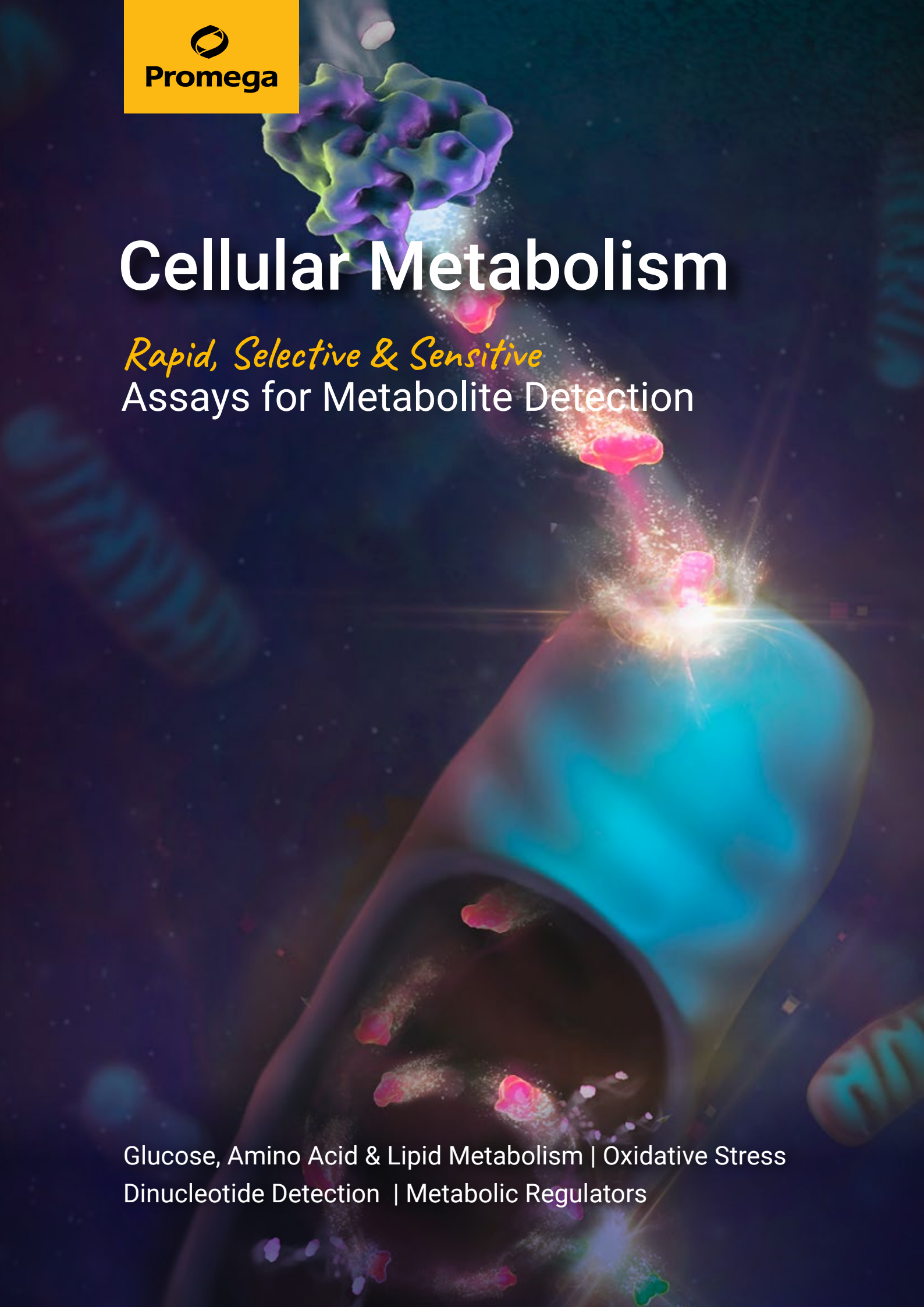
Promega

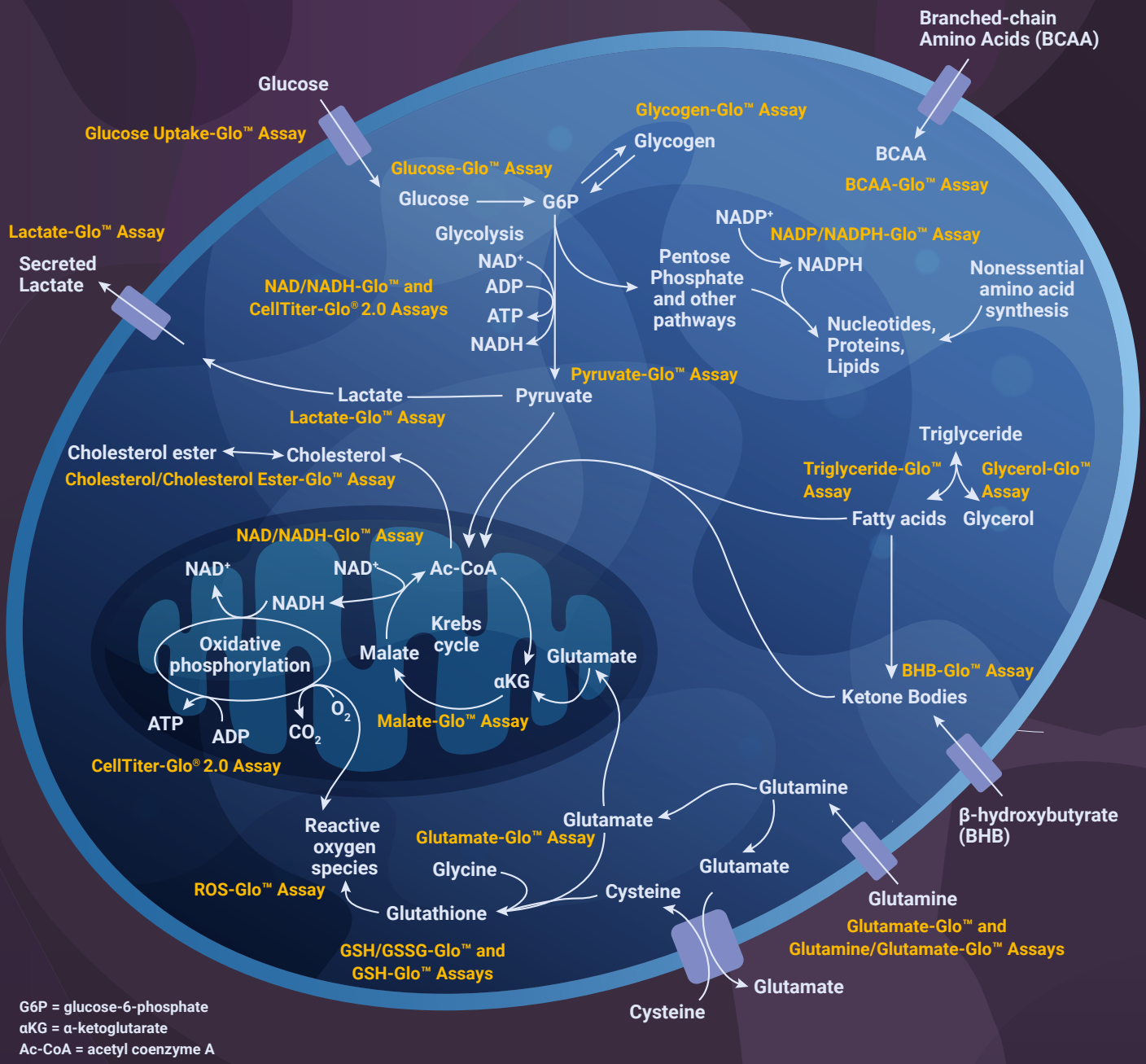
Cellular Metabolism

Rapid, Selective & Sensitive
Assays for Metabolite Detection



Glucose, Amino Acid & Lipid Metabolism | Oxidative Stress
Dinucleotide Detection | Metabolic Regulators

Cellular Energy Metabolism



G6P = glucose-6-phosphate
αKG = α-ketoglutarate
Ac-CoA = acetyl coenzyme A

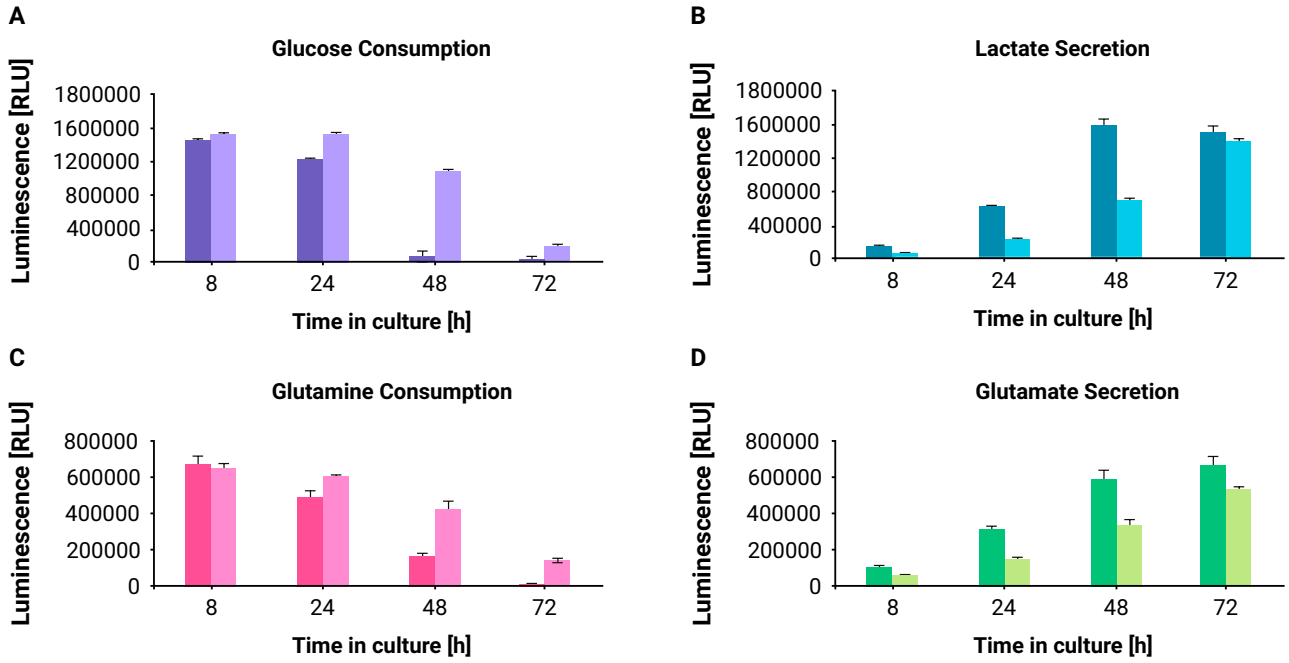
Table of Contents

Bioluminescent Detection of Glucose- & Amino Acid Metabolites

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 bioluminescent assays for robust detection of key metabolites, e.g., 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

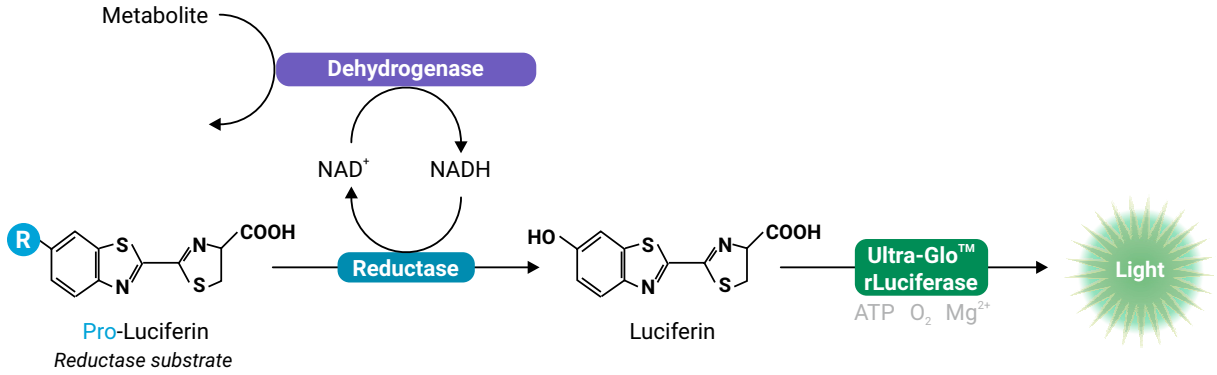
Analyze several metabolites in parallel to see which metabolic pathways are most active in your model system. Compatible sample processing of the bioluminescent metabolite assays allows the same sample to be used for detection of all four metabolites. This includes sample types such as culture media, serum, plasma and tissues.



Parallel analysis of glucose, lactate, glutamate, and glutamine using Glucose-Glo™, Lactate-Glo™, Glutamate-Glo™, and Glutamine/Glutamate-Glo™. A549 cells were plated at 5,000 (light-colored bars) and 15,000 (dark-colored bars) cells/well in medium. At indicated time points, 2.5 µl of medium was removed, diluted, and stored at –20°C before measurement.

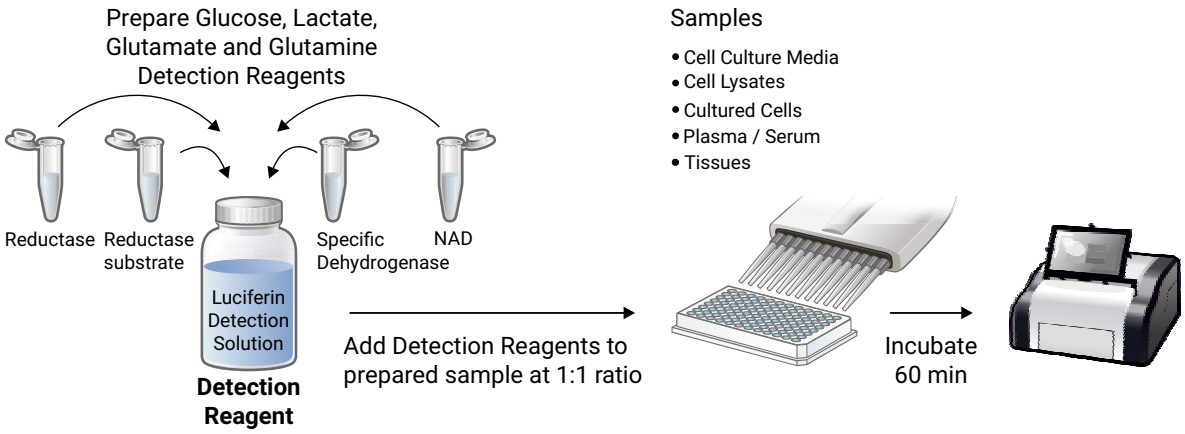
1 Glucose and Amino Acid Metabolism Assays

General assay principle



To detect most metabolites, oxidation is coupled via a metabolite-specific dehydrogenase with a concomitant reduction of $NAD(P)^+$ to NAD(P)H. The light produced is proportional to the amount of metabolite in the sample.

Simple “Add-Mix-Measure” protocol



The assays are compatible with various sample types, requiring only simple dilution and/or acid treatment followed by neutralization. This eliminates the need for cell collection, centrifugation, or spin columns. Adaptable to a 384-well format, the assays enable rapid analysis of large sample sets.

Glucose-Glo™ Assay

Cell-based

Applications

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

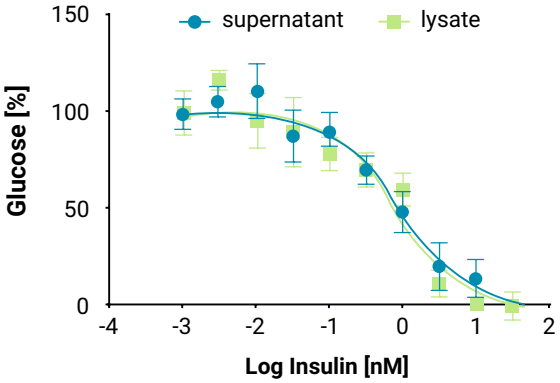
Assay principle

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 simultaneously. Glucose dehydrogenase oxidizes glucose, reducing NAD^+ to NADH. NADH then enables Reductase to convert a proluciferin substrate to luciferin, which Ultra-Glo™ Luciferase uses 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

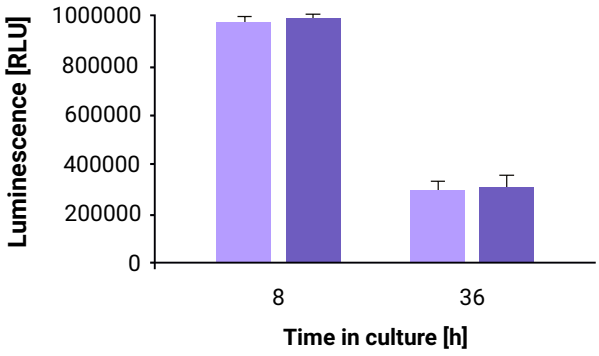
Assay type	Luminescent (glow-type), one step assay
Markers	Glucose
Applications	Monitoring of glucose levels
Sample	Cells, lysates, tissue, plasma, serum, supernatant
Time required	60 minutes
Sensitivity	5 nM with linear range up to 50 µM, S/B max > 1000
Robustness	Easily scalable from 96- to 384-well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

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



Human liver spheroids were cultured in gluconeogenesis medium to inhibit glycolysis and promote glucose production. Spheroids were then treated with increasing insulin concentrations to reduce glucose production. 25 µl of medium (blue) was collected, Glucose Detection Reagent was added, and luminescence was recorded after 60 minutes. Similar results were observed using cell lysates (green).

Glucose consumption by K562 cells



K562 cells were cultured in RPMI medium with 5 mM glucose and 10 % dialyzed FBS. After 8 and 36 hours, 1.5 ml samples were collected, diluted 10-fold in PBS, either directly (light purple) or after removing the cells by centrifugation (dark purple) and frozen at –20°C. For glucose detection samples were thawed, 20 µl was colletcted and an equal volume of Glucose Detection Reagent was added.

Lactate-Glo™ Assay

Cell-based

Applications

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

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.

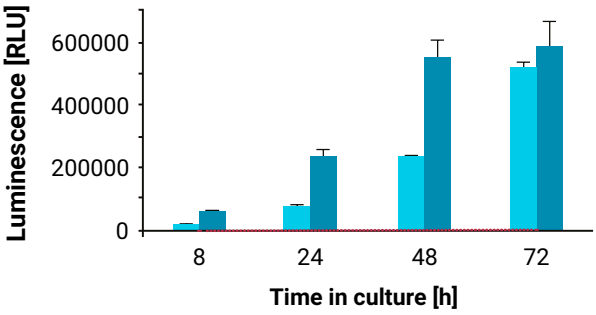
Assay principle

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 proluciferin 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

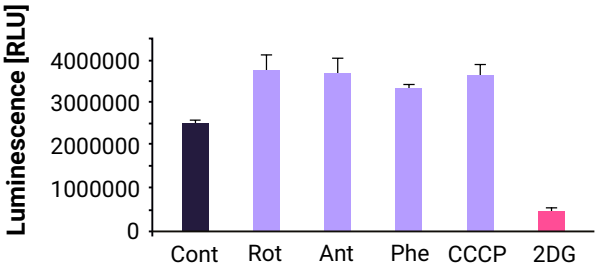
Assay type	Luminescent (glow-type), one step assay
Markers	Lactate
Applications	Monitoring of lactate levels
Sample	Cells, lysates, tissue, plasma, serum, supernatant
Time required	60 minutes
Sensitivity	100 nM with linear range up to 200 µM, S/B max > 200
Robustness	Easily scalable from 96- to 384-well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

Lactate secretion by A549 cells



A549 cells were plated at 5,000 (light blue) and 15,000 (dark blue) 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, diluted in PBS and frozen at –20°C. On the day of the assay, the samples were thawed, 25 µl of each sample was transferred to a new plate and 25 µl of Lactate Detection Reagent was added. After 60 minutes, luminescence was read. Data represent the average of four replicates.

Changes in glycolytic rate



A549 cells in DMEM 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 replaced with glucose-free medium containing mitochondria inhibitors (5 µM rotenone, 5 µM antimycin, 2.5 mM phenformin, 50 µM CCCP), a glycolysis inhibitor (10 mM 2-deoxyglucose), or no compound. After 5 minutes, 4 mM glucose was added, and samples were incubated for 1 hour before Lactate Detection Reagent was added. Data showed increased glycolysis in response to mitochondrial inhibitors and an 80 % decrease in lactate production with glycolysis inhibition by 2DG compared to control samples.

Malate-Glo™ Assay

Cell-based

Applications

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

Assay description

The **Malate-Glo™ Assay** is a bioluminescent assay for rapid, selective and sensitive detection of L-malate in biological samples. The assay couples malate oxidation and NADH production with a bioluminescent NADH detection system. The Malate-Glo™ Assay is a versatile system that is amenable to high-throughput formats and does not require sample centrifugation or spin columns. The Malate-Glo™ Assay can detect subtle changes in mitochondrial metabolism and the TCA cycle.

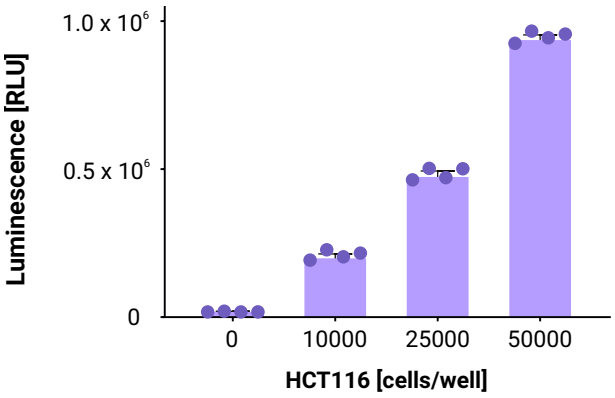
Assay principle

When Malate Detection Reagent, which contains malate dehydrogenase (MDH), 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. Malate dehydrogenase uses malate and NAD^+ to produce pyruvate and NADH. In the presence of NADH a proluciferin 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 malate in the sample and increases until all malate is consumed at which time a stable luminescent signal is achieved.

Assay features

Assay type	Luminescent (glow-type), one step assay
Markers	Malate
Applications	Monitoring of malate levels
Sample	Cells, lysates, tissue, plasma and serum
Time required	60 minutes
Sensitivity	5 nM with linear range up to 25 µM, S/B max > 200
Robustness	Easily scalable from 96- to 384-well plates

Quantification of intracellular malate



HCT116 cells in DMEM with 10 % serum were plated at 10,000, 25,000, and 50,000 cells/well. After overnight incubation, cells were washed with PBS, and malate was measured as per protocol. The average luminescence for four replicates is shown in RLU, demonstrating >10-fold signal above background with 10,000 cells/well, with signal increasing proportionally with cell numbers.

Pyruvate-Glo™ Assay

Cell-based

Applications

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

Assay description

The **Pyruvate-Glo™ Assay** is a bioluminescent assay for fast and sensitive detection of pyruvate in biological samples. The assay uses a 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 Pyruvate-Glo™ Assay is amenable to high-throughput formats, compatible with many sample types and can detect subtle changes in glycolysis and mitochondrial metabolism.

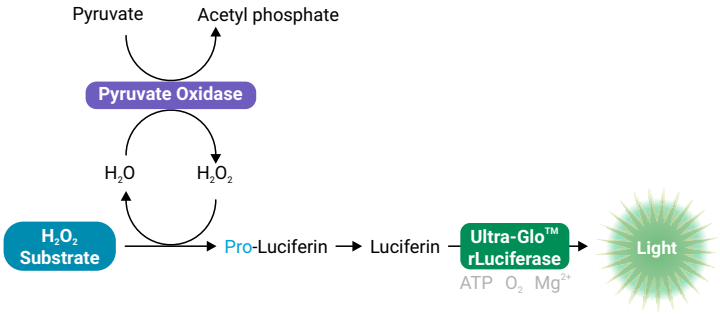
Assay principle

After sample preparation, a pyruvate oxidase catalyzes the reaction of pyruvate to acetyl phosphate, producing H_2O_2. An H_2O_2 Substrate reacts directly with the H_2O_2 to generate a luciferin precursor. By addition of the Pyruvate-Glo™ Detection Solution, the luciferin precursor is converted to luciferin, which is detected using an Ultra-Glo™ rLuciferase. The amount of light produced is proportional to the amount of pyruvate in the sample.

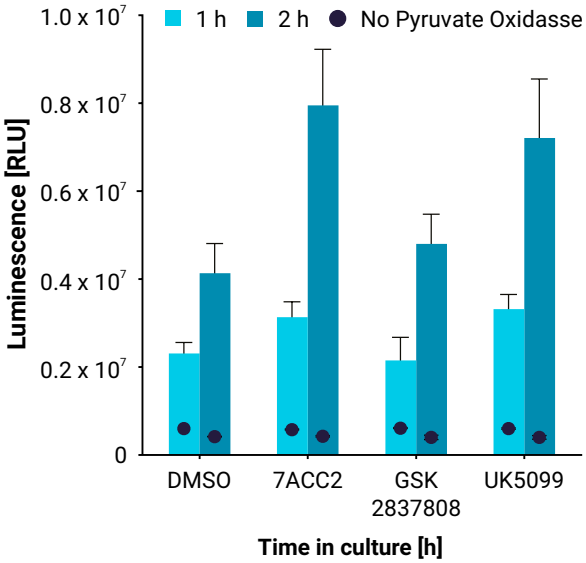
Assay features

Assay type	Luminescent (glow-type), two-step assay
Markers	Pyruvate
Applications	Monitoring of pyruvate levels
Sample	Cells, lysates, tissue, plasma, serum, supernatant
Time required	75 minutes
Sensitivity	400 nM with linear range up to 50 µM, S/B max > 150
Robustness	Easily scalable from 96- to 384-well plates

Pyruvate-Glo™ Assay principle



Monitor metabolic effects of drug treatment with the Pyruvate-Glo™ Assay



K562 cells in suspension were incubated with DMSO (control), 10 µM GSK2837808, 10 µM 7ACC2, or 10 µM UK5099 for 1–2 hours. After incubation, half the volume was analyzed with the Pyruvate-Glo™ Assay and the other half for viability using the Cell-Titer-Glo® Assay. Pyruvate levels doubled after 2 hours with UK5099 and 7ACC2 compared to DMSO, with no decrease in viability. Data represent the average of three replicates.

Glycogen-Glo™ Assay

Cell-based

Applications

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

Assay description

The **Glycogen-Glo™ Assay** is a fast and sensitive method for detecting glycogen in various biological samples. Based on bioluminescent technology, the Glycogen-Glo™ Assay can detect subtle changes in glycogen synthesis, storage and breakdown. The Glycogen-Glo™ Assay is a versatile system that is amenable to high-throughput formats and does not require sample centrifugation or spin columns.

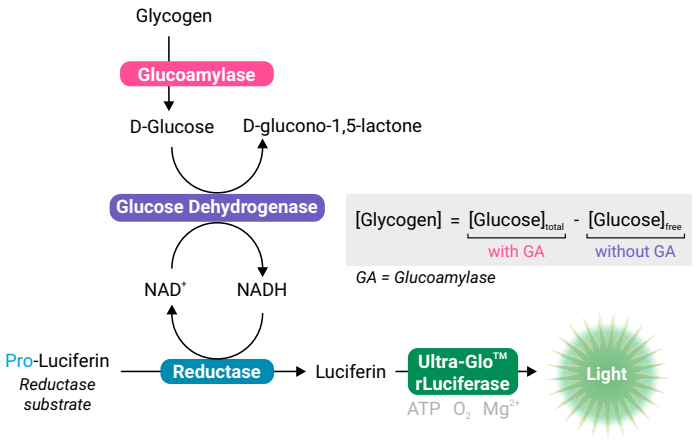
Assay principle

The Glycogen-Glo™ Assay uses two sequential steps to measure glycogen. Glycogen is first digested into glucose units by Glucoamylase enzyme. Then glucose is measured by coupling glucose oxidation and NADH production with a bioluminescent NADH detection system. The luminescent signal is proportional to the amount of glucose in the sample and increases until all glucose is consumed, at which point a stable luminescent signal is achieved. Samples that contain both glycogen and glucose require two reactions to determine the glycogen concentration. One reaction is used to measure total glucose resulting from digested glycogen plus any glucose present in the sample. The second is used to measure any glucose that was present in the sample before digestion. The difference in signal between the two reactions represents the contribution from glycogen in the sample. The effective digestion of glycogen into glucose monomers (≥70%) simplifies data interpretation and calculations.

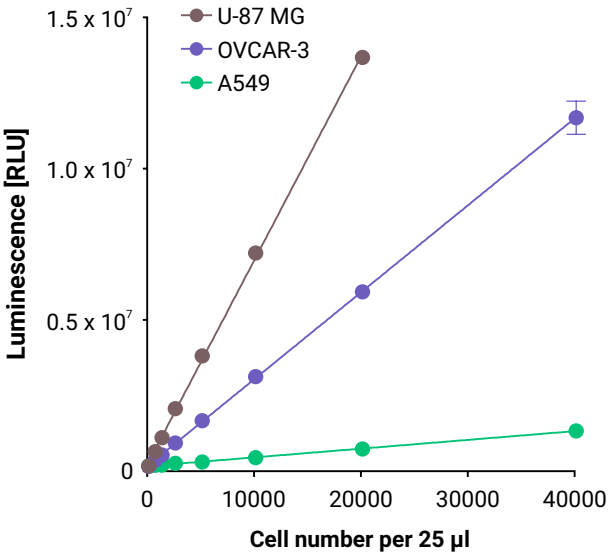
Assay features

Assay type	Luminescent (glow-type), one step assay
Markers	Glycogen
Applications	Monitoring of glycogen levels
Sample	Cells, lysates, tissue, plasma and serum
Time required	2 hours
Sensitivity	Linear range up to 20 µM, S/B max > 150
Robustness	Easily scalable from 96- to 384-well plates

Glycogen-Glo™ Assay principle



Cellular glycogen content varies greatly depending on cell type and growth conditions



The high sensitivity and wide linearity of the Glycogen-Glo™ Assay accommodate measurement of glycogen in cells, regardless of whether they have low or high glycogen levels. The data show analysis of intracellular glycogen content of three cell lines using the Glycogen-Glo™ Assay.

Glutamate-Glo™ Assay

Cell-based

Applications

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

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.

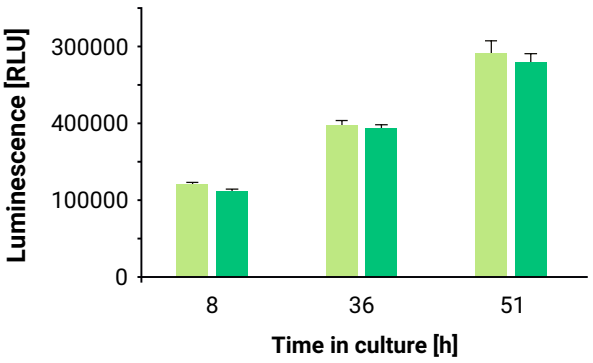
Assay principle

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 a-ketoglutarate and NADH. In the presence of NADH a proluciferin 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

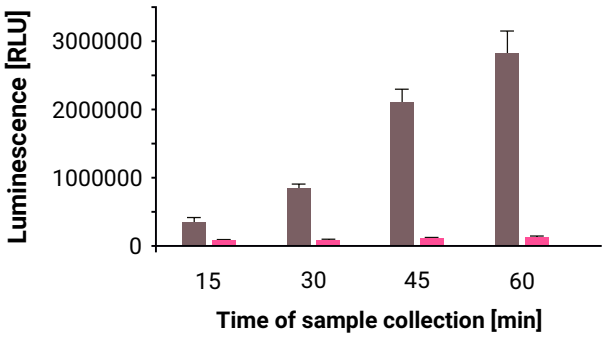
Assay type	Luminescent (glow-type), one step assay
Markers	Glutamate
Applications	Monitoring of glutamate levels
Sample	Cells, lysates, tissue, plasma, serum, supernatant
Time required	60 minutes
Sensitivity	5 nM with linear range up to 50 µM, S/B max > 100
Robustness	Easily scalable from 96- to 384-well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

Glutamate secretion by K562 cells



K562 cells were cultured in RPMI with 5 mM glucose and 10% dialyzed FBS. Samples were collected, diluted 10-fold in PBS (either directly (light green) or after centrifugation (dark green)), and frozen. For glutamate detection, samples were thawed, 20 µl was transferred to an assay plate, Glutamate Detection Reagent was added, and luminescence was read. Results indicate that a small volume of suspension cell culture can be removed, diluted and assayed directly without centrifugation or treating 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 medium. After overnight incubation, 30 µl of DMEM with (pink bars) or without (brown bars) 27 µM BPTES was added, followed by 10 µl of DMEM with glucose and glutamine. At set times, cell metabolism was stopped with 5 µl of Inactivation Solution, followed by 5 µl of Neutralization Solution. Glutamate was then measured with 50 µl of Glutamate Detection Reagent. Cells without BPTES produced increasing glutamate over time, while BPTES-treated cells showed glutaminase inhibition.

Glutamine/Glutamate-Glo™ Assay

Cell-based

Applications

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

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.

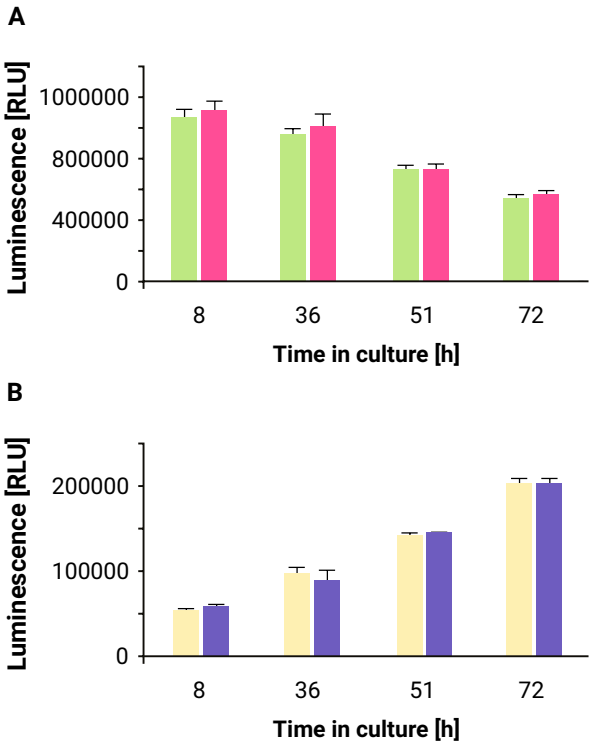
Assay principle

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 enzymecoupled 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, serum, supernatant
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. Cells were cultured in RPMI with 5 mM glucose and 10 % dialyzed FBS. Samples were collected, diluted in PBS (directly (green and yellow bars) or post-centrifugation (pink and lilac bars)), and frozen. For analysis samples were thawed and diluted with PBS. 10 µl aliquots were incubated with Glutaminase Buffer or Glutaminase Enzyme Solution, followed by Glutamate Detection Reagent. Luminescence was measured after 60 minutes. Results show that small samples can be assayed directly without pelleting the cells or treating the samples for protein inactivation and NAD(P)H degradation.

BCAA-Glo™ Assay

Cell-based

Applications

Monitoring of branched-chain amino acids (leucine, isoleucine, valine) in cells, lysates, tissue, plasma, serum and supernatant.

Assay description

The **BCAA-Glo™ Assay** is a bioluminescent assay for rapid, selective and sensitive detection of branched-chain amino acids (BCAA) in biological samples. The term branched-chain amino acids refers to three amino acids: leucine, isoleucine and valine. The workflow is compatible with 96- and 384-well plate formats, does not require sample centrifugation or spin columns, and is well-suited for rapidly analyzing multiple samples.

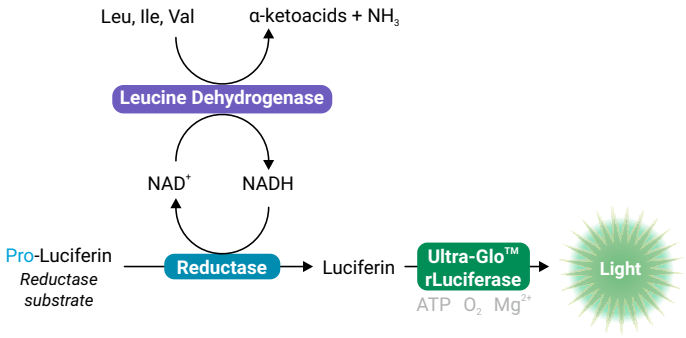
Assay principle

The BCAA-Glo™ Assay couples BCAA oxidation and NADH production with a bioluminescent NADH detection system. When BCAA detection reagent is added to a sample at a 1:1 ratio, the coupled-enzyme reactions are initiated and run simultaneously. The luminescent signal is proportional to the amount of BCAA in the sample, and increases until all BCAA is consumed, at which point a stable luminescent signal is achieved. The Leucine Dehydrogenase enzyme recognizes all three BCAA with similar efficiency. A single amino acid, Leucine, 10 mM, is provided to serve as a positive control.

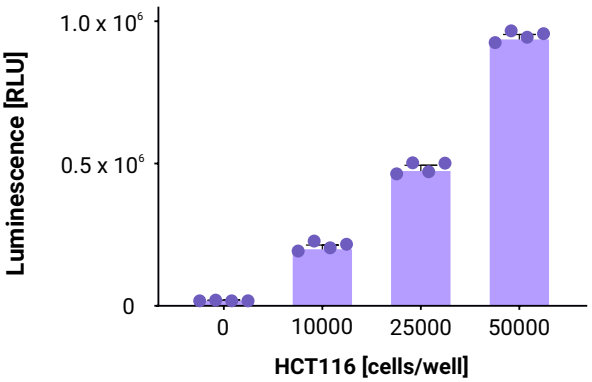
Assay features

Assay type	Luminescent (glow-type), one step assay
Markers	Branched-chain amino acids (BCAA)
Applications	Monitoring of BCAA levels
Sample	Cells, lysates, tissue, plasma, serum, supernatant
Time required	60 minutes
Sensitivity	5 nM with linear range up to 25 µM, S/B max > 300
Robustness	Easily scalable from 96- to 384-well plates

BCAA-Glo™ Assay principle



Measuring BCAA in tissues



Frozen mouse liver tissue samples were homogenized. For BCAA detection, samples were serially diluted dilution buffer (PBS + 0.6N HCl + Neutralization Buffer, 5:1:1) and 50 µl was added to an assay plate. Positive and negative controls containing 50 µl of homogenization buffer with and without 1 µM leucine were included. After adding 50 µl of BCAA Detection Reagent and a 60-minute incubation, luminescence was recorded. Dilutions in the 1:2 to 1:16 range were tested. BCAA concentrations were approximately 9.1 µM in the homogenates, with data averaged from four replicates (shown as dots).

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).

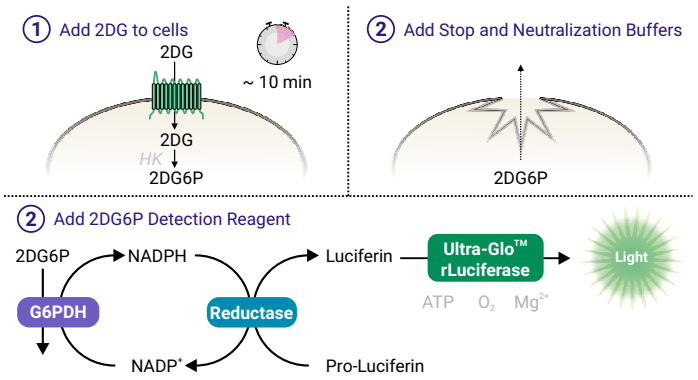
Assay principle

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.

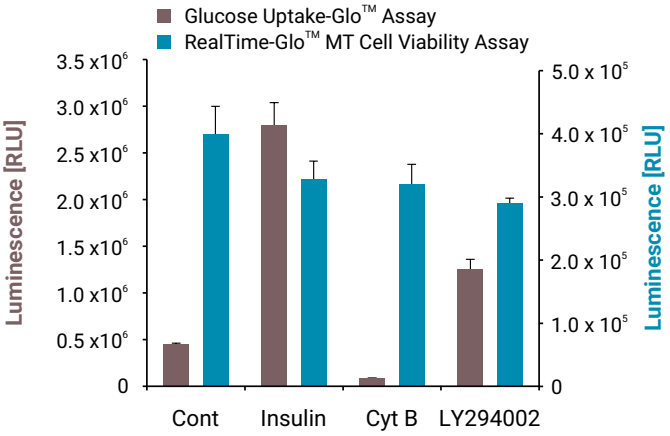
Assay features

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



Glucose uptake and viability in adipocyte cells

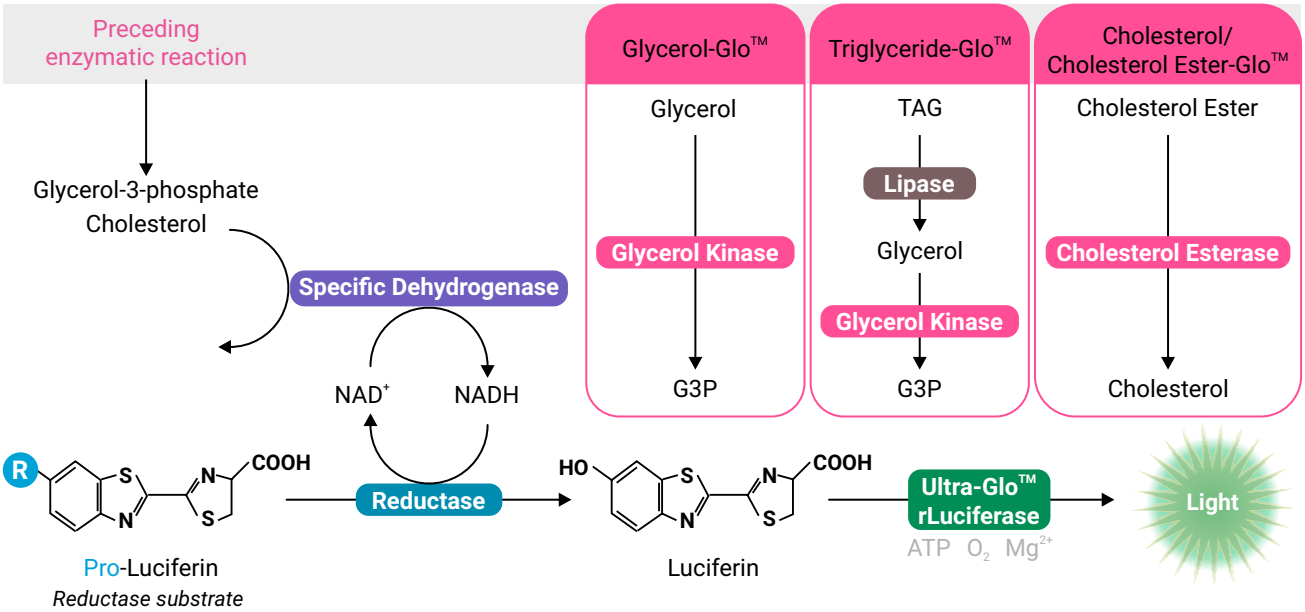


"Cont" (control) indicates the basal level of glucose uptake. Insulin increases glucose uptake by inducing the translocation of glucose transporters to the cell surface. Cytochalasin B, a glucose transporter inhibitor, decreases glucose uptake, while LY294002, a phosphatidylinositol 3-kinase (PI3K) inhibitor, reduces glucose uptake relative to insulin alone. Despite significant changes in glucose uptake, cell viability remains largely unaffected, as shown by the RealTime-Glo™ Assay.

Bioluminescent Detection of Lipid Metabolites

The Triglyceride-Glo™, Glycerol-Glo™ & Cholesterol/ Cholesterol Ester-Glo™ are bioluminescent assays for rapid and sensitive measurement of glycerol and triglyceride or cholesterol and cholesterol ester from a variety of sample types. The assays are sensitive with a large linear range, reducing the number of sample dilutions required and increasing the quantitative power to discriminate small changes in lipid metabolite levels. The bioluminescent signal eliminates signal interference that colorimetric and fluorescent assays suffer from, and there is no need for organic extraction steps. The metabolite assays are using our core luminescent technology in combination with enzyme-coupled reactions to quantitate the specific metabolites in culture medium, cell lysates, 3D structures, tissues, plasma and serum in 96-, 384-well plates.These assays can be either used for the quantification of lipolysis and lipogenesis, among other biological processes or for the detection of metabolic changes and evaluating biological activity of insulin and insulin analogues in cellular models. The BHB-Glo™ (Ketone Body) Assay is a rapid and sensitive method for detecting β-hydroxybutyrate (BHB) from various biological samples, such as cultured cells, media, tissue homogenates and serum. Based on bioluminescent technology, the BHB-Glo™ Assay can detect subtle changes in ketosis as well as ketone body consumption and secretion.

Assay principle of Triglyceride-Glo™, Glycerol-Glo™ & Cholesterol/Cholesterol Ester-Glo™



Triglyceride-Glo™ Assay

Cell-based

Applications

Measuring triglyceride accumulation and clearance under normal and pathological conditions.

Assay description

The **Triglyceride-Glo™ Assay** provides a rapid and sensitive luminescent method for measuring triglycerides in cultured cell lysates and other biological samples such as cell culture medium, serum and tissue homogenates. The assay can be performed in 96- and 384-well plates where the detection reagents are added directly to samples without the need for organic extraction, making it amenable to high-throughput applications.

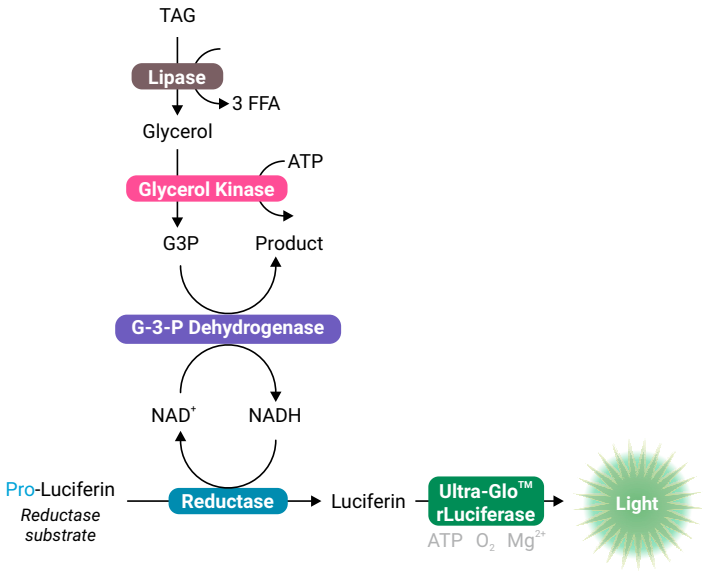
Assay principle

The Triglyceride-Glo™ Assay detects triglyceride levels by measuring glycerol that is released from an enzymatic reaction with a lipase: one mole of glycerol per mole of triglyceride. Glycerol is measured in a coupled reaction scheme that links the production of NADH to the activation of a proluciferin that produces light with luciferase. The amount of triglyceride is determined from the difference of glycerol measured in the absence (free glycerol) and presence (total glycerol) of lipase. Lipase converts triglyceride (TAG) to glycerol. Glycerol kinase and glycerol-3-phosphate dehydrogenase are used to generate NADH. In the presence of NADH, Reductase enzymatically reduces a proluciferin Reductase Substrate to luciferin. Luciferin is detected in a luciferase reaction using Ultra-Glo™ Luciferase and ATP, and the amount of light produced is proportional to the amount of glycerol in the sample.

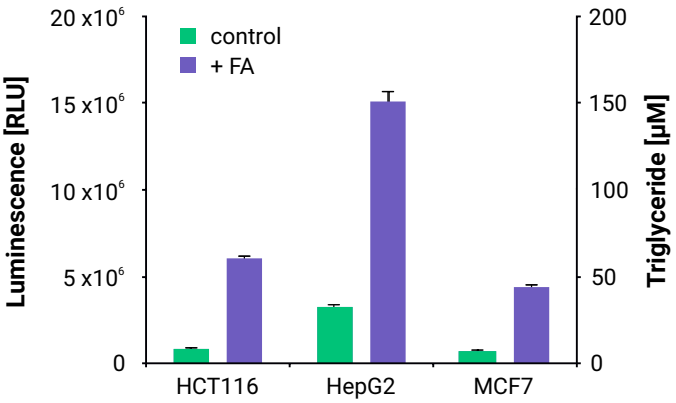
Assay features

Assay type	Luminescent (glow-type), two step assay
Markers	Triglycerides
Sample	Cells, 3D-microtissues, plasma, serum
Time required	< 2 hours
Sensitivity	1 – 5 pmole/sample with broad linear range 0.1 – 80 µM
Robustness	No organic extraction required, easily scalable from 96- to 384-well plates

Triglyceride-Glo™ Assay principle



Triglyceride levels in cancer cell lines



Ten thousand cells of three cell lines (HCT116: colon cancer cell line; HepG2: hepatocarcinoma cell line; MCF7: breast cancer cell line) were plated overnight in the absence (control) or presence (+ FA) of 0.3 mM linoleic & oleic acid bound to BSA. After media removal, cell washing and lipase treatment, 12.5 µl aliquots of the treated samples were diluted into 37.5 µl of Glycerol Lysis Solution and assayed per the standard protocol.

Glycerol-Glo™ Assay

Cell-based

Applications

Measuring changes in glycerol concentration, both extracellular and intracellular.

Assay description

The **Glycerol-Glo™ Assay** is a bioluminescent assay for rapid and sensitive measurement of glycerol in a variety of biological samples such as cell culture medium, serum and tissue lysates. Glycerol is often measured as the product of lipolysis, where it is released from triglycerides. Glycerol is also a substrate or product of many other enzymatic or metabolic processes that can be studied with the assay.

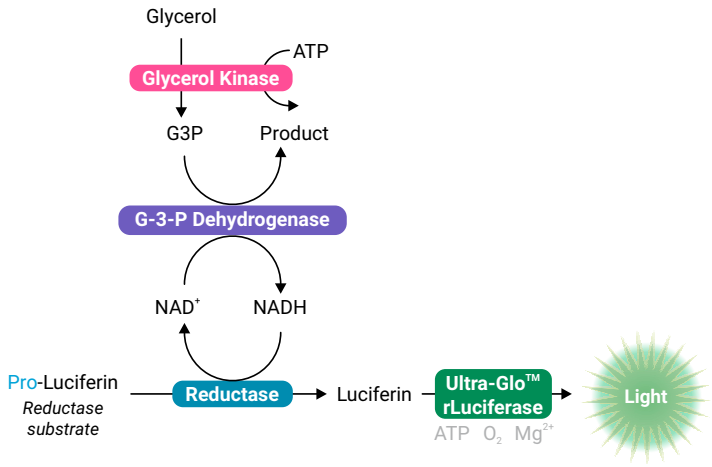
Assay principle

The Glycerol-Glo™ Assay measures glycerol in a coupled reaction scheme that links the production of NADH to the activation of a proluciferin that produces light with luciferase. Glycerol kinase and glycerol-3-phosphate dehydrogenase are used to generate NADH. In the presence of NADH, Reductase enzymatically reduces a proluciferin Reductase Substrate to luciferin. Luciferin is detected in a luciferase reaction using Ultra-Glo™ Luciferase and ATP and the amount of light produced is proportional to the amount of glycerol in the sample.

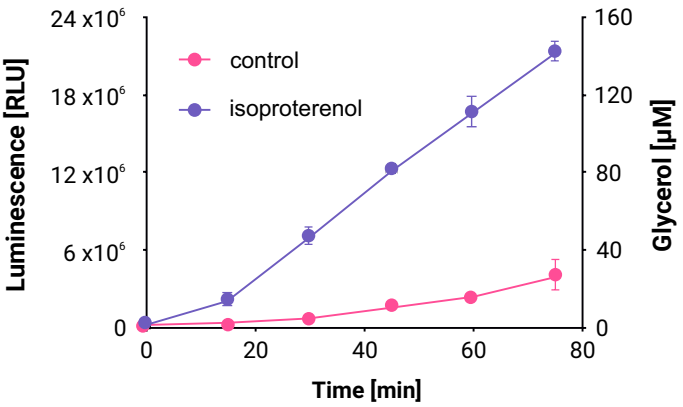
Assay features

Assay type	Luminescent (glow-type), two step assay
Markers	Glycerol
Sample	Cells, lysates, 3D-microtissues, plasma, serum
Time required	< 2 hours
Sensitivity	1 – 5 pmole/sample with broad linear range 0.1 – 80 µM
Robustness	Easily scalable from 96- to 384-well plates

Glycerol-Glo™ Assay principle



Glycerol release from adipocytes



Adipocytes were differentiated from 3T3L1-MBX fibroblasts. Cells were washed twice with PBS, then RPMI with 2% fatty acid-free BSA and 5 µM triacsin C was added to promote extracellular glycerol accumulation. The medium contained 0 or 10 µM isoproterenol for control or stimulated conditions. Medium aliquots were removed over time, diluted with Glycerol Lysis Solution, and assayed for glycerol.

Cholesterol/Cholesterol Ester-Glo™ Assay

Cell-based

Applications

Detection of even small changes of cholesterol or cholesterol esters in biological samples.

Assay description

The **Cholesterol/Cholesterol Ester-Glo™ Assay** is a bioluminescent assay for rapid and sensitive method for measuring cholesterol and cholesterol esters in cultured cell lysates and other biological samples, such as lipoprotein fractions, cell culture medium, serum and tissue homogenates. Cholesterol is an essential lipid involved in steroidogenesis, bile acid synthesis, cell signaling and maintenance of membrane structure.

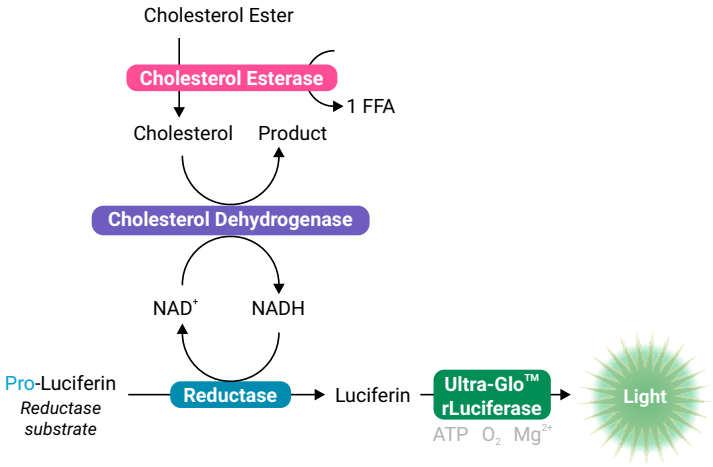
Assay principle

The Cholesterol/Cholesterol Ester-Glo™ Assay measures cholesterol using a cholesterol dehydrogenase that links the presence of cholesterol to the production of NADH to the activation of a proluciferin that produces light with luciferase. In the presence of NADH, Reductase enzymatically reduces a proluciferin Reductase Substrate to luciferin. Luciferin is detected in a luciferase reaction using Ultra-Glo™ Luciferase and ATP, and the amount of light produced is proportional to the amount of cholesterol in the sample. For measuring cholesterol ester, the assay includes a cholesterol esterase that will remove the fatty acid from cholesterol esters to produce one molecule of cholesterol per molecule of ester. The amount of cholesterol esters is determined from the difference of cholesterol measured in the absence (free cholesterol) and presence (total cholesterol) of esterase.

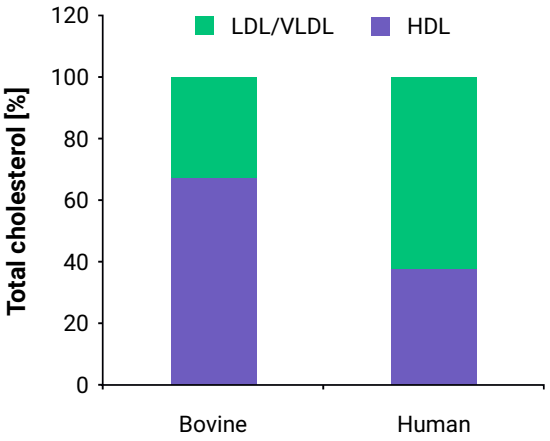
Assay features

Assay type	Luminescent (glow-type), two step assay
Markers	Cholesterol and cholesterol esters
Sample	Cells, lysates, 3D-microtissues, plasma, serum
Time required	< 2 hours
Sensitivity	1 – 5 pmole/sample with broad linear range 0.1 – 80 µM
Robustness	No organic extraction required, easily scalable from 96- to 384-well plates

Cholesterol/Cholesterol Ester-Glo™ Assay principle



Measuring cholesterol and cholesterol esters in lipoproteins



Relative amounts of high vs. low and very low density lipoproteins (VLDL) in sera. Samples of bovine and human sera were mixed 5:2 with 20 % PEG8000, incubated for 20 minutes at room temperature and ce n-trifuged for 30 minutes. HDL in the supernatant was transferred to a fresh tube and the LDL/ VLDL in the precipitate was resuspended in PBS. Each lipoprotein fraction was diluted 100-fold into Cholesterol Lysis Solution and assayed for total cholesterol (unesterified + esterified).

BHB-Glo™ Assay

Cell-based

Applications

Monitoring of β-hydroxybutyrate (BHB) levels in cells, lysates, tissue, plasma, serum and supernatant.

Assay description

The **BHB-Glo™ (Ketone Body) Assay** is a bioluminescent assay for rapid, selective and sensitive detection of β-hydroxybutyrate (BHB) in biological samples. β-hydroxybutyrate is a ketone body that is synthesized in the liver from fatty acids during low glucose conditions, such as starvation or prolonged exercise. The assay is amenable to high-throughput formats and does not require sample centrifugation or spin columns.

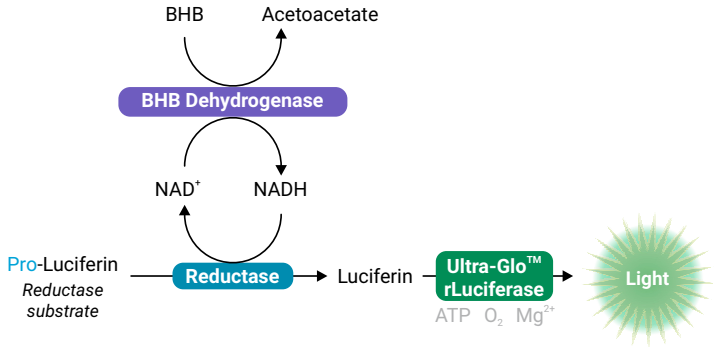
Assay principle

The BHB-Glo™ (Ketone Body) Assay couples BHB oxidation and NADH production with a bioluminescent NADH detection system. When BHB Detection Reagent is added to a sample at a 1:1 ratio, the coupled-enzyme reactions are initiated and run simultaneously. The luminescent signal is proportional to the amount of BHB in the sample and increases until all BHB is consumed, at which point a stable luminescent signal is achieved.

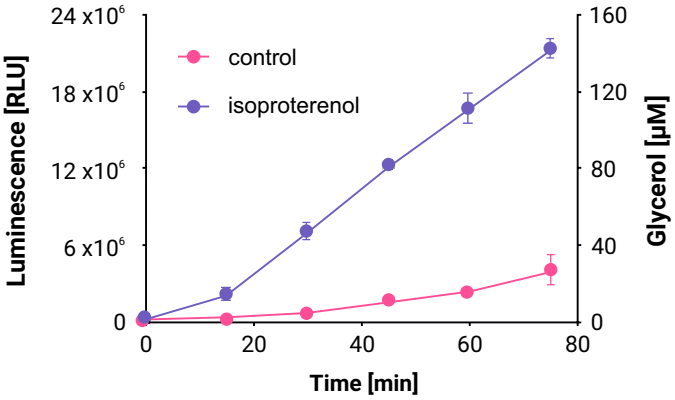
Assay features

Assay type	Luminescent (glow-type), one step assay
Markers	BHB
Applications	BHB
Sample	Cells, lysates, tissue, plasma, serum, supernatant
Time required	60 minutes
Sensitivity	50 nm with linear range up to 25 µm, S/Bmax >200M
Robustness	Easily scalable from 96- to 384-well plates

BHB-Glo™ Assay principle



BHB secretion in cell medium



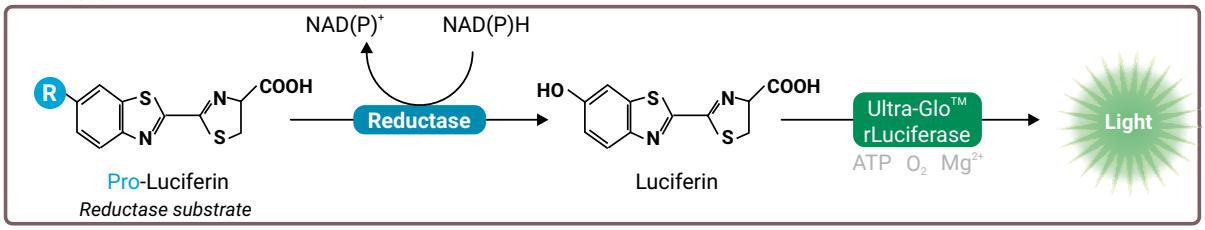
HepG2 cells were plated at 50,000 cells/well in DMEM with 10% FBS. After washing with PBS to remove BHB from medium, cells were incubated in DMEM without FBS but with 5 mM glucose and 2 mM glutamine. Medium samples were collected after 6 and 24 hours, frozen, then assayed for BHB. Results show a >sixfold increase in extracellular BHB above background at 24 hours, with levels rising from 3.1 µM at 6 hours to 7.1 µM at 24 hours. Data are the average luminescence of three replicates.

Bioluminescent Detection of NAD(P)H/NAD(P)+

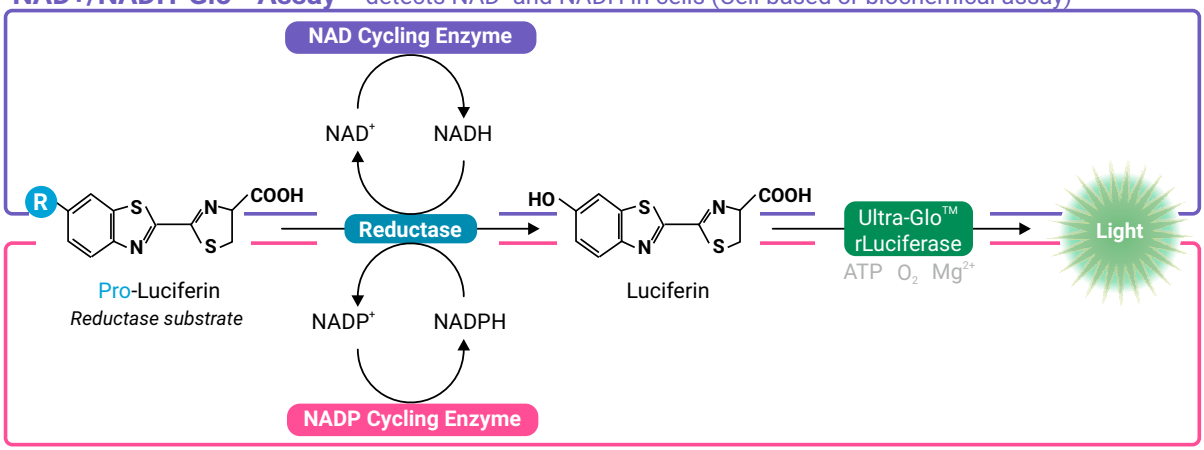
Cancer is a disease of uncontrolled cell growth that requires cancer cells to alter metabolic pathways to survive and proliferate. The principal mechanisms underlying this metabolic reprogramming by oncogenes and tumor suppressor genes are still poorly understood. Nicotinamide adenine dinucleotides (NAD^+, 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.

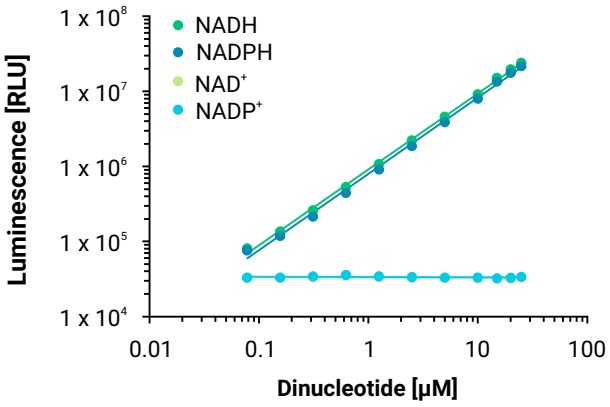
Assay principle

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.

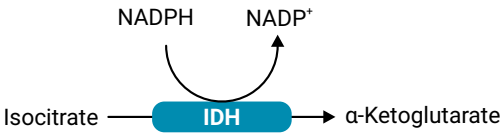
Assay features

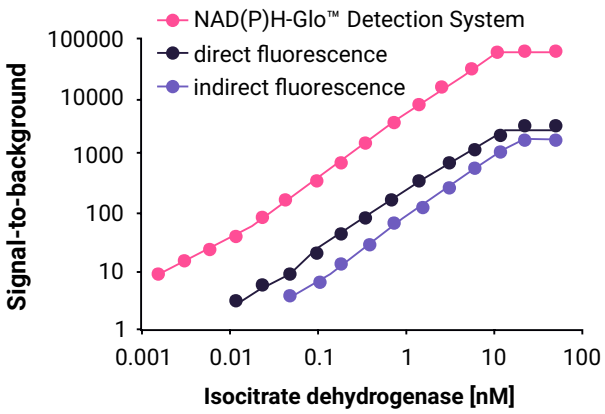
Assay type	Luminescent (glow-type; $T_{1/2}$ > 2 h)
Markers	NADH, NADPH
Applications	Monitoring the activity of enzymes that produce or use NAD(P)H.
Sample	Enzyme preparations
Implementation	Homogeneous, one-step assay with flexible storage capability
Sensitivity	Broad linear range (25 nM – 50 µM) and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

Linear range and specificity of the NAD(P)H-Glo™ Assay



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

In the following section, only **NAD⁺/NADH-Glo™ Assay** is described, since assay principle and assay features are equal to **NADP⁺/NADPH-Glo™ Assay** (see also p.20).

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.

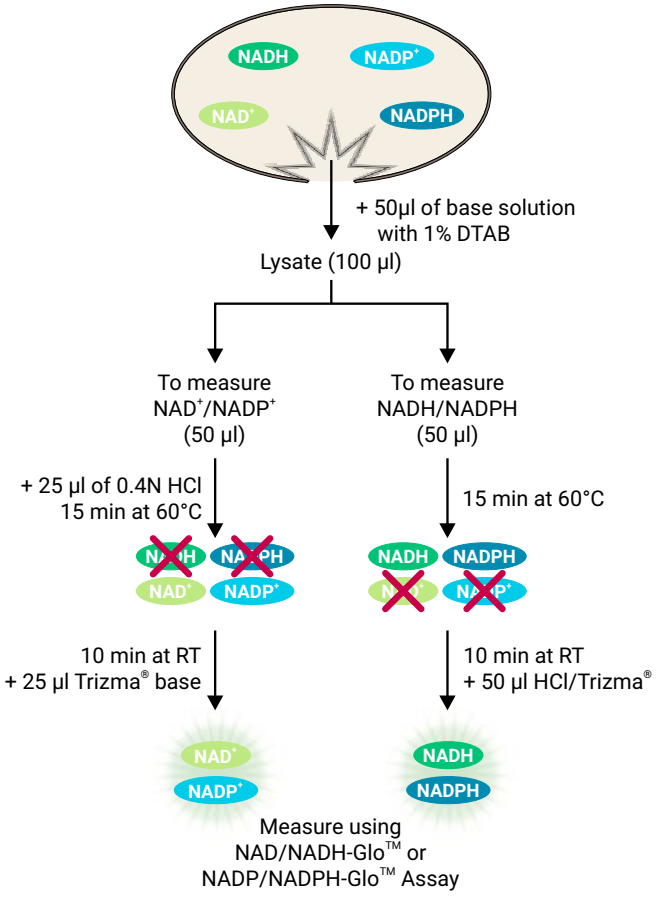
Assay description

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 non-phosphorylated NAD⁺ and NADH compared to the phosphorylated forms NADP⁺ and NADPH. The NAD/NADH-Glo™ Assay detects 1 nM 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

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.

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

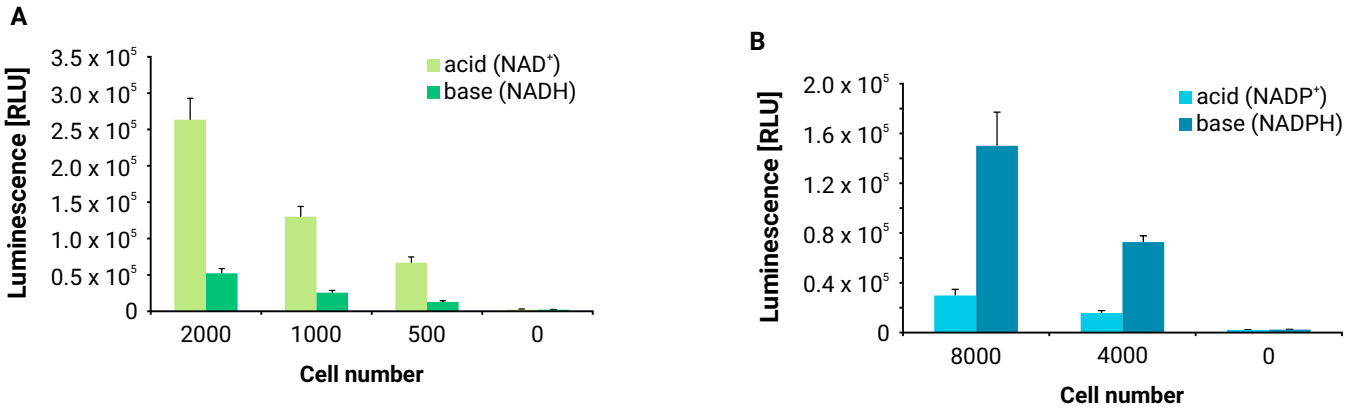


3 Dinucleotide Detection Assays

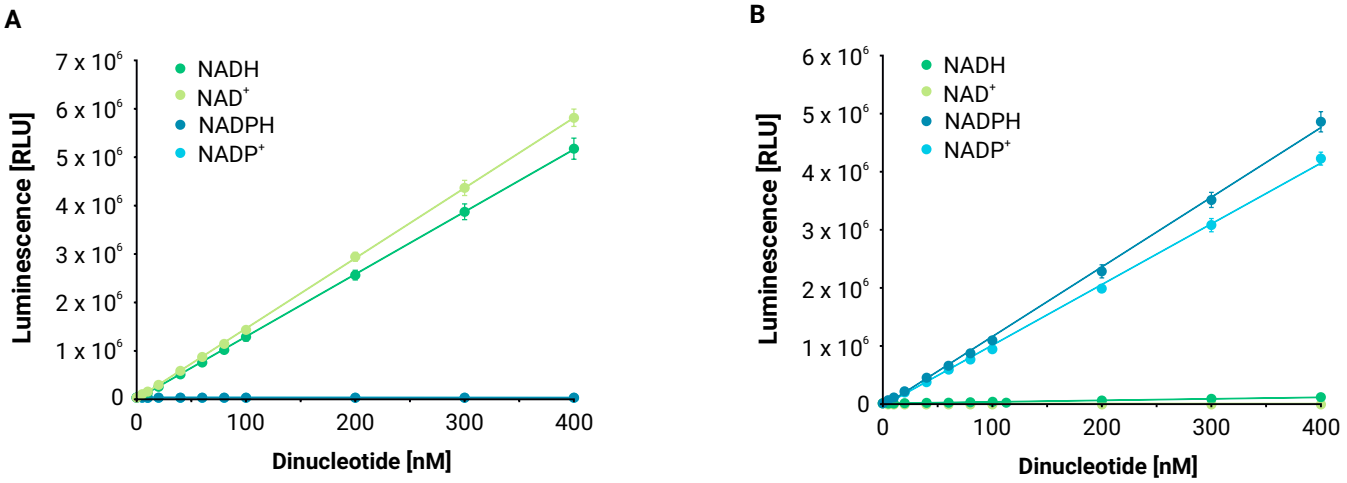
Assay features

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)	1 nM (25 fmol/25 µl)
Linearity	1 – 500 nM
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.



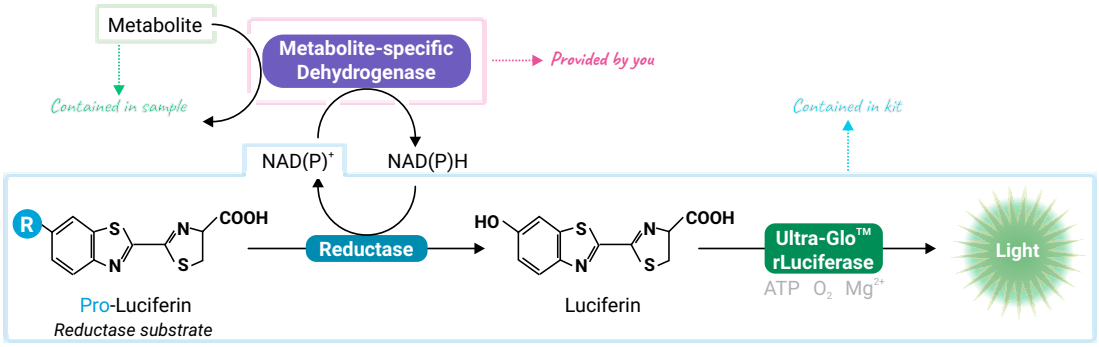
Linear range and specificity of A. $NADP^+$/NADPH- and B. NAD^+/NADH-Glo™ Assay



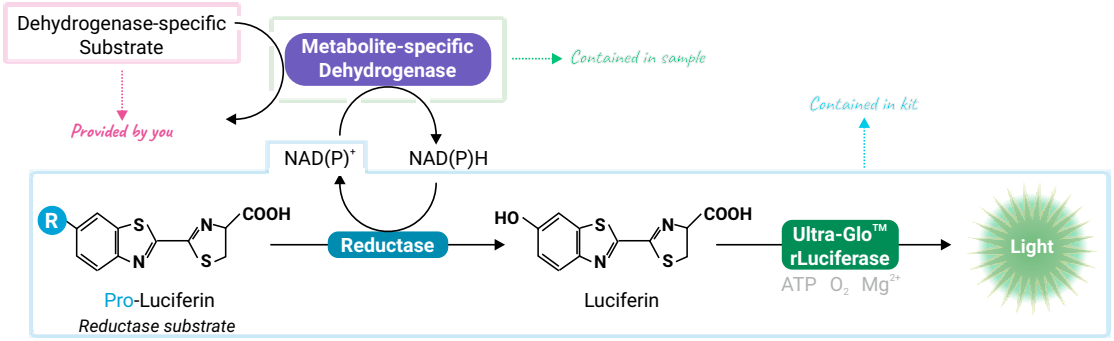
4 Build-Your-Own Metabolism Assays

For advanced metabolic readouts beyond the Promega catalog, our do-it-yourself assay kits offer NAD(P)H detection reagents for either customized metabolite detection (Metabolite-Glo™ Detection System) or functional pathway analysis (Dehydrogenase-Glo™ Detection System).

Metabolite-Glo™ Detection System – Toolkit to customize metabolite detection for a specific target



Dehyrogenase-Glo™ Detection System – Toolkit for functional analysis of all major metabolite pathways



Comparison of Metabolite-Glo™ and Dehydrogenase-Glo™ Assay:

	Metabolite-Glo™	Dehydrogenase-Glo™
Scientific question:	How much metabolite is present?	How active/abundant is the endogenouse dehydrogenase?
Detects:	NAD(P)H	NAD(P)H
Luminescence proportional to:	Metabolite level	Dehydrogenase activity
Metabolic pathway analysis:	Indirect	Direct
Requires addition of:	Dehydrogenase	Substrate
Calibration:	Metabolite standard	NAD(P)H Standard
Compatible sample types:	Cells, media, tissue, serum	Cell lysate, tissue homogenates, biochemical samples

Metabolite-Glo™ Detection System

Cell-based

Applications

Monitoring metabolites of interest in cells, lysates, tissue, plasma and serum.

Assay description

The **Metabolite-Glo™ Detection System** is a do-it-yourself toolkit to enable metabolite detection beyond Promega's catalog. It can be used to build a bioluminescent metabolite detection assay customized to a specific target. The assay couples metabolite oxidation and NAD(P)H production with bioluminescent NAD(P)H detection. The Metabolite-Glo™ Detection System provides only the reagents required for bioluminescence NAD(P)H detection. To build your own metabolite assays, a metabolite-specific NAD- or NADP-dependent dehydrogenase needs to be identified and sourced by the user.

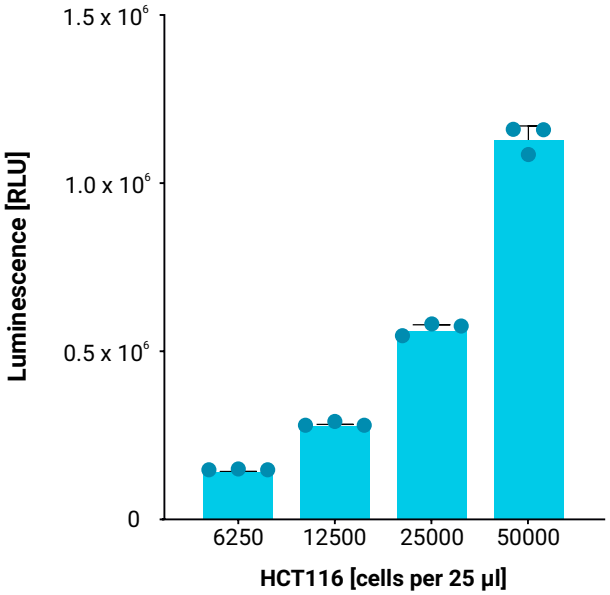
Assay principle

A metabolite-specific dehydrogenase (not provided) catalyzes the oxidation of the metabolite with concomitant reduction of NAD^+ to NADH or $NADP^+$ to NADPH. In the presence of NADH or NADPH, reductase enzymatically reduces a proluciferin reductase substrate to luciferin. Luciferin is detected using Ultra-Glo™ Recombinant Luciferase, and the amount of light produced is proportional to the amount of metabolite in the sample.

Assay features

Assay type	Luminescent (glow-type), one step assay
Markers	NAD(P)H
Applications	Monitoring metabolite of interest, NAD(P)H Screening.
Sample	Cells, lysates, tissue, plasma and serum
Time required	60 min (with optimized protocol)
Sensitivity	Between 40 nM–25 µM
Robustness	High-throughput application (384-well plate)

Isocitrate detection using the Metabolite-Glo™ Detection System



HCT116 cells were collected, washed with cold PBS and diluted two-fold from 50,000 to 6,250 cells in 25 µl. Cells (25 µl) were transferred into a 96-well assay plate, then isocitrate was measured as described in Application Note GFN219. Wells with no cells (PBS only) were included for assay background control. The average luminescence for three replicates (gray dots) without background (no cells, PBS only) is shown in relative light units (RLU). Application Note GFN219.

Dehydrogenase-Glo™ Detection Assay

Cell-based

Applications

Monitoring dehydrogenase activity in cell lysates, tissue homogenates, and biochemical samples

Assay description

The **Dehydrogenase-Glo™ Detection System** provides a core bioluminescent NAD(P)H detection technology that can be rapidly implemented to measure endogenous dehydrogenase activity in cell lysates, tissue homogenates and other biological samples. The Dehydrogenase-Glo™ Detection System provides only the reagents required for bioluminescent NAD(P)H detection. To build a specific dehydrogenase assay, the user will need to identify and source the dehydrogenase substrate. The sensitivity and robustness of the Dehydrogenase-Glo™ Detection System make it well-suited for high-throughput applications, including inhibitor screening.

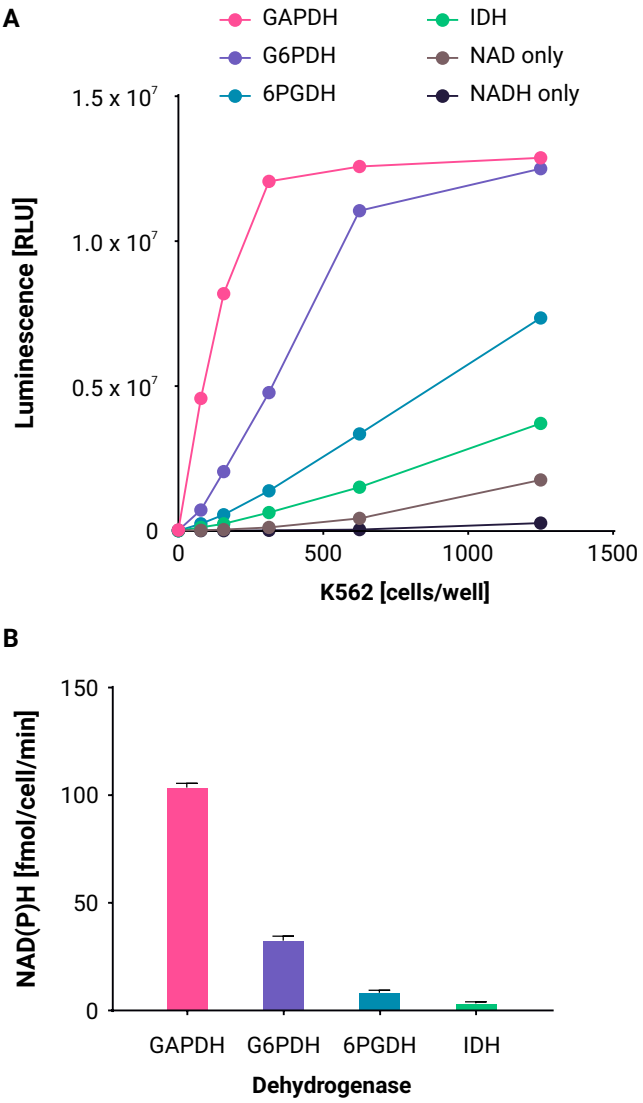
Assay principle

An endogenous dehydrogenase catalyzes the oxidation of a dehydrogenase-specific substrate with concomitant reduction of $NAD(P)^+$ to NAD(P)H. In the presence of NAD(P)H, Reductase enzymatically reduces a proluciferin Reductase Substrate to luciferin. Luciferin is detected using Ultra-Glo™ Recombinant Luciferase, and the amount of light produced is proportional to the NAD(P)H produced through specific dehydrogenase activity in the sample.

Assay features

Assay type	Luminescent (glow-type), one step assay
Markers	NAD(P)H detection
Applications	Monitoring dehydrogenase activity, functional readout of metabolic pathways
Sample	Cells, lysates, tissue, plasma and serum
Time required	30 – 90 min
Sensitivity	Detection from just 10s of cells
Robustness	High-throughput application (384-well plate)

Measuring dehydrogenase activity in K562 cells



K562 cells were diluted twofold starting at 2.5 × 10^4 cells/ml in PBS, and 50 µl was transferred into a 96-well assay plate. Dehydrogenase Detection Reagent was prepared for each dehydrogenase, and 50 µl was added to each well. No-substrate controls (NAD only and NADP only) were included to determine background from nonspecific enzyme activity in K562 cells. Panel A. Luminescence was measured for dehydrogenases and controls after 60 minutes at different cell densities. Panel B. Specific activity for each dehydrogenase was calculated. Data represents the average of three replicates with error bars indicating one standard deviation.

Bioluminescent Detection of ROS and GSH/GSSG

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 aging 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.

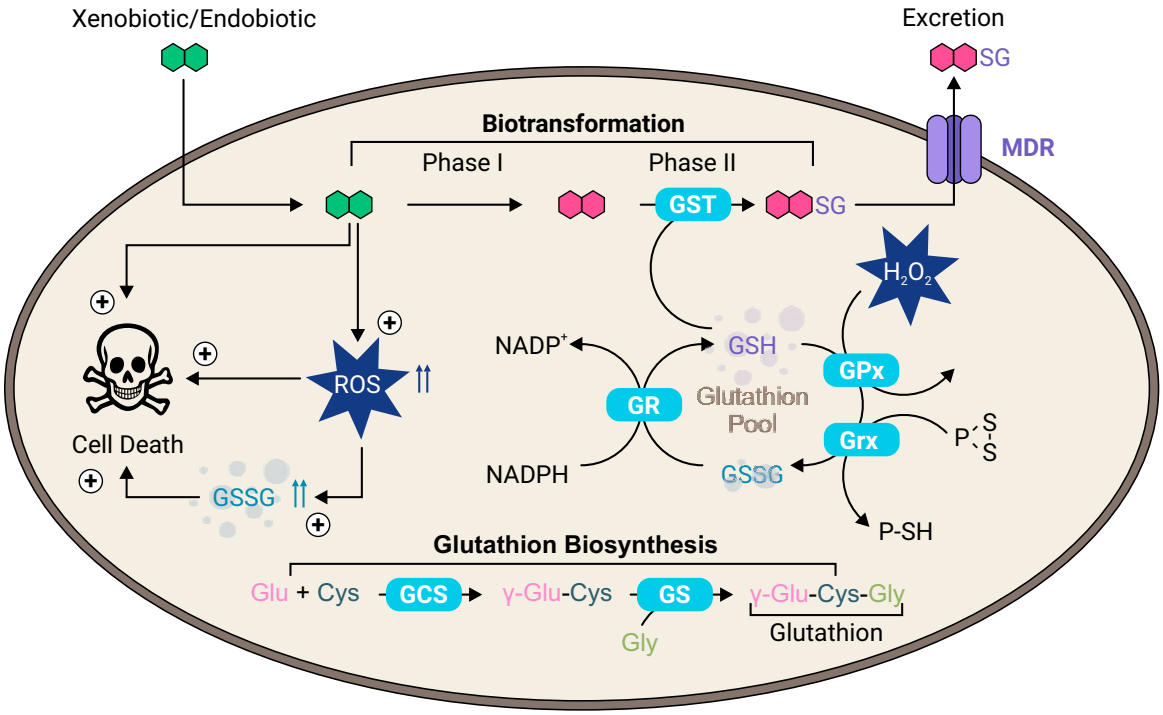
For protection from oxidative stress caused, e.g., by reactive oxygen species (ROS), glutathione is oxidized and switches from its reduced monomeric form to its oxidized dimeric form GSSG. Two molecules of GSH are regenerated from GSSG by glutathione reductase, with energy being consumed in the process.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

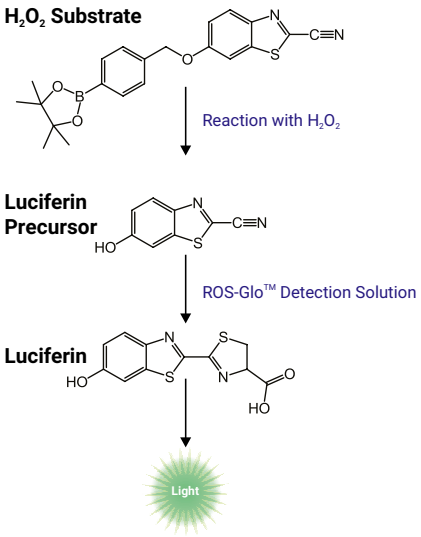
Assay principle

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.

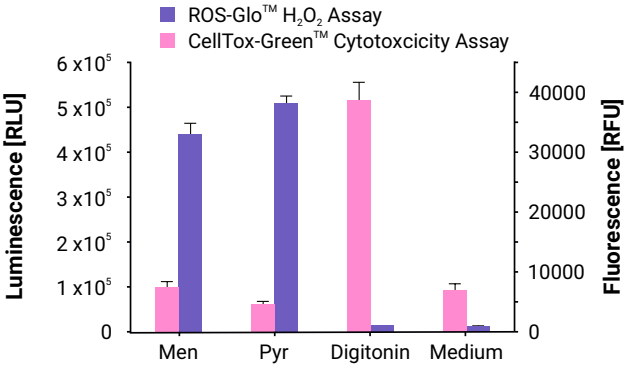
Assay features

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



Multiplex with real-time CellTox™ Cytotoxicity Assay



HepG2 cells were plated at 2,000 cells/well in a 384-well plate. The cells were 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 cul ture 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.

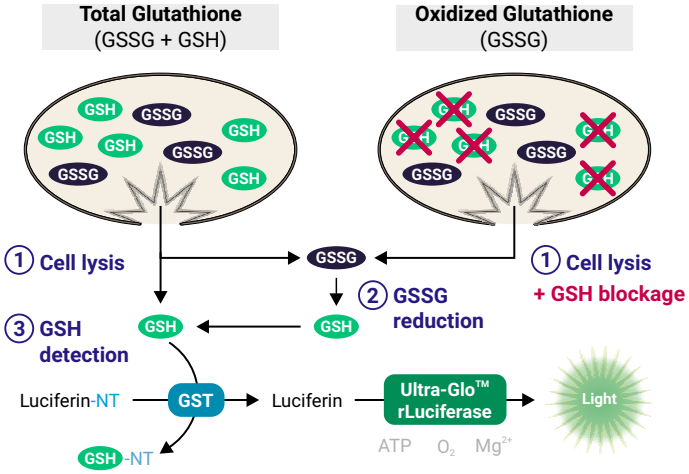
Total glutathione and GSSG are determined in two parallel reactions. The GSH level can be calculated by subtracting the GSSG level from the total glutathione level.

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.

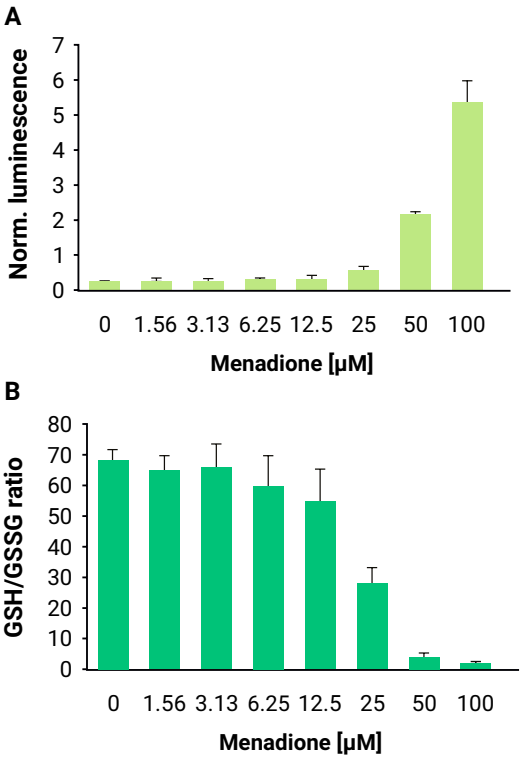
In both runs, the remaining GSH is coupled to the luciferase reaction. The glutathione-S-transferase (GST) in the reagent detaches a non-functional group from a luciferin derivative and couples this to the reduced glutathione. The free luciferin enters the luciferase reaction, the light signal being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

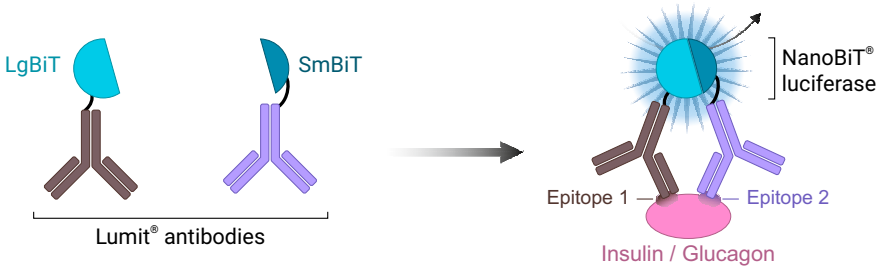


Panel A. 5,000 cells/well were treated with menadione dilutions (60 min, 37°C). GSSG data were normalized to CellTiter-Glo™ (CTG) viability to account for menadione toxicity and experimental variations, showing toxicity at the highest concentrations. Panel B. The GSH/GSSG ratio in A549 cells was measured via GSSG and total glutathione, revealing menadione's significant impact on redox status at higher concentrations.

Bioluminescent Detection of Glucagon and Insulin

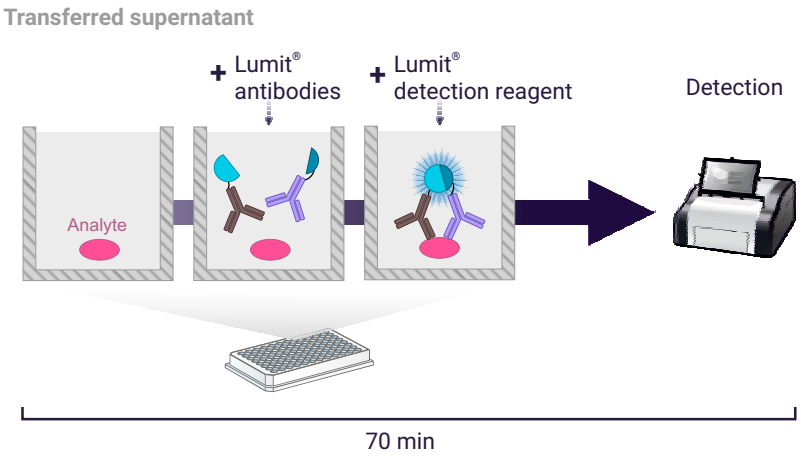
Insulin and glucagon are key target analytes measured in metabolism research. Secreted from β and α cells of pancreatic islets, respectively, the two hormones are vital for regulating glucose levels in the body. The Lumit® Immunoassays for metabolic regulators quantitatively measure insulin and glucagon in cell culture samples with a simple, time-saving assay protocol. These assays are ideally suited to process samples from glucose-stimulated insulin secretion and glucagon secretion experiments, including high-throughput perfusion assays.

Assay principles of Lumit™ Insulin and Glucagon Immunoassays



Specific primary antibodies are used to detect the analyte of interest. The antibodies are labeled with the small and large subunits of the NanoBiT® Luciferase, known as SmBiT and LgBiT, respectively. In the presence of the analyte, the subunits are brought together to reconstitute an active luciferase enzyme. Upon addition of the luciferase substrate, a bright luminescent signal is generated.

Simple "Add-mix-measure" protocol



Lumit® technology delivers high-quality data from cell-based secretion experiments in just 70 minutes with a simple, wash-free protocol. Scalable for any plate size, it enables high-throughput glucose-stimulated insulin secretion (GSIS) experiments in the morning and insulin/glucagon data analysis by afternoon, accelerating diabetes research.

Lumit® Immunoassays for Detection of Metabolic Regulators

Cell-based

Applications

Monitoring of insulin and glucagon in cell culture samples.

Assay description

Lumit® Insulin Immunoassay and **Lumit® Glucagon Immunoassay** are assays for the bioluminescent immunodetection of insulin or glucagon, respectively. The assays detect insulin or glucagon in supernatant samples. The protocol simply requires addition of labeled antibodies and detection reagent (which includes the luciferase substrate) and reading of the luminescent signal. The entire immunoassay is completed in less than 70 minutes.

Assay principle

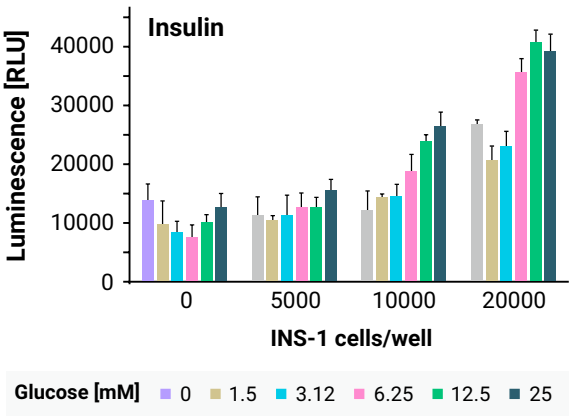
Lumit® Immunoassays are based on the NanoLuc® Binary Technology (NanoBiT®). Primary antibodies are labeled with the LgBiT and SmBiT subunits of the NanoBiT® luciferase. Upon binding of the antibodies to the analyte, the subunits are brought together to build an active luciferase and produce light proportional to the amount of analyte with the addition of the Lumit® Substrate. Both Lumit® assays and analyze insulin and glucagon side-by-side to gain more information on islet function.

Assay features

Assay type	Luminescent
Markers	Insulin or glucagon
Applications	Quantitative measurement of insulin or glucagon
Cell types	Transferred supernatant of monolayer cell cultures, 3D islet microtissues, isolated islets
Implementation	Homogeneous assay
Time required	70 minutes or less
Sensitivity	LOD of 58 pg/ml for insulin and 3.4 pg/ml for glucagon with a broad dynamic range of 58 pg/ml – 46.4 ng/ml or 3 pg/ml – 6.8 ng/ml, respectively
Robustness	Scalable from 96- to 384-well plates

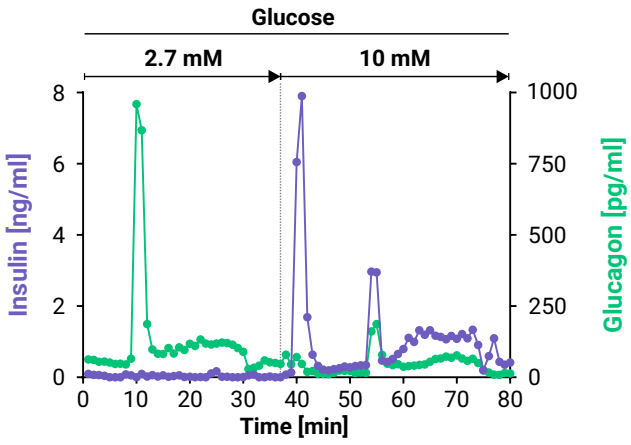
For more information about Lumit® Immunoassays, download brochure: www.promega.com/lumit-brochure

Measuring hormone secretion in perfusion experiments



INS-1 rat insulinoma cells plated at different cell numbers in 96-well plates were stimulated with glucose at different concentrations for 60 minutes. Insulin secretion was determined by removing 10 µl of supernatant and assayed with the Lumit® Insulin Immunoassay Kit in 384-well plates.

Monitoring insulin secretion in response to glucose



In a perfusion chamber, 80 mouse islets were treated with glucose in combination with an amino acid (AA) mixture. Low and high blood glucose levels were simulated with 2.7 mM glucose and 10 mM glucose respectively. Perfusate aliquots were collected every minute. Insulin and glucagon levels were detected in 10 µl perfusate in a 384-well plate.

Overview of Products

Glucose and Amino Acid Metabolism Assays

Product	Quantity	Catalog No.
Glucose-Glo™ Assay	5 ml	J6021
	50 ml	J6022
Lactate-Glo™ Assay	5 ml	J5021
	50 ml	J5022
Malate-Glo™ Assay	5 ml	JE9100
	50 ml	JE9200
Pyruvate-Glo™ Assay	5 ml	J4051
	50 ml	J4052
Glycogen-Glo™ Assay	5 ml	J5051
	50 ml	J5052
Glutamate-Glo™ Assay	5 ml	J7021
	50 ml	J7022
Glutamine/Glutamate-Glo™ Assay	5 ml	J8021
	50 ml	J8022
BCAA-Glo™ Assay	5 ml	JE9300
	50 ml	JE9400
Glucose Uptake-Glo™ Assay	5 ml	J1341
	10 ml	J1342
	50 ml	J1343

Lipid Metabolism Assays

Product	Quantity	Catalog No.
Triglyceride-Glo™ Assay	5 ml	J3160
	50 ml	J3161
Glycerol-Glo™ Assay	5 ml	J3150
	50 ml	J3151
Cholesterol/Cholesterol Ester-Glo™ Assay	5 ml	J3190
	5 ml	J3191
BHB-Glo™ Assay	5 ml	JE9500
	5 ml	JE9600

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

Build-Your-Own Metabolism Assay

Product	Quantity	Catalog No.
Metabolite-Glo™ Detection System	5 ml	J9030
	50 ml	J9040
Dehydrogenase-Glo™ Detection System	5 ml	J9010
	50 ml	J9020

Oxidative Stress

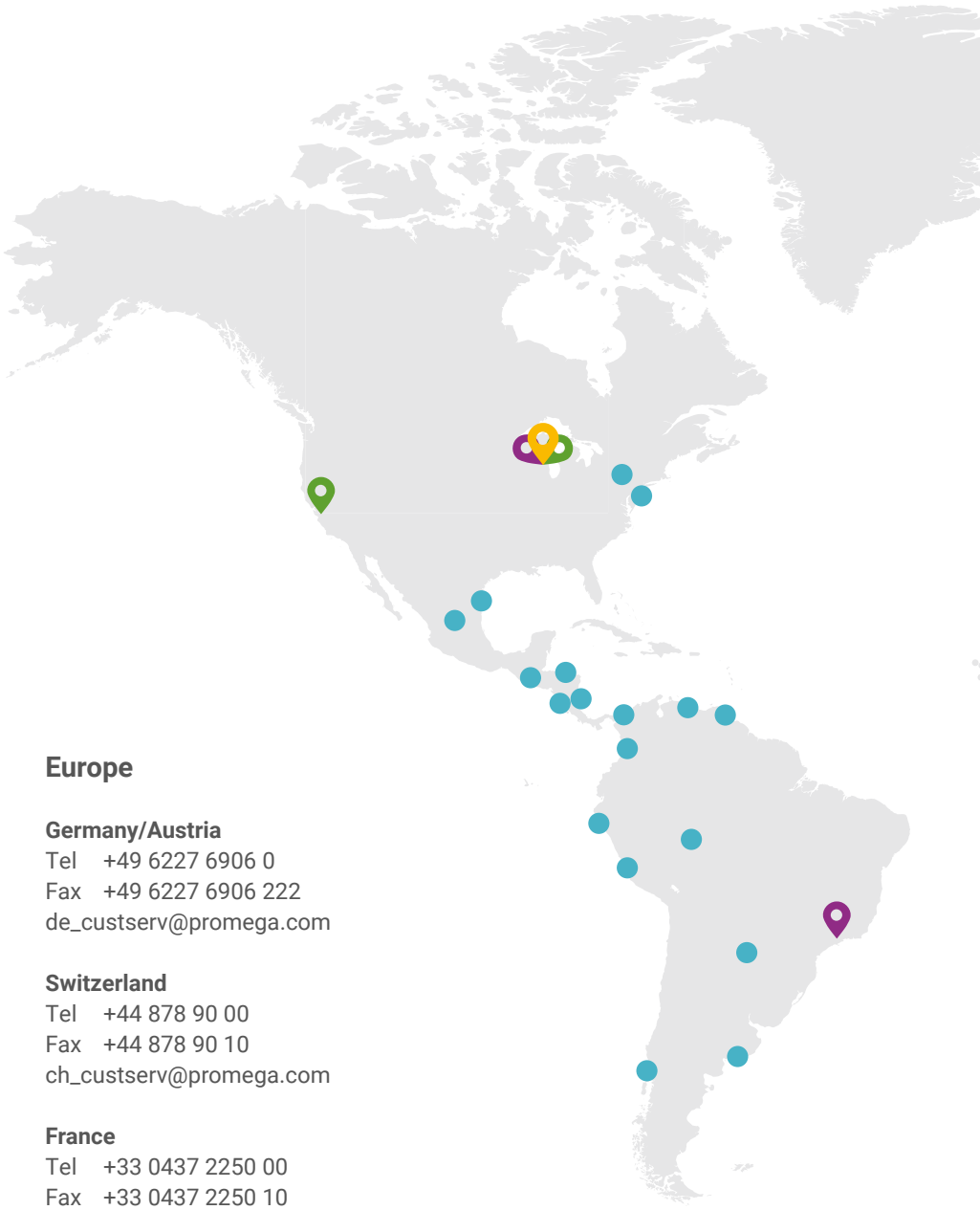
Product	Quantity	Catalog No.
ROS-Glo™ H_2O_2 Assay	10 ml	G8820
	50 ml	G8821
GSH/GSSG-Glo™ Assay	10 ml	V6611
	50 ml	V6612

Metabolite Regulators Detection Assays

Product	Quantity	Catalog No.
Lumit® Glucagon Immunoassay	100 assays	W8020
	500 assays	W8022
Lumit® Insulin Immunoassay	100 assays	CS3037A05
	500 assays	CS3037A07

Contact

- Headquarters
- R&D and Production
- Branches
- Distributors



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