminescent signal that is proportional to the concentration of 2DG6P.

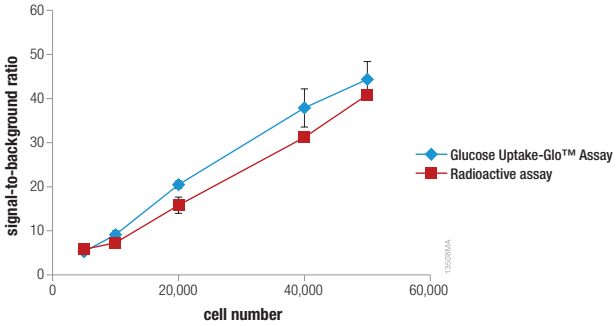
Assay features

Assay type	Luminescent
Markers	2-deoxyglucose (2DG)
Applications	Monitoring glucose uptake in mammalian cells; insulin-sensitive cell types and cancer cells
Cell type	Mammalian cells
Implementation	Homogeneous, after addition of 2DG, there are no wash steps—all steps are additions.
Time required	0.5–5 hours
Sensitivity	0.5 to 30 µM 2DG6P and generates a signal-to-background ratio > 3 with as few as 5,000 cells
Robustness	Z' factors > 0.5, scalable for use in 96- and 384-well plates

Glucose Uptake-Glo™ Assay principle

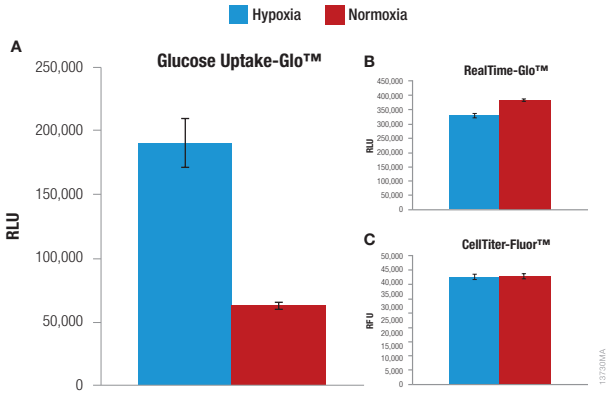


Equivalent Performance as the Radioactive Method



Comparison of a radioactive method and the Glucose Uptake-Glo™ Assay. The Glucose Uptake-Glo™ Assay (blue diamonds) and the standard radioactive method (red squares) were performed using varying amounts of HCT116 colon cancer cells. Signal-to-background ratios were comparable for both methods.

Cancer Model: Glucose Uptake in Hypoxia



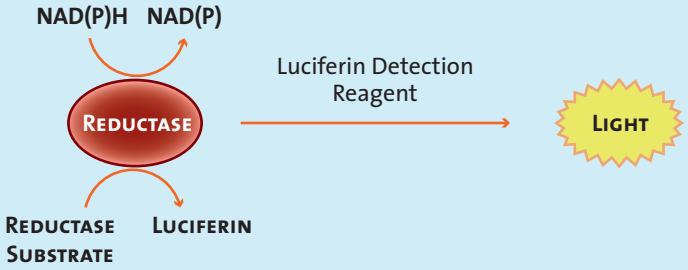
When cells are oxygen-starved, the hypoxic conditions shift cellular metabolism from oxidative phosphorylation to glycolysis. This results in increased glucose uptake.
MCF7 cells grown under hypoxia (1% oxygen) show an increase in glucose uptake (Panel A), indicating an increased glycolytic rate. The same cells demonstrate no significant change in viability using the RealTime-Glo™ (Panel B) and CellTiter-Fluor™ (Panel C) Assays.

Bioluminescent Nicotinamide Adenine Dinucleotide Detection Assays

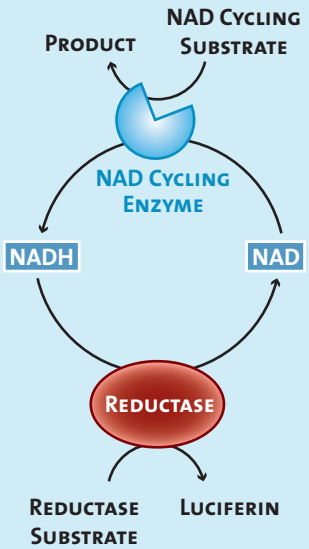
Cancer is a disease of uncontrolled cell growth that requires cancer cells to alter metabolic pathways to survive and proliferate. The principal mechanisms underlying this metabolic reprograming by oncogenes and tumor suppressor genes is still poorly understood. Nicotinamide adenine dinucleotides (NAD^+, NADH, $NADP^+$ and NADPH) are fundamental co-factors of cellular energy metabolism. These dinucleotides are essential for macromolecule biosynthesis and the maintenance of the cellular redox potential. In addition NAD-dependent signaling pathways (e.g., mono- and poly- ADP ribosylation, protein deacetylation) are involved in regulating other processes linked to cancer development, including epigenetic regulation, cell cycle progression, DNA repair, and circadian rhythm. The central role of NAD^+, NADH, $NADP^+$ and NADPH in cellular energy metabolism and signaling makes them important target-independent nodes that link the metabolic state of cells with energy homeostasis and gene regulation. Rapid, easy-to-use assays for measuring these dinucleotides provide a convenient tool for investigating their role in these processes.

Promega offers three bioluminescence assays for rapid and sensitive measurement of redox defining co-factors NAD^+, NADH, $NADP^+$ and NADPH.

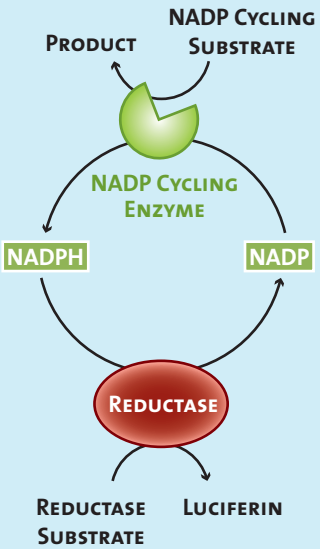
NAD(P)H-Glo™ Detection System – detects NADH and NADPH (Biochemical assay)



NAD⁺/NADH-Glo™ Assay – detects NAD and NADH in cells (Cell-based or biochemical assay)



NADP⁺/NADPH-Glo™ Assay – detects NADP and NADPH in cells (Cell-based or biochemical assay)



NAD(P)H-Glo™ Detection System

Biochemical

Applications

Monitoring the activity of enzymes that produce or use NAD(P)H; measuring NAD(P)H production or consumption in high-throughput screen formats.

Assay description

The *in vitro* enzyme-based **NAD(P)H-Glo™ Detection System** is a homogeneous, bioluminescent assay that quantitatively monitors the concentration of the reduced forms of NADH and NADPH, and does not discriminate between them. The oxidized forms, NAD^+ and $NADP^+$, are not detected and do not interfere with quantitation. The assay is rapid, requiring only a 40- to 60-minute incubation, has a broad linear range and high signal-to-background ratio. The NAD(P)H-Glo™ Detection System detects 25 nM to 50 µM NAD(P)H. Reactions are scalable and can be performed at low volumes in 96-, 384- and 1536-well plates.

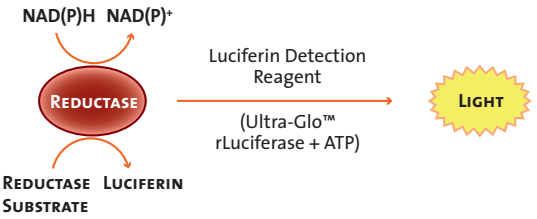
Assay principle

In the presence of NAD(P)H, a reductase enzyme reduces a proluciferin Reductase Substrate to form luciferin. Luciferin then is quantified using Ultra-Glo™ Recombinant Luciferase, and the light signal produced is proportional to the amount of NAD(P)H in the sample. The reductase and luciferase reactions are initiated by adding an equal volume of a single reagent, which contains reductase, proluciferin reductase substrate and Ultra-Glo™ Recombinant Luciferase, to a NAD(P)H-containing sample.

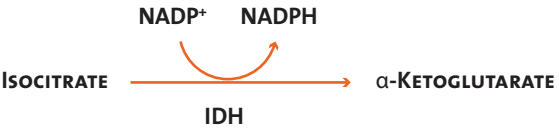
Assay features

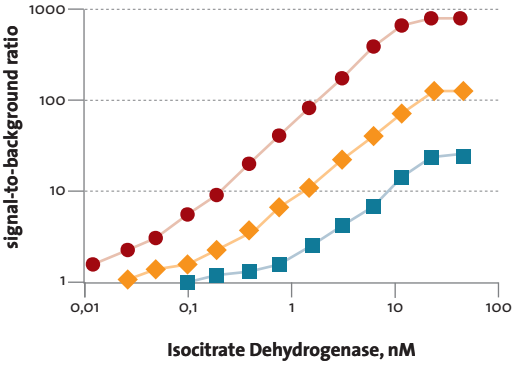
Assay type	Luminescent (glow-type; $T_{1/2}$ > 2 h)
Markers	NADH, NADPH
Applications	Monitoring the activity of enzymes that produce or use NAD(P)H.
Sample	Enzyme preparations
Implementation	Homogeneous, one-step assay with flexible storage capability
Sensitivity	Broad linear range (25 nM – 50 µM) and high signal-to-background ratio (~400)
Robustness	Z' factor > 0.7

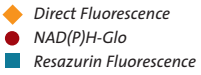
NAD(P)H™ Detection System Assay-Principle

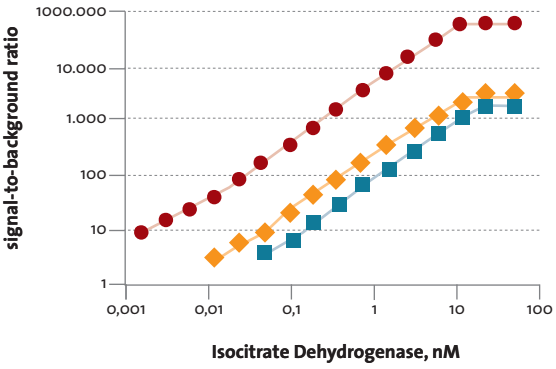


The luminescent NAD(P)H-Glo™ Detection System is more sensitive than alternative fluorescent methods









Isocitrate dehydrogenase (IDH) at the indicated concentrations was incubated with 100 µM NADP and 100 µM isocitrate for 30 minutes. The manufacturer's protocol for each of the fluorescent assays was followed using 100 µl of the reaction for the direct fluorescence NADH detection method and 50 µl for the indirect fluorescence NADH detection method (diaphorase conversion of resazurin).

NAD⁺/NADH-Glo™ Assay | NADP⁺/NADPH-Glo™ Assay

Cell-based/Biochemical

Both assays are using the same technology (see Figure):

- Convert oxidized dinucleotides to reduced forms using Cycling Enzymes (Dehydrogenases)
- Cycling Enzymes provide specificity for nonphosphorylated or phosphorylated
- Cycling reaction increases sensitivity
- Cycling enzyme, reductase, and luciferase reactions occur in one reagent

In the following section, only **NAD⁺/NADH-Glo™ Assay** is described, since assay principle and assay features are equal to **NADP⁺/NADPH-Glo™ Assay**.

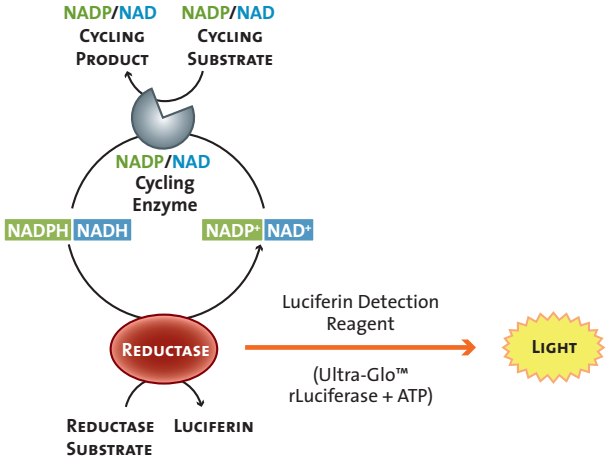
Applications

Monitoring changes in cellular levels of total NAD⁺ and NADH; determining NAD⁺/NADH ratios; monitoring the effects of small molecule compounds on NAD⁺ and NADH levels in enzymatic reactions or directly in cells in high-throughput formats.

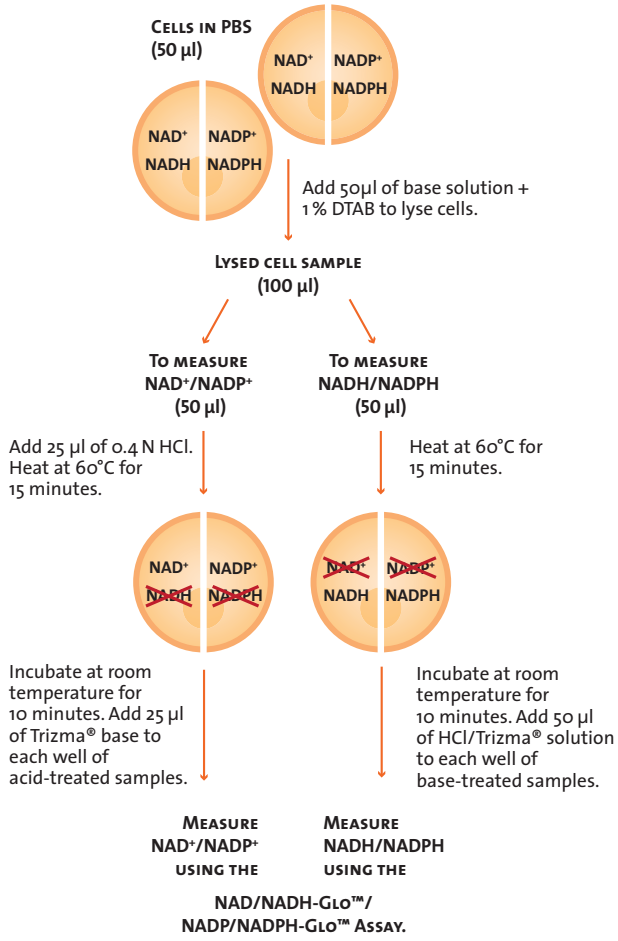
Assay description

The NAD⁺/NADH-Glo™ Assay is a bioluminescent, homogeneous single-reagent-addition assay for detecting total oxidized and reduced nicotinamide adenine dinucleotides (NAD⁺ and NADH, respectively) and determining their ratio in biological samples or in defined enzyme reactions. Cycling between NAD⁺ and NADH by the NAD Cycling Enzyme and Reductase increases assay sensitivity and provides selectivity for the nonphosphorylated NAD⁺ and NADH compared to the phosphorylated forms NADP⁺ and NADPH. The NAD/NADH-Glo™ Assay detects 1nM to 500 nM NAD⁺ or NADH. The simple add-mix-read protocol and scalable assay chemistry make the NAD⁺/NADH-Glo™ Assay well suited to monitor effects of small molecule compounds on NAD⁺ and NADH levels in high-throughput screen formats.

Assay Principle of NAD/NADH-Glo™ Assay and NADP/NADPH-Glo™ Assay



Schematic diagram of the sample preparation protocol for measuring a) NAD⁺ and NADH and b) NADP⁺ and NADPH individually.



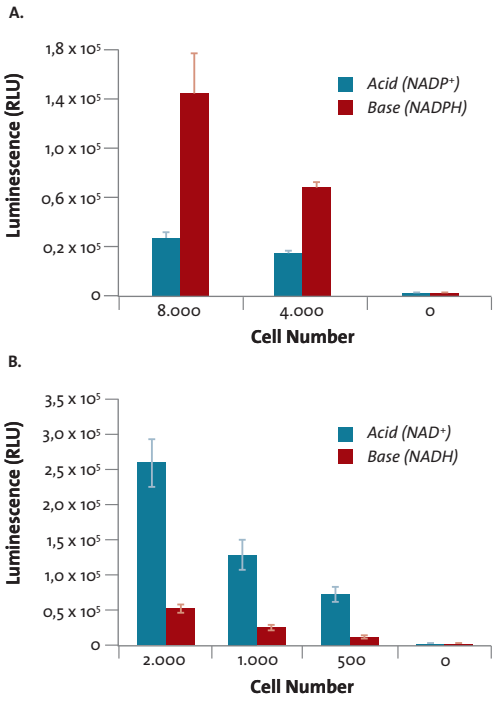
Assay principle

The NAD Cycling Enzyme, Reductase and luciferase reactions are initiated by adding an equal volume of NAD⁺/NADH-Glo™ Detection Reagent, which contains NAD Cycling Enzyme and Substrate, Reductase, Reductase Substrate and Ultra-Glo™ Recombinant Luciferase, to an NAD⁺- or NADH-containing sample. An NAD Cycling Enzyme is used to convert NAD⁺ to NADH. In the presence of NADH, the provided reductase enzyme reduces a proluciferin reductase substrate to form luciferin. Luciferin then is quantified using Ultra-Glo™ Recombinant Luciferase, and the light signal produced after an incubation of 30–60 minutes is proportional to the amount of NAD⁺ and NADH in the sample. Detergent present in the reagent lyses cells, allowing detection of total cellular NAD⁺ and NADH in a multiwell format with addition of a single reagent. An accessory protocol is provided to allow separate measurements of NAD⁺ and NADH, and calculation of the NAD⁺ to NADH ratio.

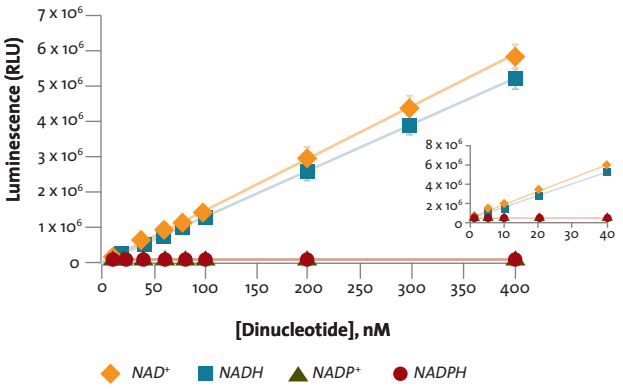
Assay features

Assay type	Luminescent (glow-type)
Markers	NAD⁺ and NADH
Applications	Monitoring changes in cellular levels of total NAD⁺ and NADH; determining NAD⁺/NADH ratios; monitoring the effects of small molecule compounds on NAD⁺ and NADH levels in enzymatic reactions or directly in cells in high-throughput formats.
Cell type/Sample	Cells and Enzyme preparations
Implementation	Homogeneous, one-step assay with flexible storage capability. The luminescent format avoids fluorescent interference due to reagents and test compounds sometimes seen in fluorescent assays.
Limit of Detection (LOD)	1nM (25fmol/25µl)
Linearity	1–500nM
Signal-to-background ratio (S/B max)	~250
Robustness	Z' factor > 0.7

Separate measurement of cellular A. NADP⁺/NADPH and B. NAD⁺/NADH from a single cell sample.



Linear range and specificity of the NAD/NADH-Glo™ Assay



Individual purified nicotinamide adenine dinucleotides were assayed following the protocol. NADH, NADPH, NAD⁺ and NADP⁺ stocks were prepared freshly from powder (Sigma Cat.# N6660, N9910, N8285 and N8035, respectively) and diluted to the indicated concentrations in phosphate-buffered saline (PBS). 50µl microliter samples at each dinucleotide concentration were incubated with 50 µl of NAD/NADH-Glo™ Detection Reagent in white 96-well luminometer plates.

Oxidative stress

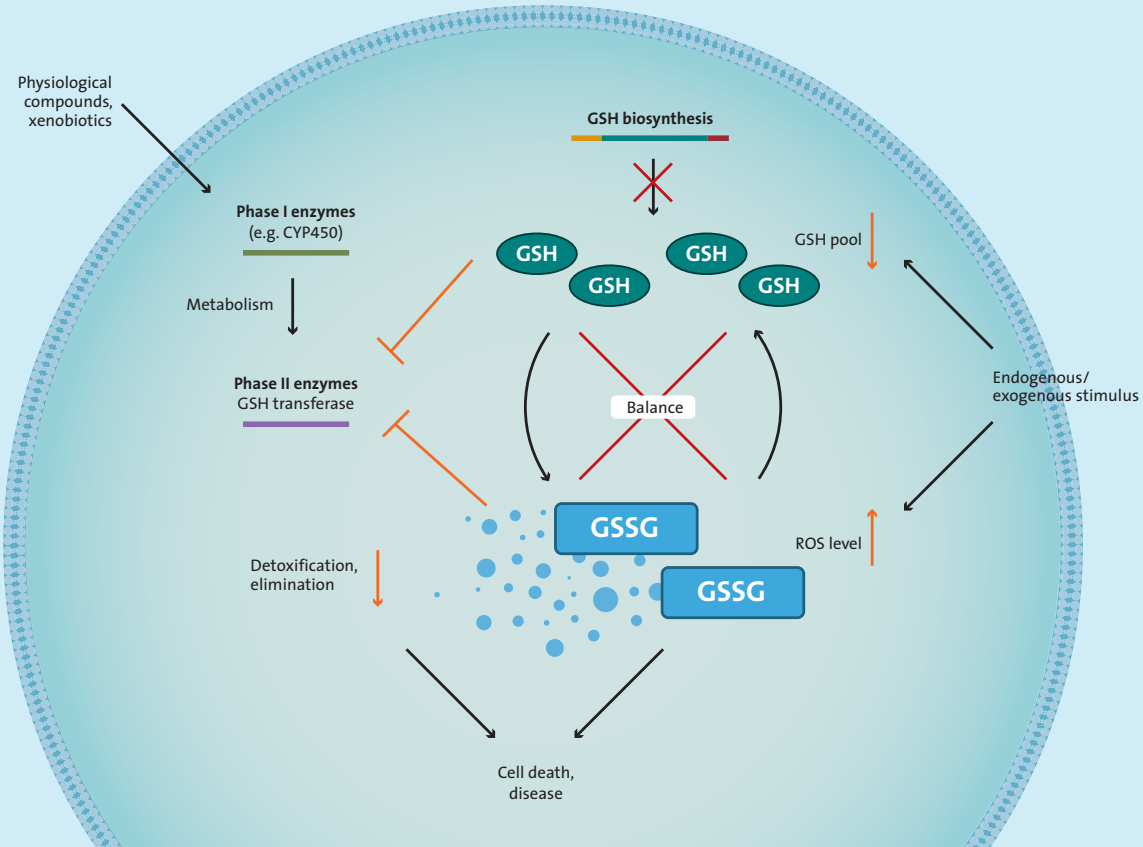
Glutathione is the most important and most powerful antioxidant in a cell. Glutathione is also involved in phase II biotransformation. It can occur in the reduced form as a monomer (GSH) or in the oxidized form as a dimer (GSSG). The ratio of reduced GSH to oxidized GSSG is an indicator of oxidative stress, which can lead to apoptosis or cell death. Acute degenerative diseases such as stroke, arteriosclerosis, diabetes, Alzheimer's disease and Parkinson's disease can develop as a result of this. Findings concerning the effects of glutathione levels on cellular signaling pathways offer new methods for intervention in ageing processes and the treatment of degenerative diseases.

Glutathione consists of the three amino acids glutamic acid, cysteine and glycine. Besides functioning as the main component of the reductive pool, GSH probably constitutes the most important reserve of the amino acid cysteine.

For protection from oxidative stress caused e.g. by reactive oxygen species (ROS), glutathione is oxidized and switches from its reduced monomeric form to its oxidized dimeric form GSSG. Two molecules of GSH are regenerated from GSSG by glutathione reductase, with energy being consumed in the process. 98% of glutathione in the body occurs in the reduced form GSH.

Promega offers two assays for analyzing GSH and one assay for the detection of H_2O_2-levels:

1. **ROS-Glo™ H_2O_2 Assay** for the detection of reactive oxygen species (H_2O_2) in cells.
2. **GSH/GSSG-Glo™ Assay** for measuring total glutathione levels (GSH+GSSG) and for measuring oxidized GSSG. The GSH:GSSG ratio serves as an indicator of the redox status of a mammalian cell or tissue. It depends on the cell type used and under physiological conditions normally lies between 50:1 and 100:1. Changes in the GSH:GSSG ratio allow more specific conclusions to be drawn about possible stress conditions and toxicity mechanisms in the cell or cell group.
3. **GSH-Glo™ Glutathione Assay** for quantitatively determining reduced GSH.



ROS-Glo™ H_2O_2 Assay

Cell-based/Biochemical

Applications

Measure changes in hydrogen peroxide (H_2O_2) levels directly in cell culture samples; measure the activity of enzymes that generate or eliminate H_2O_2; identify small molecule inhibitors or inducers that alter reactive oxygen species (ROS) levels either in cells in culture or in enzyme assays.

Assay description

The **ROS-Glo™ H_2O_2 Assay** is a homogeneous, fast and sensitive bioluminescent assay that measures the level of H_2O_2, a reactive oxygen species (ROS), directly in cell culture or in defined enzyme reactions. The ROS-Glo™ H_2O_2 Substrate reacts directly with H_2O_2, obviating the need for horseradish peroxidase (HRP) as a coupling enzyme and thus eliminating false hits associated with HRP inhibition. The assay can be used to screen compounds in both cell-based and enzyme-based formats (96- to 384-well plate formats). Multiplexing with a real-time cytotoxicity assay (CellTox™ Green Cytotoxicity Assay), in the same well or with a viability assay, results in more informative data.

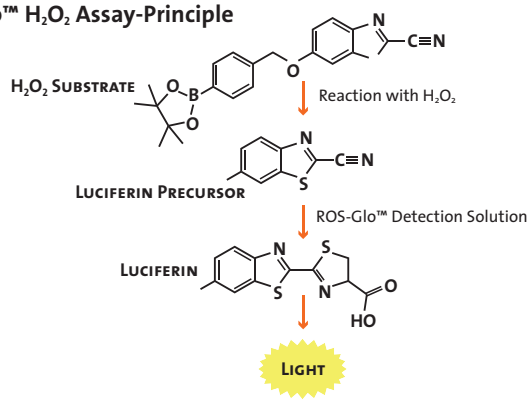
Assay principle

The homogeneous assay is performed following a simple two-reagent-addition protocol that does not require sample manipulation. A H_2O_2 substrate is incubated with sample and reacts directly with H_2O_2 to generate a luciferin precursor. Addition of ROS-Glo™ Detection Solution converts the precursor to luciferin and provides Ultra-Glo™ Recombinant Luciferase to produce light signal that is proportional to the level of H_2O_2 present in the sample. The assay can be completed in less than 2 hours after reagent addition.

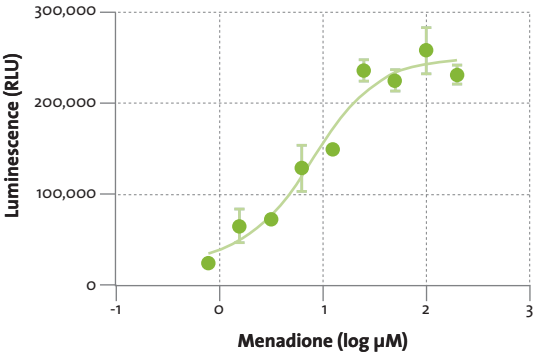
Assay features

Assay type	Luminescent (glow-type), two-step assay, obviating the need for HRP
Markers	H_2O_2
Applications	Measure changes in H_2O_2 levels directly in cell culture samples.
Cell type/Sample	Cell lines and enzyme preparations, low molecular weight substances
Time required	2 hours
Robustness	Easily scalable from 96- to 384-well plates

ROS-Glo™ H_2O_2 Assay-Principle

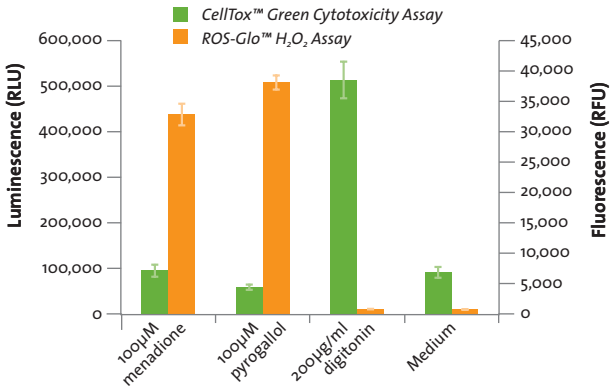


ROS induction in cultured cells



K562 cells were treated with menadione and the ROS-Glo™ H_2O_2 Assay was used to determine ROS production. Menadione resulted in a concentration-dependent ROS increase.

Multiplex with real-time CellTox™ Cytotoxicity Assay



HepG2 cells were plated at 2,000 cells/well in a 384-well plate and incubated overnight. The cells were then treated with either 100 µM menadione, 100 µM pyrogallol or 200 µg/ml digitonin and incubated at 37°C in 5% CO_2 for 2 hours. 1X CellTox™ Green Dye and 25 µM H_2O_2 Substrate were added to the cell culture at the time of dosing. After incubation the CellTox™ Green fluorescence signal was first measured and afterwards H_2O_2 levels using ROS-Glo™.

GSH/GSSG-Glo™ Assay

Cell-based

Applications

Determination of the GSH/GSSG ratio in cells as an indicator of oxidative stress; quantification of total glutathione (reduced and oxidized) in cells as an indicator of cell viability.

Assay description

The assay enables quantification of three parameters:

1. Total glutathione (GSH + GSSG)
2. Oxidised glutathione (GSSG)
3. Ratio of GSH to GSSG

Since the cells are not transferred, the loss of GSH and GSSG is minimal compared with that in conventional assays.

Assay principle

The GSH/GSSG-Glo™ assay can readily be adapted for 96-well and 384-well formats.

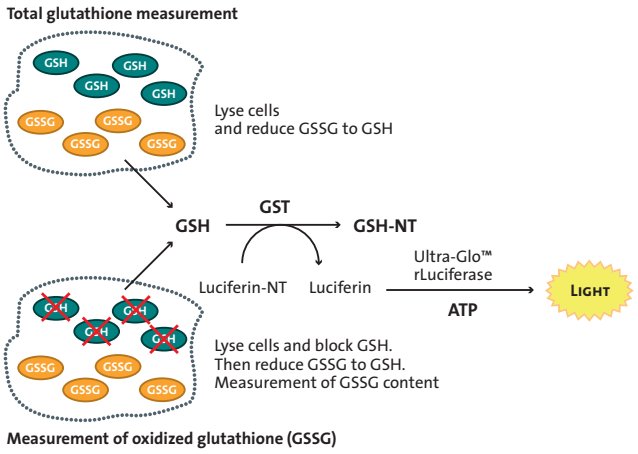
Total glutathione and GSSG are determined in two parallel reactions. The GSH level can be calculated by subtracting the GSSG level from the total glutathione level.

1. In the first mixture, after addition of the total glutathione reagent, total cellular glutathione (GSSG and GSH) is converted into GSH.
2. In the parallel second mixture, after the addition of the oxidized glutathione reagent, the cellular GSH is first blocked. The GSSG remains intact and is then reduced to GSH.

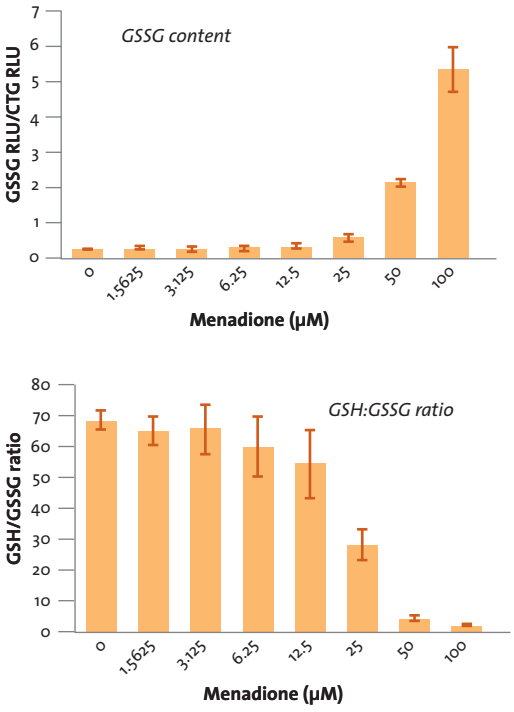
In both runs, the remaining GSH is coupled to the luciferase reaction. The glutathione-S-transferase (GST) in the reagent detaches a non-functional group from a luciferin derivative and couples this to the reduced glutathione. The free luciferin enters the luciferase reaction, the light signal being directly proportional to the level of GSH in the cell. In order to determine the level of reduced glutathione (GSH), the GSSG level is subtracted from the total glutathione level.

Assay features

Assay type	Luminescent (glow-type)
Markers	Total glutathione and GSSG
Applications	Determination of the GSH/GSSG ratio
Sample material	Cells, tissue extracts or blood samples
Implementation	Homogeneous, two-step assay automatable
Time required	45 minutes



Measurement of oxidative stress in A549 lung carcinoma cells



Top: 5,000 cells/well were treated with a series of dilutions of menadione (60 minutes, 37°C). After measurement of GSSG, the data was normalized against a viability measurement using CellTiter-Glo™ (CTG) in order to compensate for the influence of toxic effects due to menadione and of experimental fluctuations. Menadione has a toxic effect at the two highest concentrations shown after 60 min.
Below: Determination of the GSH/GSSG ratio by measuring GSSG and total glutathione in A549 cells. At higher concentrations, menadione has a significant effect on the redox status of the cells.

Overview of products

Bioluminescent Energy Metabolite Assays for Glucose, Lactate, Glutamate and Glutamine Detection

Product	Quantity	Catalog No.
Glucose-Glo™ Assay	10 ml	J6021
	50 ml	J6022
Lactate-Glo™ Assay	10 ml	J5021
	50 ml	J5022
Glutamate-Glo™ Assay	10 ml	J7021
	50 ml	J7022
Glutamine/Glutamate-Glo™ Assay	10 ml	J8021
	50 ml	J8022
Glucose Uptake-Glo™ Assay	5 ml	J1341
	10 ml	J1342
	50 ml	J1343

Bioluminescent Nicotinamide Adenine Dinucleotide Detection Assays

Product	Quantity	Catalog No.
NADP/NADPH-Glo™ Assay	10 ml	G9081
	50 ml	G9082
NAD/NADH-Glo™ Assay	10 ml	G9071
	50 ml	G9072
NAD(P)H-Glo™ Detection System	10 ml	G9061
	50 ml	G9062

Oxidative stress

Product	Quantity	Catalog No.
ROS-Glo™ H_2O_2 Assay	10 ml	G8820
	50 ml	G8821
GSH/GSSG-Glo™ Assay	10 ml	V6611
	50 ml	V6612

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