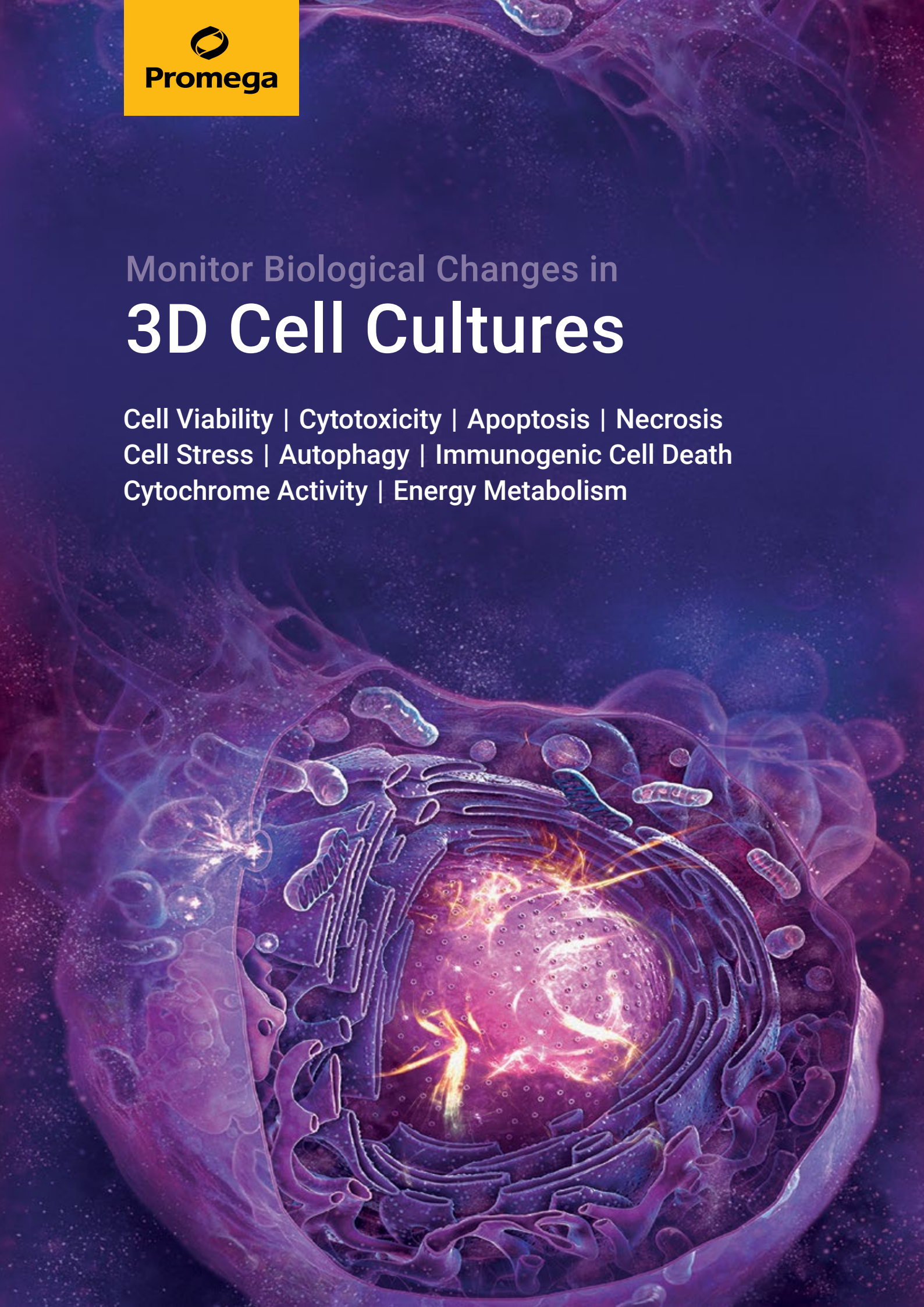
Promega

Monitor Biological Changes in

3D Cell Cultures

Cell Viability | Cytotoxicity | Apoptosis | Necrosis
Cell Stress | Autophagy | Immunogenic Cell Death
Cytochrome Activity | Energy Metabolism



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- Kinetic measurements
- Cellular metabolim assays
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- Immunoassays (ELISA, Lumit™)
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GloMax DISCOVER

A high-performance, easy-to-use multimode plate reader for luminescence, fluorescence, absorbance, BRET, and FRET applications

For more information, visit www.promega.com/GloMax

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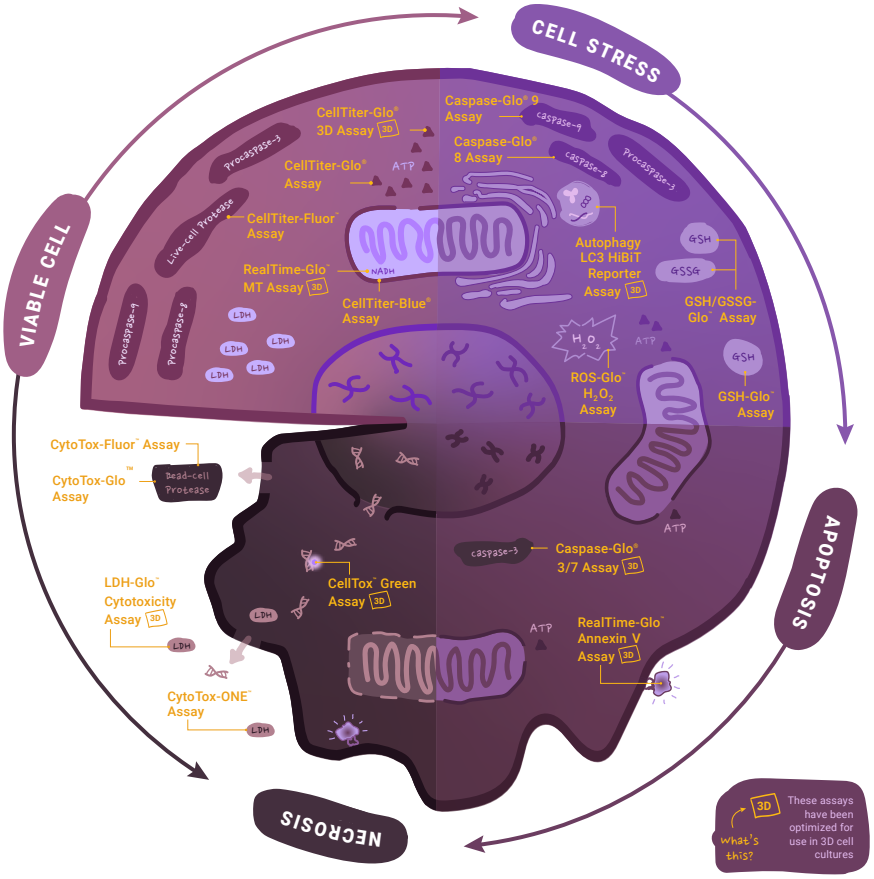
Cell Health Analysis in 3D Cultures

Cell Viability, Cytotoxicity, Apoptosis, Necrosis, Cell Stress, Autophagy, Immunogenic Cell Death, Cytochrome Activity

Three-dimensional (3D) cell cultures are widely employed from basic research to drug discovery/development because of their physiological relevance. The use of assays originally designed for cell monolayers (2D) or suspensions, however, may yield inaccurate or misleading results.

Many Promega assays have already been validated to show good performance and produce accurate results with different 3D cell culture models. Others were specifically optimized with adapted reagents (e.g., CellTiter-Glo® 3D Cell Viability Assay) or modified protocols (e.g., Caspase-Glo® 3/7 3D Assay) for higher capacity to penetrate 3D structures.

We help you identify the ideal assay to meet your needs!



3D-Validated Cell Health Assays – These Are Your Benefits:

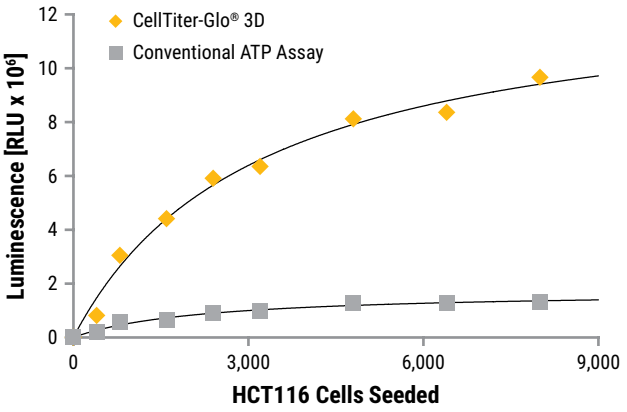
- Compatible with **various 3D culture systems** (with or without scaffold)
- **Add-mix-measure protocols** for easy implementation
- **Real-time live-cell assays** for repeated measurement of the same well
- **Multiplexing options** to determine several parameters from the same well
- **High-throughput-** and **automation-compatible**

Cell Viability

CellTiter-Glo® 3D Cell Viability Assay

Monitor cell viability through ATP measurement with an assay specifically designed for 3D cell cultures. The assay reagent is based on the same reliable chemistry as the classical **CellTiter-Glo® Assay** but features a significantly higher lytic capacity. This enables a more thorough cell lysis, resulting in complete ATP release and thus more accurate quantification. The assay is compatible with a variety of 3D culture models, including spheroid and Matrigel® 3D cultures.

- Lytic assay (endpoint measurement)
- Luminescent

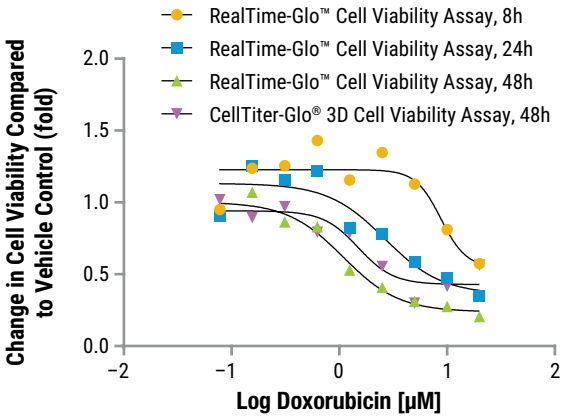


Comparison of CellTiter-Glo® 3D Cell Viability Assay vs. a conventional ATP assay. HCT116 colon cancer spheroids were grown for 4 days in a 96-well hanging drop plate. After addition of reagents, shaking für 5 minutes, and 30 minutes incubation, the luminescence signal was recorded. The CellTiter-Glo® 3D Assay shows improved lytic capacity and ATP detection compared to competitor ATP assay.

RealTime-Glo™ MT Cell Viability Assay

Monitor cell viability in real-time to obtain time and dose-dependent information about your treatment. The non-lytic chemistry allows the continuous analysis of the same sample well for up to 72 hours. The **RealTime-Glo™ MT Cell Viability Assay** is ATP-independent and determines the number of viable cells in culture by measuring their reducing potential. The sensitive assay is compatible with 3D cell cultures, and the results correspond well with the CellTiter-Glo® 3D Cell Viability Assay.

- Non-lytic assay (real-time measurement)
- Luminescent



Analysis of doxorubicin-treated HCT116 spheroids with RealTime-Glo™ MT Cell Viability and CellTiter-Glo® 3D Cell Viability Assay. HCT116 colon cancer spheroids were grown for 4 days in a 96-well hanging drop plate. The RealTime-Glo™ Reagent was added to a subset of wells, and cell viability was monitored at various time points for up to 48 hours. At 48 hours, the CellTiter-Glo® 3D Reagent was added to a parallel set of samples. The graph shows the fold change in cell viability of doxorubicin-treated cells compared to the vehicle control at each concentration.

Download Application Note

Assaying Cell Health in 3D Cell Culture with the GloMax® Discover System

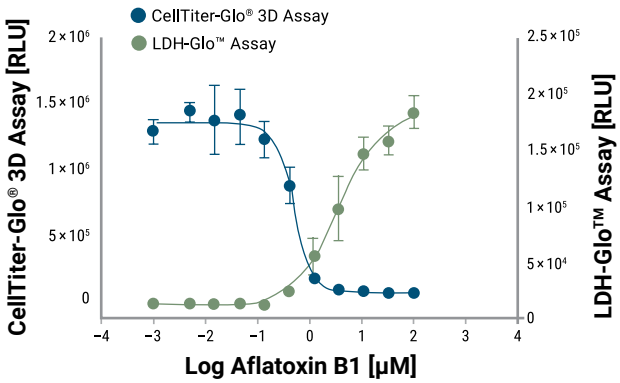
www.promega.de/GloMax-3D-AppNote

Cytotoxicity

LDH-Glo™ Cytotoxicity Assay

Measure cytotoxicity by quantifying lactate dehydrogenase release from 3D cell culture systems. The high sensitivity of this assay enables analysis of small media aliquots (2–5 µl). Thus, cytotoxicity can be kinetically assessed from the same well by sampling cell culture supernatant at different time points. Remaining media or cells are available for downstream analysis with other assays, e.g., for cell viability or metabolism.

- Non-lytic assay (sampling of culture medium)
- Luminescent



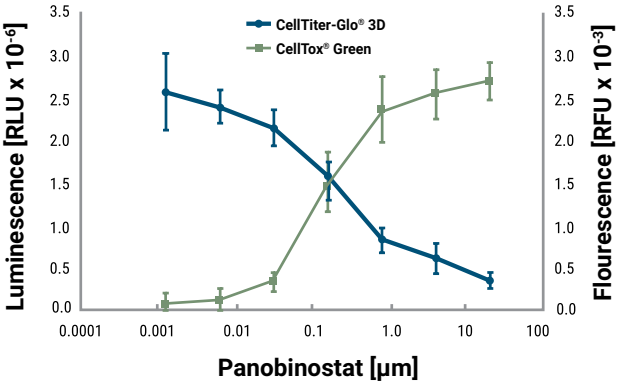
	LDH Assay	CTG 3D Assay
EC50	3.52	0.4999

Multiplexing LDH-Glo™ Assay with CellTiter-Glo® 3D Cell Viability Assay. Human liver microtissue spheroids were treated with aflatoxin B1 for 48 hours. Media samples were collected and assayed with the LDH-Glo™ Assay. The remaining cells were assayed for viability with the CellTiter-Glo® 3D Assay.

CellTox™ Green Cytotoxicity Assay

The **CellTox™ Green Cytotoxicity Assay** measures changes in membrane integrity as a result of cell death by staining the DNA of dead cells. The non-toxic assay is tolerated by a wide range of cells, including 3D cell cultures, and allows continuous measurement of the same sample well for up to 72 hours. The fluorescent read-out enables multiplexing with bioluminescent cell health assays, e.g., RealTime-Glo™ MT Cell Viability Assay for live/dead cell monitoring or can be used upstream of the CellTiter-Glo® 3D Cell Viability Assay.

- Non-lytic assay (real-time measurement)
- Fluorescent



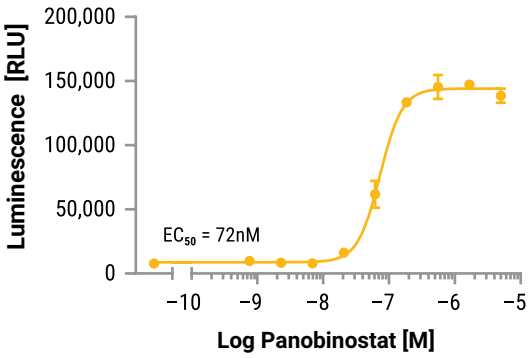
Multiplexing CellTox™ Green Cytotoxicity Assay with CellTiter-Glo® 3D Cell Viability Assay. HCT116 colon cancer spheroids were grown for 4 days in a 96-well hanging drop plate. Panabinostat was added along with CellTox Green and samples were incubated for 48 hours. After recording fluorescence, an equal volume of CellTiter-Glo® 3D was added, the plate was shaken for 5 minutes, and the luminescence recorded after 30 minutes incubation.

Apoptosis/Necrosis

Caspase-Glo® 3/7 3D Assay

Measure caspase-3 and -7 activities in apoptotic 3D cultures. This lytic endpoint assay is based on a luminogenic caspase-3/7 substrate. A one-step addition of the reagent results in cell lysis followed by caspase-mediated cleavage of the substrate and generation of a luminescent signal, which is proportional to the amount of caspase 3/7 activity. The 3D-adapted assay was validated with different 3D cell culture models, e.g., spheroids or cells embedded in Matrigel®.

- Lytic assay (endpoint measurement)
- Luminescent

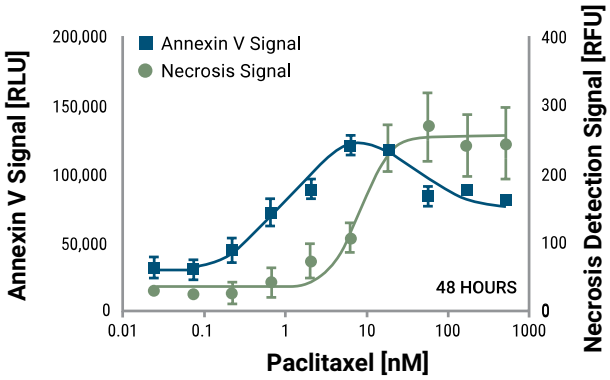


Caspase-Glo® 3/7 3D Assay verified with Matrigel® embedded cultures. HCT116 cells were embedded in a 4.5 mg/ml Matrigel® solution, allowed to grow for 4 days and then treated with a serial dilution of panobinostat. Caspase-Glo® 3/7 3D Assay Reagent was added to determine caspase 3/7 activity

RealTime-Glo™ Annexin V Apoptosis and Necrosis Assay

The **RealTime-Glo™ Annexin V Apoptosis and Necrosis Assay** measures the translocation of phosphatidylserine (PS) to the outer leaflet of the cellular membrane, a late event during apoptosis. The assay also includes a cell-impermeant, pro-fluorescent DNA binding dye to detect necrosis. The assay enables time course analysis of 3D cell cultures of up to 48 hours.

- Non-lytic assay (real-time measurement)
- Fluorescent/Luminescent



Measurement of apoptosis and secondary necrosis. HepG2 spheroids were treated for 48 hours with different concentrations of paclitaxel. The RealTime-Glo™ Annexin V Apoptosis and Necrosis Assay was used to monitor real-time apoptosis progression. Luminescence and fluorescence were measured on a GloMax® Discover Instrument.

Download Scientific Poster

Real-Time Apoptosis and Necrosis Detection in 3D Spheroid Cell Models

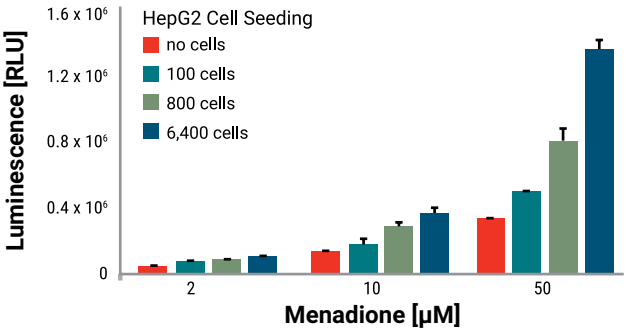
www.promega.de/3Dposter

Cell Stress

ROS-Glo™ H_2O_2 Assay

The **ROS-Glo™ H_2O_2 Assay** is a homogeneous and bioluminescent assay that determines oxidative stress by measuring the level of hydrogen peroxide (H_2O_2), a reactive oxygen species (ROS), directly in cell culture. The assay is based on a proluciferin substrate that directly reacts with H_2O_2. It thereby obviates the need for horseradish peroxidase (HRP) eliminating false hits associated with HRP inhibition.

- Lytic (endpoint measurement) or non-lytic assay options (sampling of culture medium)
- Luminescent

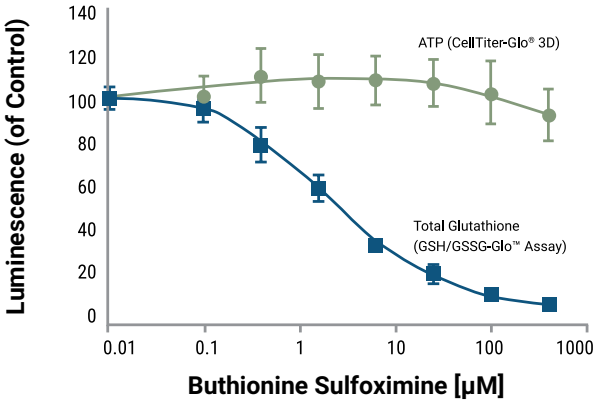


Response of HepG2 spheroids to ROS-inducing menadione. Response of HepG2 spheroids of differing diameters to different levels of menadione. Cells were incubated for 4 days in ultra-low attachment plates. H_2O_2 levels were measured with the ROS-Glo™ H_2O_2 Assay and detected on a GloMax® Discover Instrument.

GSH/GSSG-Glo™ Assay

The **GSH/GSSG-Glo™ Assay** is a luminescence-based system to detect and quantify total glutathione (GSH +GSSG), GSSG and GSH/GSSG ratios in cultured cells. The ratio is calculated from two parallel measurements to determine total (GSH+ GSSG) and oxidized (GSSG) glutathione. Minor changes of the protocol, i.e., increasing the incubation time from 5 to 30 minutes allow easy transfer from 2D to 3D culture models.

- Lytic assay (endpoint measurement)
- Luminescent



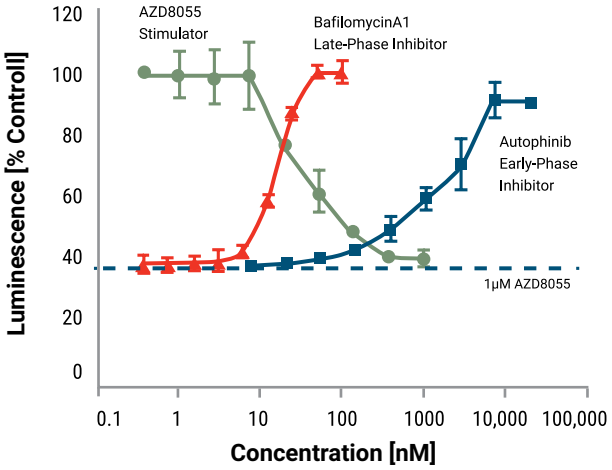
Monitoring total glutathione and cell viability in parallel. HCT116 spheroids formed over 4 days in a hanging drop platform were treated for 48 hours with buthionine sulfoximine. Total glutathione was measured with the GSH/GSSG-Glo™ Assay after addition of the lysis reagent and shaking for 30 minutes. In parallel cell viability was determined by ATP measurement with the CellTiter-Glo® 3D Assay.

Autophagy/Immunogenic Cell Death

Autophagy LC3 HiBiT Reporter Assay System

Monitor autophagic flux with a bioluminescent plate-based assay. Stably transfect cells with the LC3 HiBiT Reporter, grow in 3D culture, and determine changes in LC3 level using the Nano-Glo® HiBiT Lytic Detection System. Additionally, the reporter localization to autophagosomes can be monitored through fluorescent microscopy and LC3-I to LC3-II conversion can be assessed by HiBiT blotting.

- Lytic assay (endpoint measurement)
- Luminescent

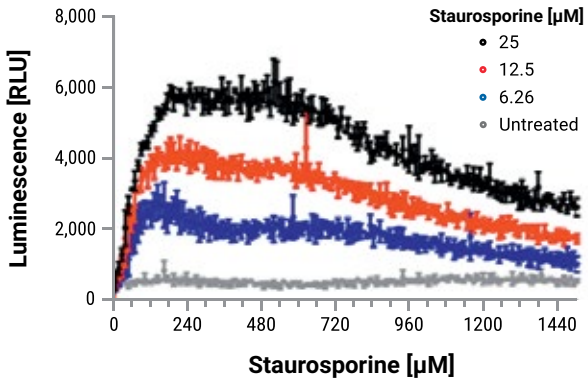


Response of HEK293 spheroids expressing the LC3 HiBiT reporter to autophagy stimulators and inhibitors. Cells were plated into 96-well ultra-low attachment round bottom, black walled plates and cultured for 4 days prior to the assay. Cells were treated with an autophagic stimulator (AZD8055) or two different autophagic inhibitors (Bafilomycin A1 and Autophinib). For inhibitor studies, cells were dosed with 1µM AZD8055 first to lower the basal autophagy signal and then treated with the autophagic inhibitors for 6 hours. For detection, the Nano-Glo® HiBiT Lytic Reagent was added.

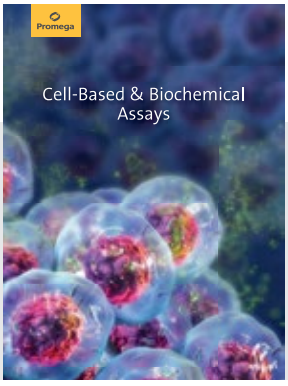
RealTime-Glo™ Extracellular ATP Assay

Quantify the release of extracellular ATP as biomarker for immunogenic cell death in real time for up to 24 hours. The assay has been validated with different 3D cell culture models (e.g., microtissues or Matrigel® embedded cells).

- Non-lytic assay (real-time measurement)
- Luminescent



Measurement of immunogenic cell death in 3D cell culture models. HCT-116 cells were grown in ultra-low attachment plates to form spheroids. Cells were treated with dilutions of staurosporine, the RealTime-Glo™ Extracellular ATP Assay Reagent was added, and the signal was monitored.



Discover the Comprehensive Portfolio of Cell-Based and Biochemical Assays.

www.promega.com/CellbasedAssays

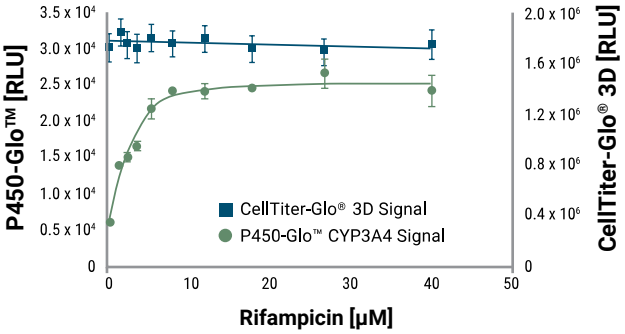
Cytochrome Activity

P450-Glo™ CYP450 Assays

Quantify cytochrome P450 (CYP) enzyme activity and identify CYP-inducing or –inhibiting drugs in cell culture. The assays use a pro-substrate which is converted to luciferin by CYP enzymes. The cell-permeable luciferin diffuses out of the cell and can be detected in the culture media. The remaining cells can be used for other cell-based assays, e.g., to assess cell viability.

Kits are available for the following CYP enzymes: CYP3A4, CYP2B6, CYP1A2, CYP1A1, CYP1B1, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP26A1, and CYP26B1.

- Non-lytic assay (sampling of culture medium)
- Luminescent



Measurement of CYP3A4 activity and viability in human liver microtissue in response to rifampicin. CYP3A4 activity was measured using the non-lytic P450-Glo™ 3A4 Assay followed by determination of viability from the same well using CellTiter- Glo® 3D Assay.

Download Application Note
P450 Glo™ CYP3A4 Assay on 3D Microtissues

www.promega.de/P450-Glo-3D-AppNote

Featured Application

Drug screening using single organoids

Read in this article how Cell Microsystems' CellRaft® Technology and Promega Cell Health Assays address two critical bottlenecks preventing the acceleration of organoid models into high-throughput screening applications by providing:

- an automated solution for generating customized, scalable and reproducible organoids that are ready to use in plate-based assays and
- assays that have been optimized and validated for 3D cell culture models.

Read article
www.promega.de/Drug-Screening-Organoids-Article

Multiplexing

Get More Data From a Single Well

Combine 3D-validated assays to maximize data output from a single well. Obtain reliable results and draw valid conclusions by easy data confirmation and interpretation. Improve data quality by data normalization.

Examples of 3D-validated assays that can be combined:

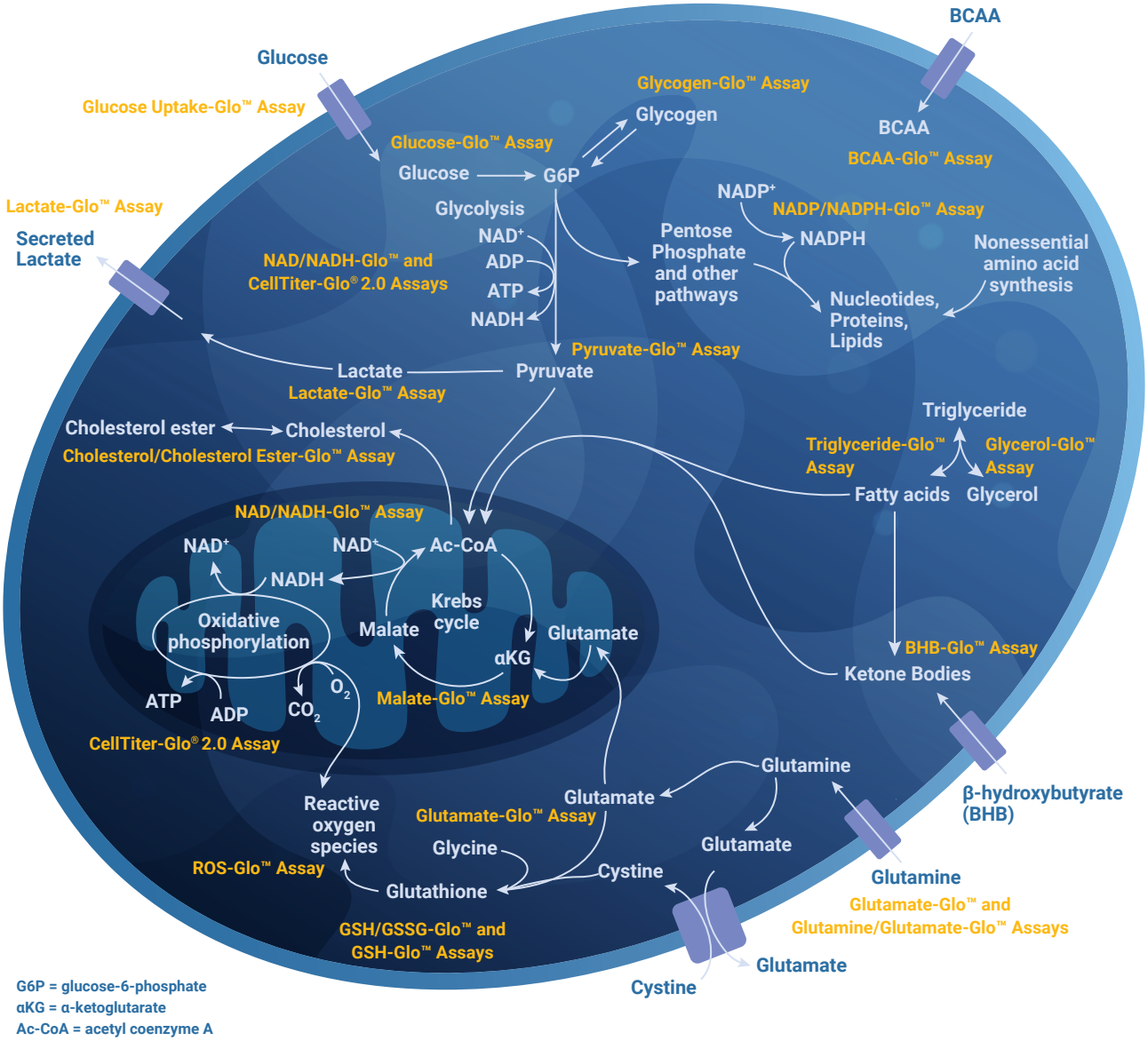
1st Assay:	Combine with 2nd Assay:
RealTime-Glo™ MT Cell Viability Assay (non-lytic, real-time, luminescent)	**CellTox™ Green Cytotoxicity Assay** (non-lytic, real-time, fluorescent)
RealTime-Glo™ Annexin V Apoptosis and Necrosis Assay (non-lytic, real-time, luminescent/fluorescent)	**CellTiter-Glo® 3D Cell Viability Assay** (lytic, endpoint, luminescent) or **Caspase-Glo® 3/7 3D Assay** (lytic, endpoint, luminescent)
ROS-Glo™H_2O_2 Assay (non-lytic option, luminescent)	**CellTiter-Glo® 3D Cell Viability Assay** (non-lytic option, luminescent) or **RealTime-Glo™ MT Cell Viability Assay** (non-lytic, real-time, luminescent)
CellTox™ Green Cytotoxicity Assay (non-lytic, real-time, fluorescent)	Can be combined with all **Glo™ Assays and Autophagy LC3 HiBiT Reporter Assay System**
LDH-Glo™Cytotoxicity Assay (non-lytic, luminescent)	LDH-Glo™ Assay uses only 2–5 µl supernatant of cell culture medium. This allows multiple sampling from one well (e.g. for kinetic analysis) and multiplexing with other assays or downstream applications, e.g., nucleic acid extraction.
Glucose-Glo™, Lactate-Glo™, Glutamate-Glo™ and Glutamine/ Glutamate-Glo™ Assays (non-lytic option, luminescent)	Metabolite assays can be used with 2–5 µl supernatant of cell culture medium. This allows multiple sampling from one well (e.g. for kinetic analysis) and multiplexing with other assays or downstream applications, e.g., nucleic acid extraction.

For more information, please contact our technical service de_techserv@promega.com

Metabolism Analysis in 3D Cultures

Metabolite and Dinucleotide Detection

Every cell has a unique metabolic profile. Understanding changes in cellular metabolism can provide novel starting points to develop treatments for diseases, e.g., diabetes, cancer, and liver disease. We offer several assays to measure metabolic activity with optimized protocols for 3D cell culture applications.



3D-Validated Metabolism Assays – These Are Your Benefits:

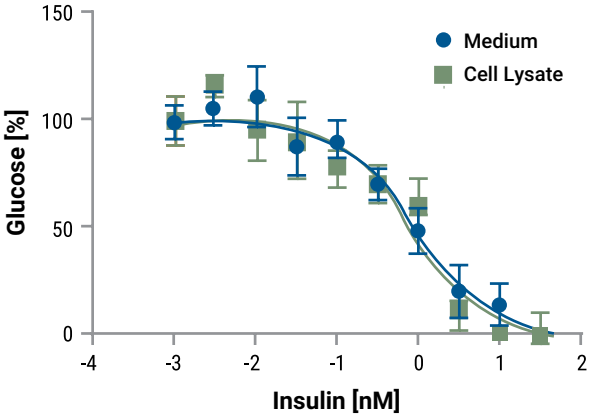
- Compatible with **different sample types** different sample types, e.g., organoids, tissue, blood, plasma
- **No radioactive** detection or **organic extraction** needed
- Simple protocols in **add-mix-measure format**
- Measure **several metabolites** from one sample
- **High-throughput screening-** and **automation-compatible**

Metabolite Detection

Glucose-Glo™ Assay
Lactate-Glo™ Assay
Glutamate-Glo™ Assay
Glutamine/Glutamate-Glo™ Assay

Monitor glucose, lactate, glutamate, or glutamine levels in 3D cell cultures. The assays couple metabolite oxidation and NADH to a sensitive bioluminescent readout. The assays require only 2–5 µl of culture medium per time point, allowing multiple aliquots to be taken from the same well, e.g., for kinetic analysis or to measure multiple metabolites in parallel.

- Lytic (endpoint measurement) and non-lytic assay options (sampling of culture medium, cell lysates, serum, plasma, tissue lysates)
- Luminescent

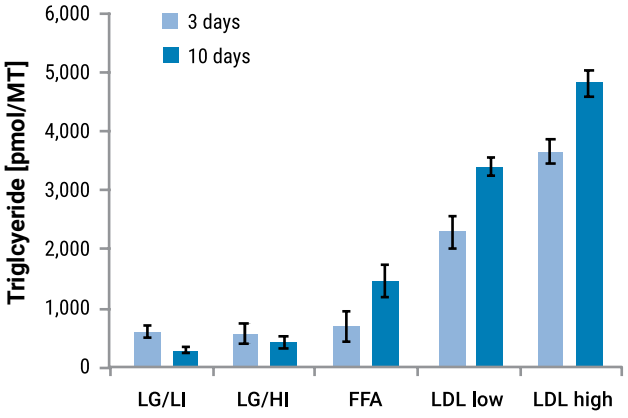


Insulin-mediated inhibition of gluconeogenesis in iPSC-derived human liver spheroids. iCell® Hepatocytes 2.0 (Cellular Dynamics, Inc.) were grown as spheroids on ultra-low attachment 96-well spheroid plates. The cells were cultured for 1.5 hours in a glucose-free gluconeogenesis medium to promote hepatic glucose production. Next, the cells were treated for 6 hours with gluconeogenesis medium with increasing insulin concentrations to inhibit glucose production. After 6 hours, 25 µl medium (blue) was removed from the wells and Glucose Detection Reagent was added. Similar results were obtained when glucose detection was performed with cell lysates (green).

Triglyceride-Glo™ Assay
Glycerol-Glo™ Assay

The **Triglyceride-Glo™ Assay** detects triglyceride levels by measuring glycerol that is released from an enzymatic reaction with a lipase. 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. The **Glycerol-Glo™ Assay** uses the same chemistry to measure free glycerol but does not include a lipase. Both assays work without organic extraction and can be used in a variety of biological samples, including cells cultured in 3D.

- Lytic (endpoint measurement) and non-lytic assay options (sampling of culture medium, serum)
- Luminescent



Triglyceride levels in human liver microtissues. 3D InSight™ Human Liver Microtissues (InSphero) were incubated for 3 and 10 days in serum-free medium containing either physiological (LG/LI) or supraphysiological (LG/HI) levels of glucose and insulin and supplementation with either free fatty acids bound to BSA (FFA) or low-density lipoprotein plasma fraction (LDL). The microtissues were washed twice in PBS and assayed for total glycerol content according to the Triglyceride-Glo™ protocol. Values were plotted as concentration of triglyceride per microtissue (MT). The data were generously provided by InSphero.

Download Technical Article
Homogenous Assays for Triglyceride Metabolism Research

www.promega.de/Triglyceride-Glo-3D-Article

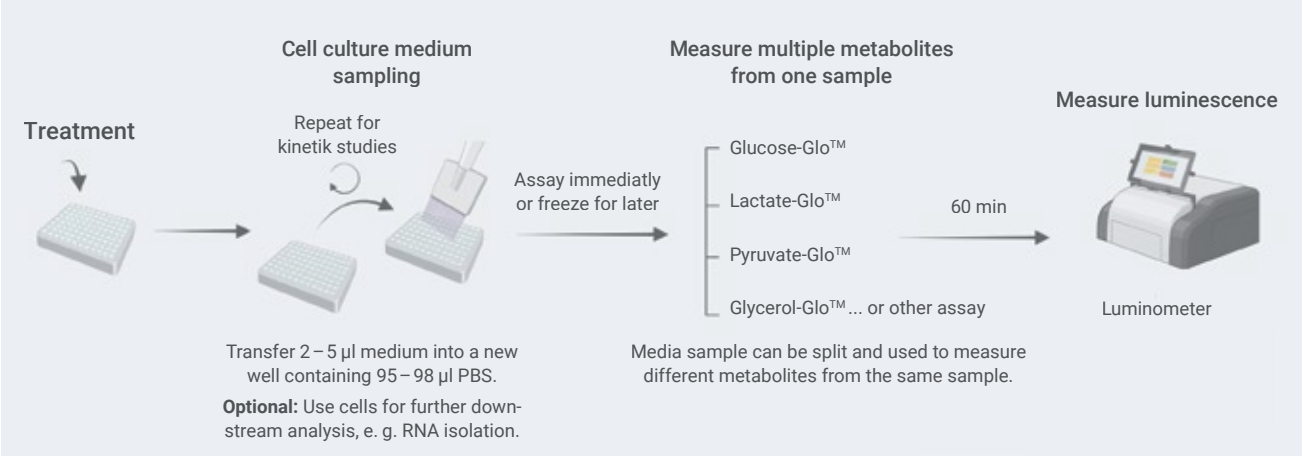
Metabolite Detection in Cell Supernatant

Monitor Multiple Metabolites from a Single Sample

Easily detect metabolites secreted in the cell supernatant with medium-compatible assays like, e.g., Glucose-Glo™, Lactate-Glo™, Glutamate-Glo™, or Glycerol-Glo™. The highly sensitive assays require only 2–5 µl sample volume allowing multiple sampling from the same well for kinetic studies. The assays also use common sample preparation protocols, so that one sample can be used to monitor several metabolites in parallel. This gives you a comprehensive understanding of which metabolic pathways are most active in your model system.

How you benefit from supernatant measurement?

- Non-lytic approach, ideal for 3D cultures, especially organoids
- Analyze multiple metabolites from a single sample
- Keep cells for downstream analysis (e.g., nucleic acid isolation, cell health analysis)



Assays that enable metabolite detection in supernatant:

Glucose Metabolism	Amino Acid Metabolism	Lipid Metabolism
Glucose-Glo™	Glutamate-Glo™	Glycerol-Glo™
Lactate-Glo™	Glutamine/Glutamate-Glo™	BHB-Glo™
Pyruvate-Glo™	BCAA-Glo™	Triglyceride-Glo™
		Cholesterol/Cholesterol Ester-Glo™

Looking for a specific metabolite?

With the **Metabolite-Glo™ Detection System** you can build your own bioluminescent metabolism assay tailored to your target.

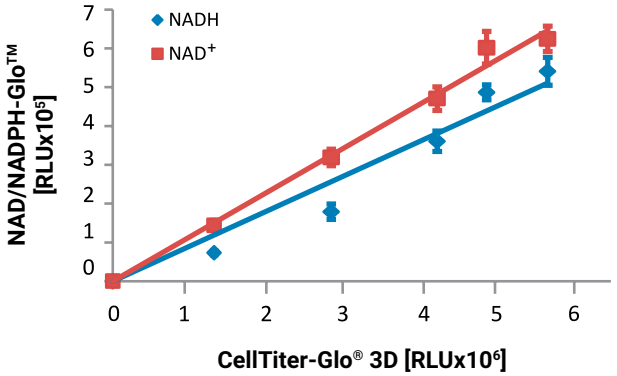
For more information about the products, please visit: www.promega.com/MetabolismAssays

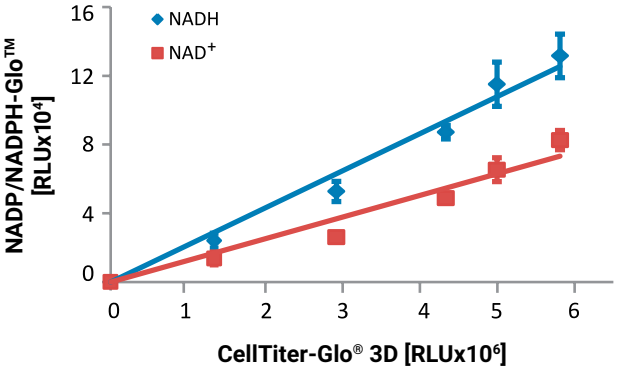
Dinucleotide Detection

NAD/NADH-Glo™
NADP/NADPH-Glo™ Assays

Monitor NAD⁺/NADH or NADP/NADPH levels in cells or enzymatic reactions. The assays are easily adapted for inhibitor screening in high-throughput formats. The sensitivity and robustness of the assay chemistry allow fewer cells to be used to detect individual dinucleotides directly in multiwell plates.

- Lytic assay (endpoint measurement)
- Luminescent





NAD/NADH and NADP/NADPH signals correlate with increasing 3D microtissue diameter. Varying numbers of HCT116 cells were seeded into GravityPLUS™ 96-well plates (InSphero) and grown for 4 days. Media was removed and replaced with PBS. Microtissues were lysed by the addition of bicarbonate buffer plus 2 % DTAB and shaking for 30 minutes. After lysis, half of the volume was transferred to a white luminescent plate for acid treatment to measure either NAD⁺ or NADP⁺. The other half went through base treatment to determine NADH or NADPH. After neutralization, the samples were divided and assayed with either the NAD/NADH-Glo™ Assay or the NADP/NADPH-Glo™ Assay. Parallel wells were assayed with the CellTiter-Glo® 3D Assay as the signal is proportional to microtissue diameter.

Want to do functional analysis of metabolic pathways?

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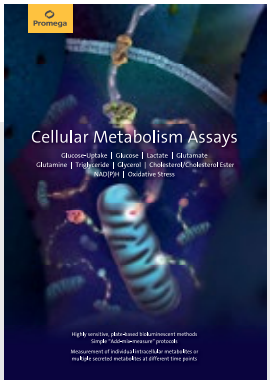

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Expression Analysis in 3D Cultures

RNA Purification, Quantitative Real-Time PCR

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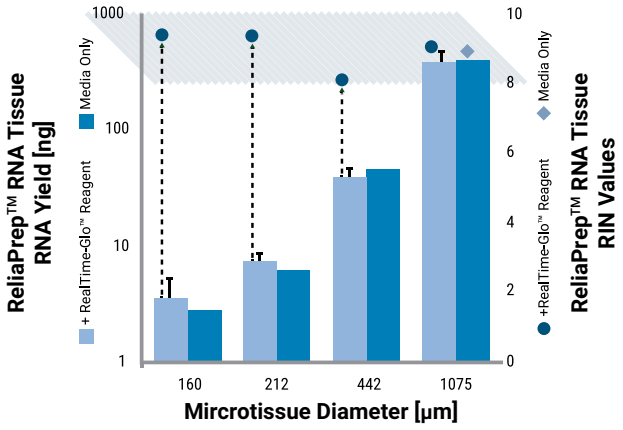

RNA extraction following the RealTime-Glo™ MT Cell Viability Assay. The Real-Time-Glo™ MT Cell Viability Assay was used to measure viability of differently sized HEK293 cell spheroids (not shown) followed by RNA extraction of the same samples using ReliaPrep™ RNA Tissue Miniprep System. The presence of the RealTime-Glo™ Reagent had no effect on the yield or quality of RNA (RIN value).

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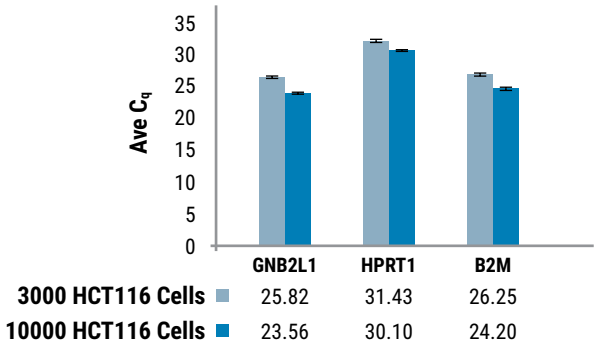
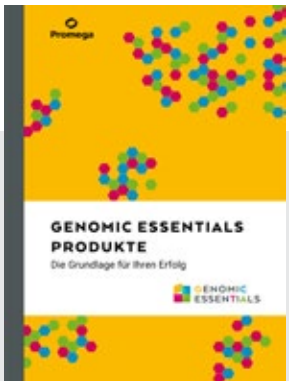


GoTaq® 1-Step RT-qPCR from 3D microtissues. HCT116 cells were seeded at two different densities on GravityPLUS™ hanging drop 96-well plates to form spheroids. Purified RNA was evaluated by looking at the gene expression levels of three genes (GNB2L1, HPRT1, and B2M) using the GoTaq® 1-Step RT-qPCR System. The GoTaq® System was able to detect all three genes from RNA purified from two different microtissue densities using the ReliaPrep™ RNA Cell Miniprep System.



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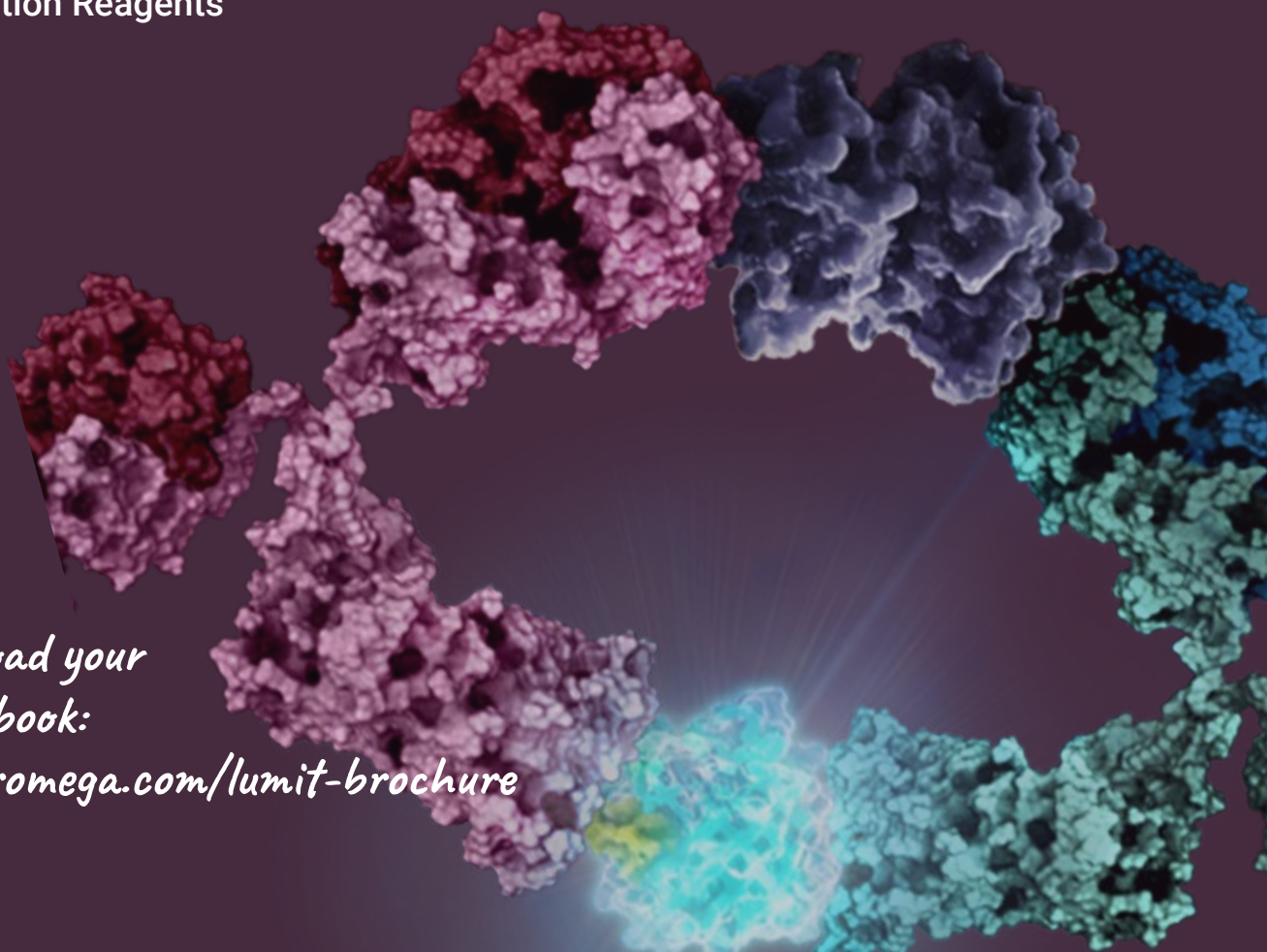

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Selected Citations

Organoids

Daoutsali, E., *et al.* (2023) Amyloid beta accumulations and enhanced neuronal differentiation in cerebral organoids of Dutch-type cerebral amyloid angiopathy patients Front. *Aging Neurosci.* 14, doi.org/10.3389/fnagi.2022.1048584. **(ReliaPrep™ RNA Cell Miniprep System)**

Bauersachs, H.G., *et al.* (2022) N-methyl-d-aspartate receptor-mediated preconditioning mitigates excitotoxicity in human induced pluripotent stem cell-derived brain organoids. *Neurosience* 484, 83–97. **(Glutamate-Glo™ Assay, LDH-Glo™ Cytotoxicity Assay)**

Cromwell. E.F., *et al.* (2022) Multifunctional profiling of triple-negative breast cancer patient-derived tumoroids for disease modeling. *SLAS Discovery* 27, 191–200 **(CellTiter-Glo® 3D Cell Viability Assay, RealTime-Glo™ Cell Viability Assay)**

Rodrigues, D., *et al.* (2022) Unravelling mechanisms of doxorubicin-induced toxicity in 3D human intestinal organoids. *Int. J. Mol. Sci.* 23, 1286 **(CellTiter-Glo® 3D Cell Viability Assay, Caspase-Glo™ 3/7 3D Assay)**

Spheroids

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Hedemann, N., *et al.* (2021) ADAM17 inhibition increases the impact of cisplatin treatment in ovarian cancer spheroids. *Cancers* 13, 2039. **(CellTox™ Green Cytotoxicity Assay, RealTime-Glo™ MT Cell Viability Assay, Caspase-Glo® 3/7 Assay)**

Peirsman, A., *et al.* (2021) MISpheroID: a knowledgebase and transparency tool for minimum information in spheroid identity. *Nat. Methods* 18, 1294–1303. **(Glucose-Glo™ Assay, Lactate-Glo™ Assay, CellTiter-Glo® 3D Cell Viability Assay)**

Ströbel, S., *et al.* (2021) A 3D primary human cell-based in vitro model of non-alcoholic steatohepatitis for efficacy testing of clinical drug candidates. *Sci Rep* 11, 22765. **(Triglyceride-Glo™ Assay, LDH-Glo™ Cytotoxicity Assay)**

Eilenberger, C., *et al.* (2019) Effect of spheroidal age on sorafenib diffusivity and toxicity in a 3D HepG2 spheroid model. *Sci. Rep.* 19; 9(1):4863. **(CellTiter-Glo® 3D Cell Viability Assay)**

Abu-Bonsrah, K.D., *et al.* (2018) Generation of adrenal chromaffin-like cells from human pluripotent stem cells. *Stem Cell Rep.* 10, 134-50. **(GoTaq® Probe qPCR Master Mix (2-step RT-qPCR))**

Kota, S., *et al.* (2018) A novel three-dimensional high-throughput screening approach identifies inducers of a mutant KRAS selective lethal phenotype. *Oncogene* 37, 4372–84 **(RealTime-Glo™ Annexin V Apoptosis and Necrosis Assay)**

Stadler, M., *et al.* (2018) Exclusion from spheroid formation identifies loss of essential cell-cell adhesion molecules in colon cancer cells. *Sci. Reports* 8, 1151. **(ReliaPrep™ RNA Cell Miniprep System)**

Wang, Y., *et al.* (2018) CPT1A-mediated fatty acid oxidation promotes colorectal cancer cell metastasis by inhibiting anoikis. *Oncogene* 37, 6025-40. **(GSH/GSSG-Glo™ Assay, NADP/NADPH-Glo™ Assay)**

Microfluidic Organ-on-a-Chip

Busek, M., *et al.* (2023) Pump-less, recirculating organ-on-a-chip (rOoC) platform. *Lab Chip* 23, 591–608. **(CellTiter-Glo® 3D Cell Viability Assay, P450-Glo™ CYP3A4 Assay)**

Cox, B., *et al.* (2022) Setup of human liver-chips integrating 3D models, microwells and a standardized microfluidic platform as proof-of-concept study to support drug evaluation. *Biomaterials and Biosystems* 7, 100054. **(LDH-Glo® Cytotoxicity Assay, CellTiter-Glo® 3D Cell Viability Assay)**

Paek, K., *et al.* (2022) A high-throughput biomimetic bone-on-a-chip platform with artificial intelligence-assisted image analysis for osteoporosis drug testing. *Bioeng. Transl. Med.* 8, e10313. **(CellTiter-Glo® 3D Cell Viability Assay)**

Bovard, D., *et al.* (2022) Impact of aerosols on liver xenobiotic metabolism: A comparison of two methods of exposure. *Toxicology in Vitro* 9, 105277. **(CellTiter-Glo® Cell Viability Assay, P450-Glo™ CYP1A1, CYP1B1, CYP1A2, CYP3A4)**

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Ordering Information

Cell Health Assays

Product	Size	Cat. #
CellTiter-Glo® 3D Cell Viability Assay	100 assays	G9681
RealTime-Glo™ MT Cell Viability Assay	100 assays	G9711
LDH-Glo™ Cytotoxicity Assay	10 ml	J2380
CellTox™ Green Cytotoxicity Assay	10 ml	G8741
Caspase-Glo® 3/7 3D Assay	100 assays	G8981
RealTime-Glo™ Annexin V Apoptosis and Necrosis Assay	100 assays	JA1011
ROS-Glo™ H_2O_2 Assay	10 ml	G8820
GSH/GSSG-Glo™ Assay	10 ml	V6611
HEK293 Autophagy LC3 HiBiT Reporter Assay	Kit *	GA1040
U2OS Autophagy LC3 HiBiT Reporter Assay	Kit *	GA1050
Autophagy LC3 HiBiT Reporter Vector	20 µg	GA2550
RealTime-Glo™ Extracellular ATP Assay	200 assays	GA5010
P450-Glo™ CYP3A4 Assay System (with Luciferin-IPA)	10 ml	V9001
P450-Glo™ CYP2B6 Assay System	10 ml	V8321
P450-Glo™ CYP1A2 Assay System	10 ml	V8771
P450-Glo™ CYP1A1 Assay System	10 ml	V8751
P450-Glo™ CYP1B1 Assay System	10 ml	V8761
P450-Glo™ CYP2C8 Assay System	10 ml	V8781
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P450-Glo™ CYP2D6 Assay System	10 ml	V8891

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Metabolism Assays

Product	Size	Cat. #
Glucose-Glo™ Assay	5 ml	J6021
Lactate-Glo™ Assay	5 ml	J5021
Pyruvat-Glo™ Assay	5 ml	J4051
Glutamate-Glo™ Assay	5 ml	J7021
Glutamine/Glutamate-Glo™ Assay	5 ml	J8021
BCAA-Glo™	5 ml	JE9300
Triglyceride-Glo™ Assay	10 ml	J3160
Glycerol-Glo™ Assay	10 ml	J3150
Cholesterol/Cholesterol-Ester-Glo™ Assay	5 ml	J3190
BHB-Glo™ (Keton Body) Assay	5 ml	JE9500
NAD/NADH-Glo™ Assay	10 ml	G9071
NADP/NADPH-Glo™ Assay	10 ml	G9081
Metabolite-Glo™ Detection System	5 ml	J9030
Dehydrogenase-Glo™ Detection System	5 ml	J9010

Expression Analysis

Product	Size	Cat. #
ReliaPrep™ RNA Cell Miniprep System	50 preparations	Z6011
ReliaPrep™ RNA Tissue Miniprep System	50 preparations	Z6111
ReliaPrep™ miRNA Cell and Tissue Miniprep System	50 preparations	Z6211
GoTaq® qPCR MasterMix	5 ml	A6001
GoTaq® Probe 1-Step RT-qPCR System	5 ml	A6020
GoTaq® Probe 2-Step RT-qPCR System	5 ml	A6010

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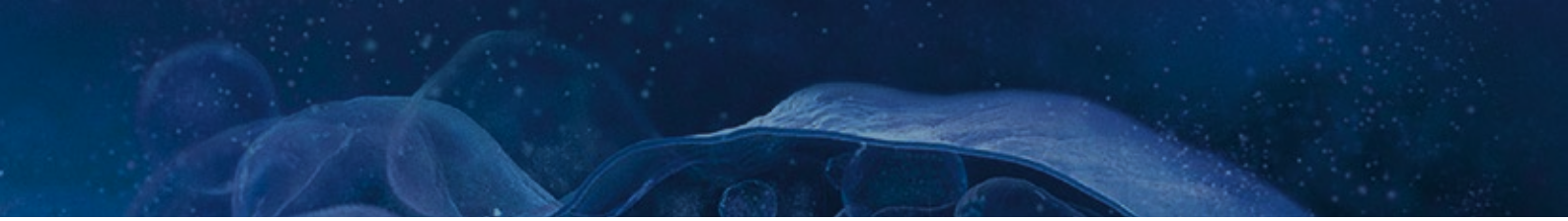

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