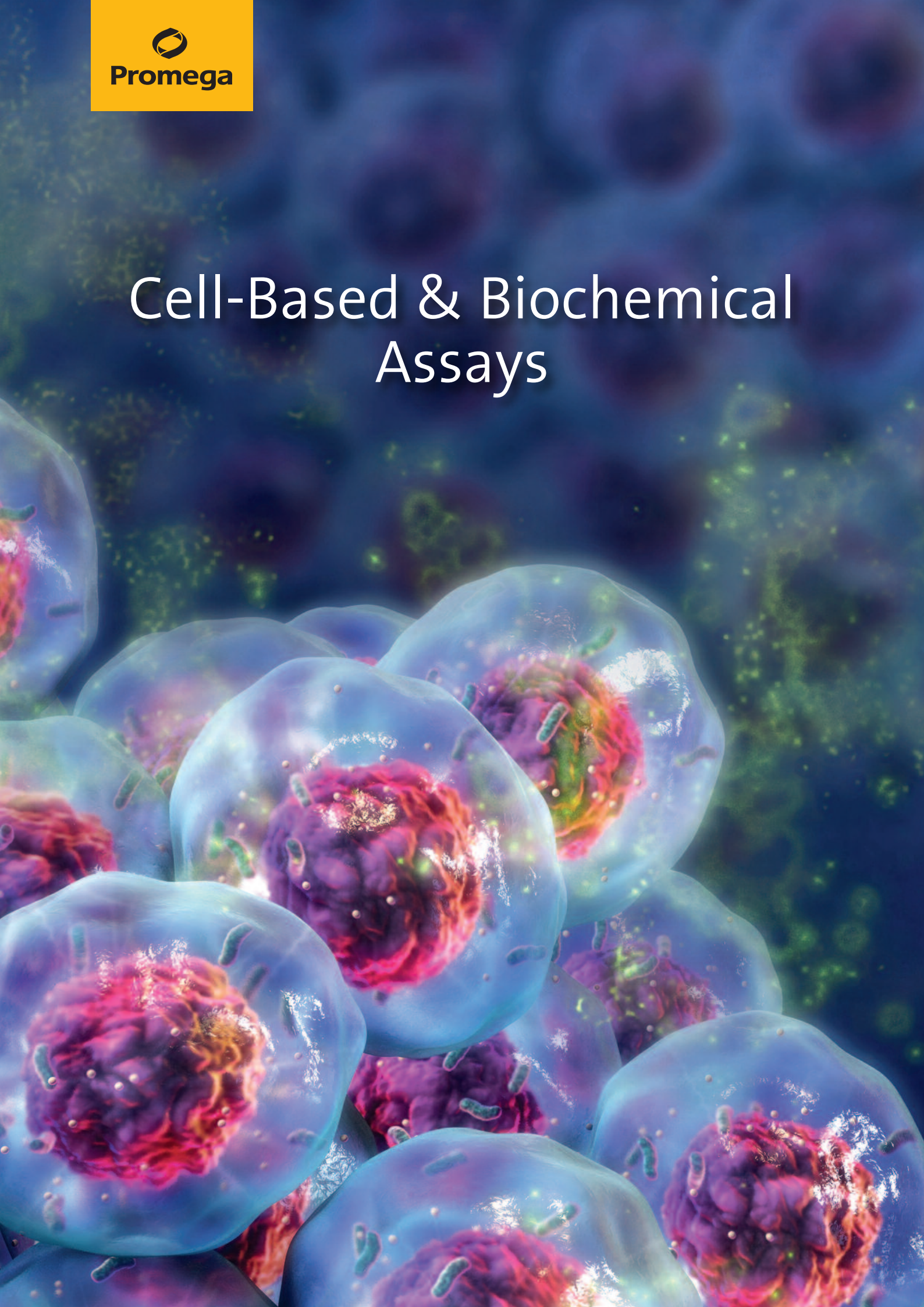


Cell-Based & Biochemical Assays



Promega is your comprehensive partner for cell-based applications and instruments!

Reporter Gene and Cell-based Assays

Detection

Analysis and Support

GloMax® Discover is an advanced multimode plate reader developed with Promega reagent chemistries to provide high-performance luminescence, fluorescence, UV-visible absorbance, BRET and FRET, two-color filtered luminescence

and kinetic measurement capabilities. GloMax® Discover can be used as a standalone plate reading instrument or integrated into high-throughput automated workflows. Results are easy to interpret using integrated data analysis software.

One instrument for numerous applications:

Reporter Gene Assays | Cell Viability, Cytotoxicity and Apoptosis Assays | Energy Metabolism & Oxidative Stress
Kinetic Measurements | Multiplexing | ELISA | Protein:Protein Interaction



For more information visit: www.promega.com/glomax-comparison

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Cell-based and biochemical assay formats

Promega offers an extensive range of products for analyzing complex cellular and biochemical processes. These cell-based and biochemical assays are used in pure and applied research, as well as in the identification and characterization of drugs in the pharmaceutical industry. In addition to the existing assays for investigating cell viability, cytotoxicity and apoptosis, there are now new assays for analyzing cellular processes in real-time and autophagy. Researchers can use these to analyze how cells react to growth factors, cytokines, hormones, mitogens, radiation, effectors and other signaling molecules. In the development of new drugs, such assays are indispensable for investigating the effectiveness and toxicity of active-substance molecules before investing in expensive animal experiments or clinical studies.

Add-mix-measure – the simple assay format

The add-mix-measure format makes our cell-based and biochemical assays particularly easy to use. Most of the assays are homogeneous and can be added to cells directly, without any washing or centrifugation steps. This minimizes potential sources of error. Furthermore, this format readily lends itself to automation.

Multiplexing: combining different assays to produce more information

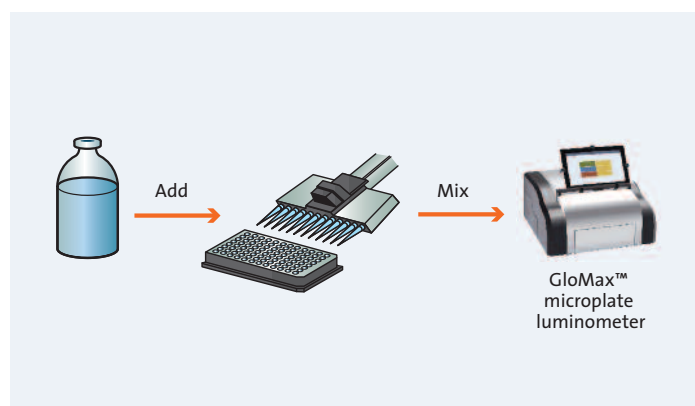
It is often helpful for scientists to be able to obtain multiple data points from a single sample. It is therefore an advantage if an assay is suitable for multiplexing – the analysis of more than

one parameter in a single sample. For example, researchers may initially use a fluorescence assay for measuring cytotoxicity or cell viability. They can then perform a luminescent caspase assay or reporter gene assay on the same sample. Multiplexing saves time, sample material, cell-culture reagents, and scarce or expensive test compounds. It also improves the quality of the data and simplifies interpretation of the results.

High-sensitivity assays

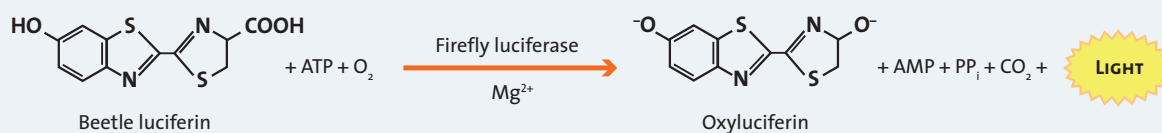
Most of Promega's assays are based on luminescence or fluorescence. In multiplexing, in particular, these two assay types are combined with one another in order to obtain as much information as possible from a sample. The fluorescent assays are based on profluorescent dyes which have been linked to recognition sequences for specific enzymes or enzyme classes. The fluorescent dye is released depending on the activity of the relevant enzymes. Luminescent assays, on the other hand, are based on the luciferase reaction of the firefly *Photinus pyralis* and a small luciferase subunit (19 kDa) from the deep sea shrimp *Oplophorus gracilirostris* (NanoLuc®). In such assays, the luciferase reaction can be used in a variety of ways for detecting, with a very high degree of sensitivity, a large number of cellular processes. The luminescent assays can be subdivided into three basic assay types:

- Type I: Measurement of luciferase expression
- Type II: Measurement of ATP content
- Type III: Measurement of luciferin release
- Type IV: Measurement of Protein:Protein Interaction (PPI) and Apoptosis using NanoBiT®
- Type V: Monitor translational and post-translational-regulation of proteins using a Protein Tagging and Detection System – NanoBiT® HiBiT



Promega's five basic assay types based on the luciferase reaction:

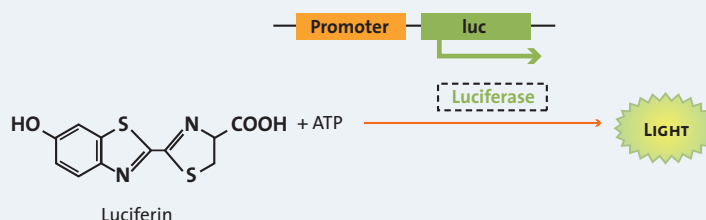
Reaction of firefly luciferase



Type I:

Measurement of luciferase expression

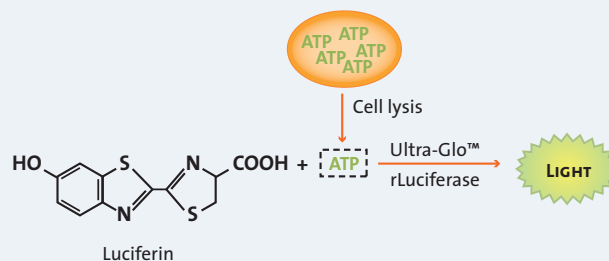
The measurement of luciferase expression is the basis of all reporter gene assays or GPCR assays. The level of luciferin expression is determined by the strength or saturation state of the promoter. The assay reagent combines an excess of luciferin and ATP and therefore the expressed luciferase is the limiting factor.



Type II:

Measurement of ATP content

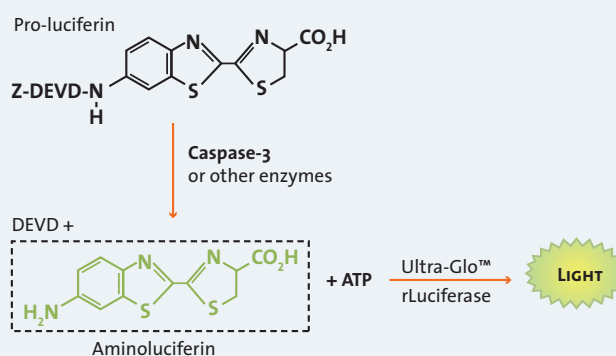
In ATP-dependent assays, the ATP content in a sample is measured. Here, ATP is the limiting factor. It is determined indirectly via the luciferase reaction. This principle is used in the determination of cell viability and in the measurement of kinase activities, for example. The assay reagent contains an excess of Ultra-Glo™ Recombinant Luciferase and luciferin.



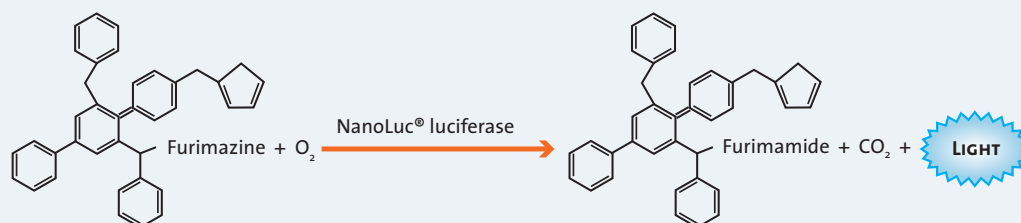
Type III:

Measurement of luciferin release

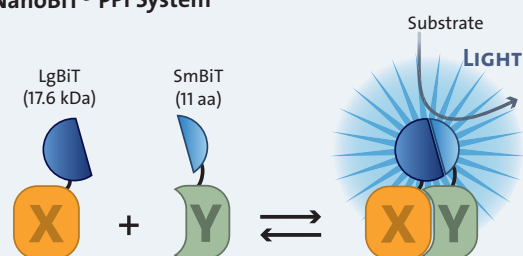
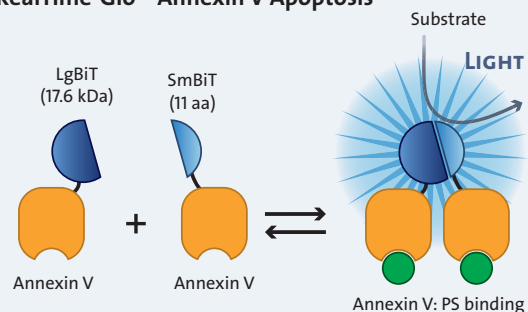
The luciferin-generating assays contain a pro-luciferin with enzyme recognition sequences, such as DEVD tetrapeptide for determining caspase-3/7 activity. Only after the substrate has been released the luminescence signal is generated by the relevant enzyme. The assay reagent contains modified luciferin and an excess of Ultra-Glo™ Recombinant Luciferase and ATP. The same principle is used for the RealTime-Glo™ Metabolic Cell Viability Assay using the NanoLuc® reaction and a Pro-Furimazine substrate.



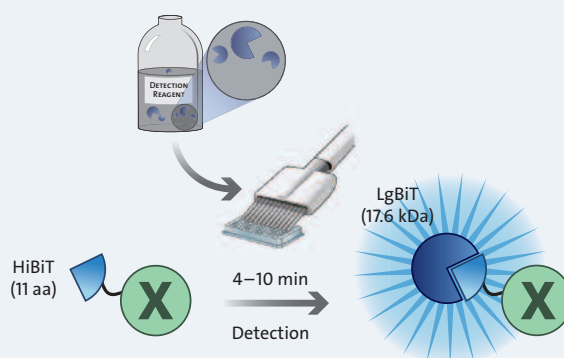
Reaction of NanoLuc® luciferase

**Type IV:****Measurement of Protein:Protein Interaction (PPI) and Apoptosis using NanoBiT®**

The NanoLuc® Binary Technology (NanoBiT®) is a complementation assay based on two optimized subunits of the NanoLuc® luciferase, i.e. LargeBiT (LgBiT) and SmallBiT (SmBiT). The features of this technology, i.e. low intrinsic affinity ($K_D = 190 \mu M$) of the LgBiT and emission of a bright signal upon complementation, make it an ideal tool to study associative and dissociative PPI or to investigate apoptosis in live cells. Assays based on this technology, e.g. the NanoBiT® PPI System or the RealTime-Glo™ Annexin V Apoptosis and Necrosis Assay, are applicable to simple endpoint measurements but also to acquire real-time data. Complementation of LgBiT and SmBiT reconstitutes a functional luciferase whose substrate conversion is accompanied by emission of light.

NanoBiT® PPI System**RealTime-Glo™ Annexin V Apoptosis****Type V:****Monitor translational and post-translational regulation of proteins using the HiBiT Protein Tagging System**

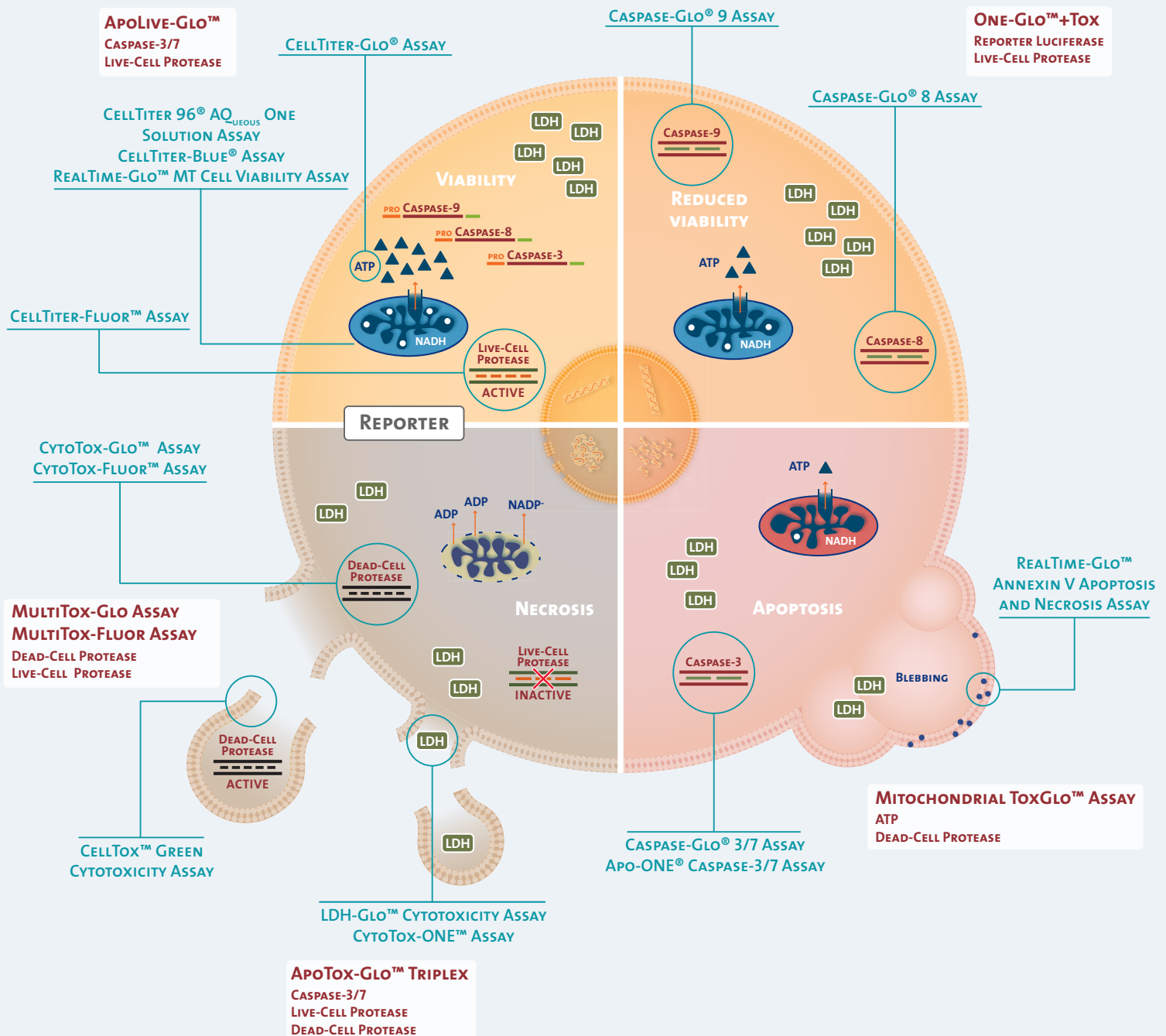
The HiBiT Protein Tagging System enables the setup of highly sensitive reporter assays on the basis of an 11 aa peptide fusion tag HiBiT. HiBiT-tagged proteins can rapidly be quantified with detection reagents containing the LgBiT subunit in an add-mix-measure workflow. Due to its exceptionally high intrinsic affinity ($K_D = 700 pM$), HiBiT spontaneously complements with LgBiT to form a functional enzyme producing luminescence. One of the many applications of HiBiT is to measure autophagy using the Autophagy LC3 HiBiT Reporter Assay System.



II Cell viability, cytotoxicity, apoptosis, autophagy and inflammation

In many areas of bioscience, the investigation and determination of cell viability, cytotoxicity, apoptosis, autophagy and inflammation are among the most important methods used. Cell-based assays are routinely used nowadays in the development of new therapeutic agents and in the identification of potential drug candidates.

There is now a range of different cell-based assays for detecting cell viability, cytotoxicity, apoptosis, autophagy and inflammation. These use both metabolic and non-metabolic markers. Combining different assays (multiplexing) makes it possible to analyze more than one parameter in a sample. This simplifies interpretation of the data and enables the user to differentiate between necrosis and apoptosis, for example.



Cell-based assays at a glance for cell viability, cytotoxicity, apoptosis, autophagy and inflammation

Assay	Parameters/Biomarkers	Time required	96-well sensitivity	Plate format	Instrument
RealTime-Glo™ MT Cell Viability Assay	Reducing capacity of the cells	0.5–72 h	< 100 cells/well in 96-well format	96/384/1536	Luminometer
CellTiter-Glo® Assay, CellTiter-Glo® 2.0 Assay	ATP	10 min	10–15 living cells	96/384/1536	Luminometer
CellTiter-Glo® 3D Assay	ATP	30 min	ND	all common 3D-microtissues formats	Luminometer
CellTiter-Fluor™ Assay	Live-cell protease	0.5–3 h	40 living cells	96/384/1536	Fluorometer, AFC 400 _{ex} /505 _{em}
CellTiter-Blue® Assay	Resazurin reduction by reducing equivalents	1–4 h	400 living cells	96/384/1536	Fluorometer, resorufin 560 _{ex} /590 _{em}
CellTiter 96® AQ _{ueous} One Solution Assay	MTS reduction by reducing equivalents	1–4 h	1,000 living cells	96/384	Spectrophotometer Abs 490 nm
BacTiter-Glo™ Assay	ATP	5 min	10 living bacteria	96/384	Luminometer
LDH-Glo™ Cytotoxicity Assay	LDH	30–60 min	< 10 dead cells	96/384/1536	Luminometer
CellTox™ Green Assay	DNA	0.25–72 h	ND	96/384/1536	Fluorometer, (485–500 _{ex} /520–530 _{em})
CytoTox-Glo™ Assay	Dead-cell protease release	15 min	10 dead cells	96/384/1536	Luminometer
CytoTox-Fluor™ Assay	Dead-cell protease release	0.5–3 h	10 dead cells	96/384	Fluorometer, R110 485 _{ex} /520 _{em}
CytoTox-ONE™ Assay	LDH release	10 min	200 dead cells	96/384	Fluorometer, resorufin 560 _{ex} /590 _{em}
Viral ToxGlo™ Assay	ATP	10 min	15 living cells (384-well)	96/384/1536	Luminometer
RealTime-Glo™ Annexin V Apoptosis and Necrosis Assay	Phosphatidylserine, DNA	0.5–48 h	ND	96/384	Luminometer, Fluorometer, (485–500 _{ex} /520–530 _{em})
Caspase-Glo® 3/7 Assay, Caspase-Glo® 3/7 3D Assay	Caspase-3/7 activity	0.5 h	100 apoptotic cells	96/384/1536	Luminometer
Apo-ONE® Caspase 3/7 Assay	Caspase-3/7 activity	1–18 h	625 apoptotic cells	96/384/1536	Fluorometer, R110,499 _{ex} /521 _{em}
Autophagy LC3 HiBiT Reporter Assay System	Human LC3	10 min–3 h	Signal-to-background values >100	96/384	Luminometer
Caspase-Glo® 1 Inflammasome Assay	Caspase-1 activity	1 h	ND	96/384	Luminometer
RealTime-Glo™ Extracellular ATP Assay	Extracellular ATP	5 min – 24 h	LOD 10 pM	96/384	Luminometer
Lumit™ HMGB1 Human/Mouse Immunoassay	HMGB1	60 – 90 min	LOD 1 ng/ml (Hu), 3 ng/ml (Mus)	96	Luminometer
MultiTox-Glo Assay	Viability + cytotoxicity; live- + dead-cell protease	0.5 h	40 living cells, 10 dead cells	96/384/1536	Fluorometer, AFC 400 _{ex} /505 _{em} Luminometer
MultiTox-Fluor Assay	Viability + cytotoxicity; live- + dead-cell protease	0.5–3 h	40 living cells, 10 dead cells	96/384/1536	Fluorometer, AFC 400 _{ex} /505 _{em} R110 485 _{ex} /520 _{em}
ApoLive-Glo™ Multiplex Assay	Viability + apoptosis; live-cell protease + caspase-3/7	1–3 h	~ 40 living cells, 100 apoptotic cells	96/384	Fluorometer, AFC 400 _{ex} /505 _{em} Luminometer
ApoTox-Glo™ Triplex Assay	Viability, cytotoxicity + apoptosis live- + dead-cell protease + caspase-3/7	1–3 h	~ 40 living cells, 100 apoptotic cells	96/384	Fluorometer, AFC 400 _{ex} /505 _{em} R110 485 _{ex} /520 _{em} Luminometer
One-Glo™+ Tox Assay	Viability + reporter gene expression; live-cell protease + luciferase activity	0.6–3 h	~ 40 living cells	96/384	Fluorometer, AFC 400 _{ex} /505 _{em} Luminometer
Mitochondrial ToxGlo™ Assay	Mitochondrial toxicity; dead-cell protease + ATP	0.6–3 h	ND	96/384	Fluorometer, R110 485 _{ex} /520 _{em} Luminometer

IIa Cell viability

Are the cells in my cell culture multiplying or not? This is usually the first question asked when cells are exposed to substances or other influences in an experiment. This question can be answered by measuring various parameters. One well-known method is to measure reducing equivalents in a cell culture sample. This value is proportional to the number of living cells in the medium. A further option is to measure the ATP content in living cells, which can be indirectly detected by means of the luciferase reaction. A new method to monitor cell viability in real time is based on a luminescent biosensor that generates more information about the mode of action of a treatment with regard to time and dose dependence. In order to distin-

guish whether a reduced cell viability is due to cytostasis, apoptosis or necrosis, it is recommended to determine membrane integrity and/or caspase activity by multiplexing.

RealTime-Glo™ MT Cell Viability Assay

CellTiter-Glo® Luminescent Cell Viability Assay

CellTiter-Glo® 2.0 Cell Viability Assay

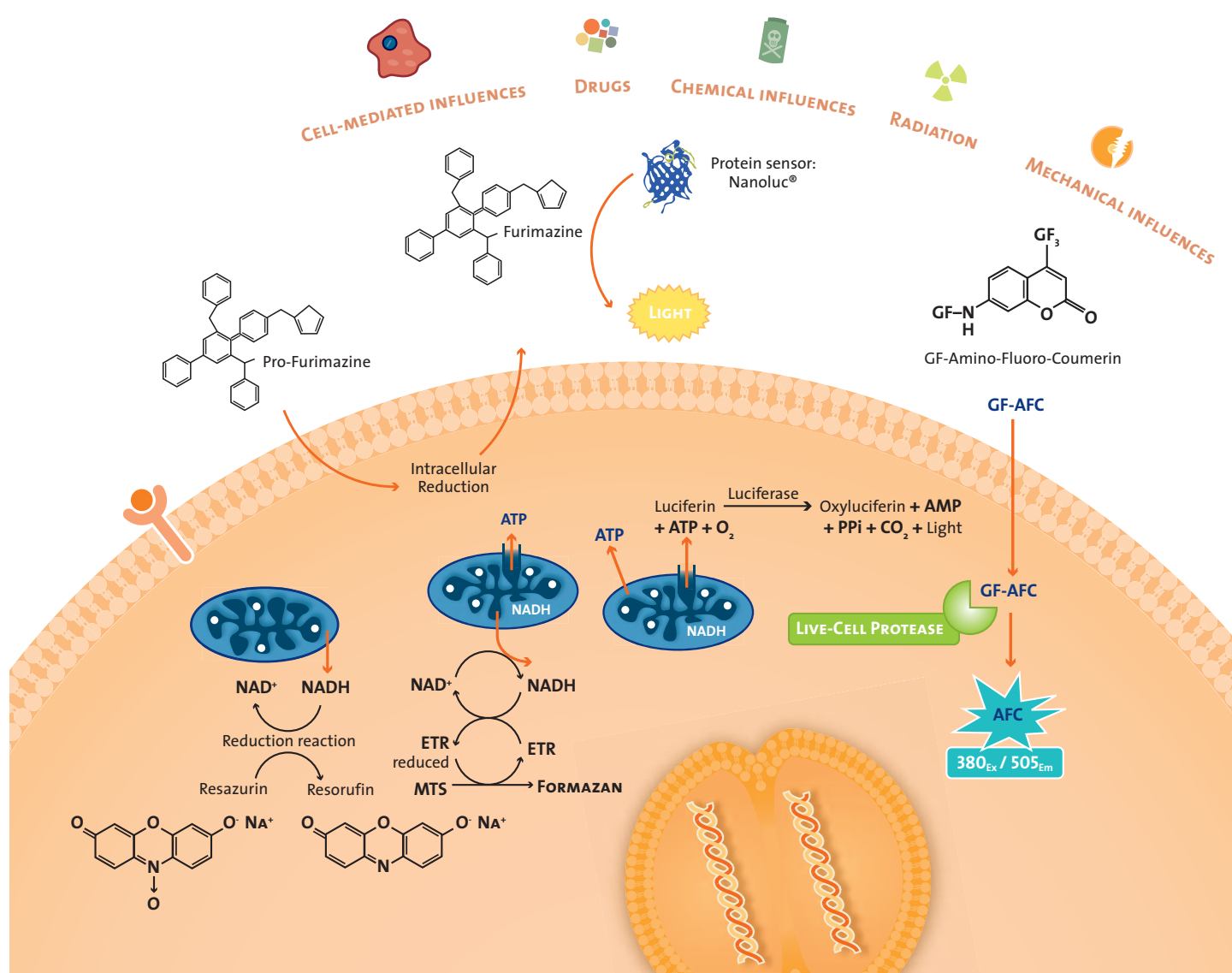
CellTiter-Glo® 3D Viability Assay

CellTiter-96® AQueous One Solution Cell Proliferation Assay (MTS)

CellTiter-Blue® Cell Viability Assay

CellTiter-Fluor™ Cell Viability Assay

BacTiter-Glo™ Microbial Cell Viability Assay



RealTime-Glo™ MT Cell Viability Assay

Cell-based

Applications

Monitor cell viability in real time to determine onset of toxicity, analyze potency versus efficacy over time and analyze differential cell growth, multiplexing with other assays

Assay description

The **RealTime-Glo™ MT Cell Viability Assay** is a non-lytic, homogeneous, bioluminescent method to determine in real time the number of viable cells in culture by measuring the reducing potential of cells and thus metabolism (MT). The reagent is stable and nontoxic to cells for up to 72 hours. No cell washing, removal of medium or further reagent addition is required to determine the number of viable cells. The bioluminescent assay provides a greater signal-to-background ratio and higher sensitivity in less time compared to colorimetric or fluorometric viability assays that are based on the reducing potential of cells. The assay is compatible with automated and high-throughput protocols.

Assay principle

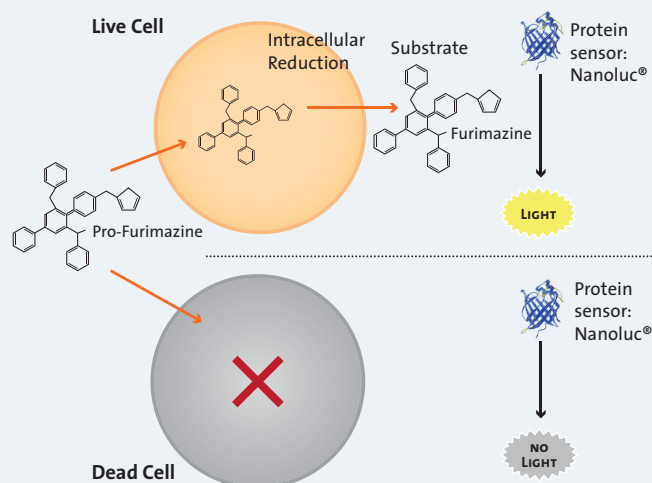
The assay involves adding NanoLuc® luciferase protein sensor and a cell-permeant pro-furimazine substrate to cells in culture. Viable cells reduce the proprietary pro-substrate to generate a substrate for NanoLuc® luciferase. This substrate diffuses from cells into the surrounding culture medium, where it is rapidly used by the NanoLuc® enzyme to produce a luminescent signal. The signal correlates with the number of viable cells, making the assay well suited for cytotoxicity studies. Real time measurements can be performed by adding reagents when cells are plated, when test compound is added to the cells or at any time point when cell viability measurements are needed.

Assay features

Assay type	Luminescent
Markers	Reducing capacity of the cell
Applications	Cell viability in real time, onset of toxicity
Cell type	Cell lines, primary cells and 3D cultures
Implementation	Homogeneous, no-step assay
Time required	10–60 minutes (after adding the reagent)
Sensitivity	< 100 cells/well in 96-well format, < 10 cells/well in low-volume 384-well format
Robustness	Reactions are scalable in 96-, 384- and 1,536-well plates

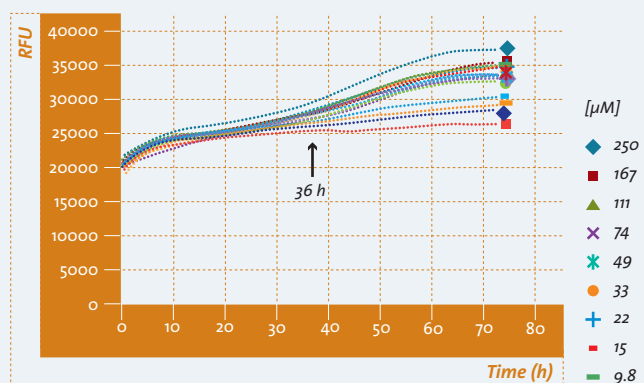
Suitable for
3D-microtissues

Multiplexing with
CellTox™ Green

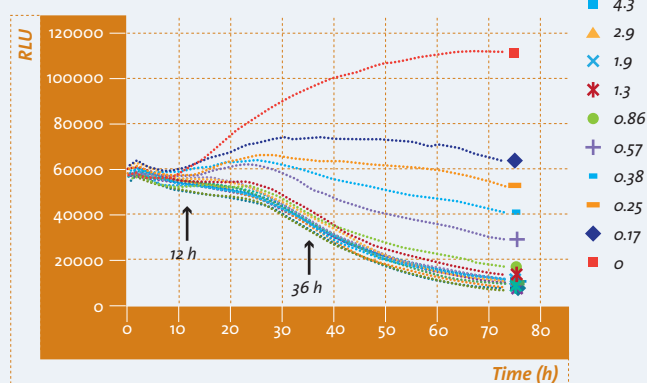


RealTime-Glo™ utilizes NanoLuc® luciferase and a novel pro-furimazine substrate to determine the number of viable cells through measurement of the reducing capacity of the cell.

Cytotoxicity: CellTox™ Green



Viability: RealTime-Glo™



MCF cells (500 cells/well) dosed with etoposide, RealTime-Glo™ MT Cell Viability reagents and CellTox Green in media. Fluorescence and luminescence measured on Tecan M200 with Gas Control Module (37C/5% CO₂) every 1 h for 72 h from the same sample wells.

CellTiter-Glo® Luminescent Cell Viability Assay

Cell-based

Suitable for
primary Screening

Applications

Cell viability; proliferation; cytotoxicity.

Assay description

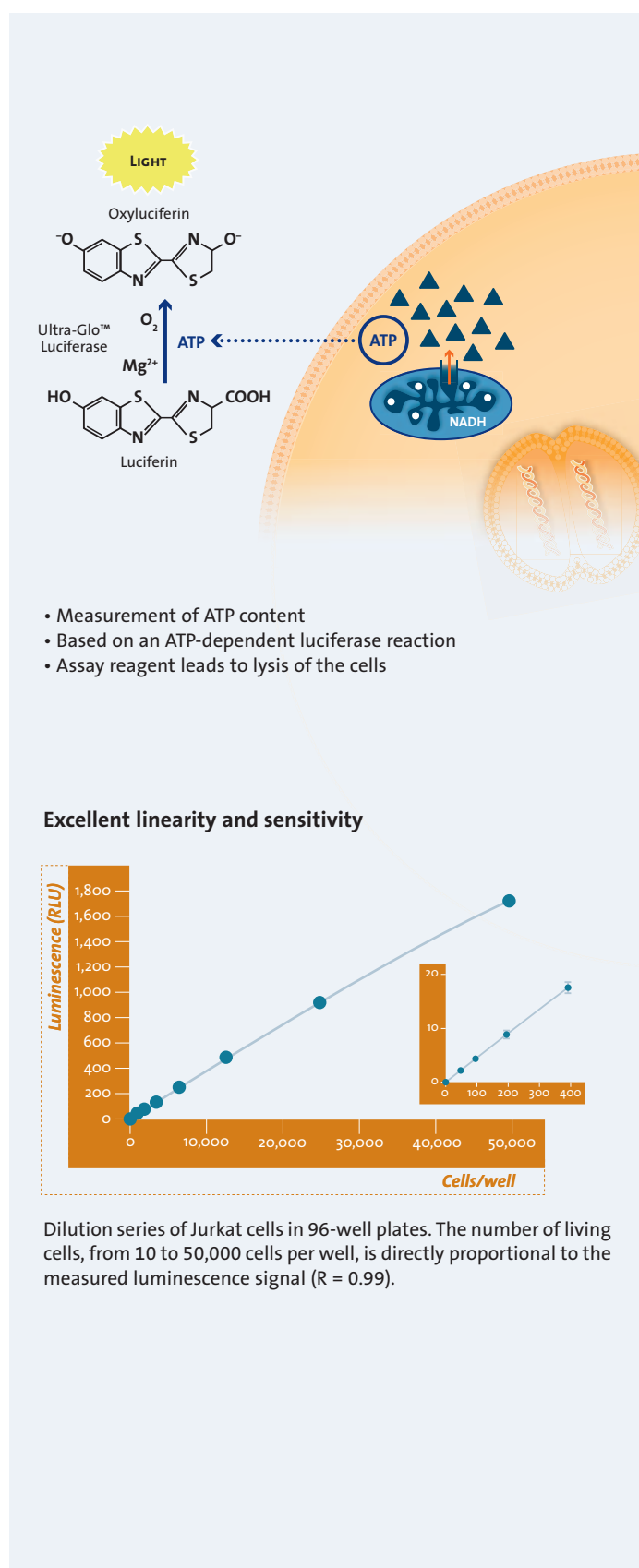
CellTiter-Glo® Assay is the most sensitive cell-based assay for detecting cell viability. It is therefore particularly suitable for use in studies on primary cells. Notable features of the assay include the ease and speed with which it can be performed and the reproducibility of the data (Z' factor > 0.63 in a 1536-well format), as well as the extremely wide linear measurement range of 10–50,000 cells. The assay is provided as two components, lyophilized substrate and buffer, that are combined to make a single addition reagent.

Assay principle

The assay is based on the measurement of ATP content in an ATP-dependent luciferase reaction. ATP content is a measure of the metabolic activity of cells. Conversion of luciferin by a recombinant luciferase (Ultra-Glo™ Luciferase) produces oxyluciferin and light. The light signal can be measured both in a luminometer and with the aid of a CCD camera and is proportional to the number of living cells. The assay reagent is added directly to the cells and leads to lysis of the cells.

Assay features

Assay type	Luminescent (glo-type; $T_{1/2}$ > 5 h)
Markers	ATP
Applications	Cell viability, proliferation, cytotoxicity
Cell type	Cell lines and primary cells (adherent or in suspension)
Implementation	Homogeneous, one-step assay
Time required	10 minutes
Sensitivity	10 living cells (96-well format)
Linearity	10–50,000 cells
Robustness	High Z' factor, Reactions are scalable in 96-, 384- and 1536-well formats



CellTiter-Glo® 2.0 Cell Viability Assay

Cell-based

Applications

Cell viability; proliferation; cytotoxicity.

Assay description

The **CellTiter-Glo® 2.0 Assay** is based on the original CellTiter-Glo® Assay chemistry that detects ATP as indicator for cell viability but with improved storage convenience for easy implementation. The CellTiter-Glo® 2.0 Assay is provided as a single ready-to-use reagent that can be stored at 4°C for up to 1 month with >90% activity remaining or at room temperature for 1 week with >85% activity remaining. The assay is designed for use with multiwell plate formats, making it ideal for automated high-throughput screening (HTS), for cell proliferation and cytotoxicity assays. The system detects as few as 15 cells/well in a 384-well format in 10 minutes after adding reagent.

Assay principle

The homogeneous assay procedure involves adding the single reagent (CellTiter-Glo® 2.0 Reagent) directly to cells cultured in serum-supplemented medium. Cell washing, removal of medium and multiple pipetting steps are not required. The “add-mix-measure” format results in cell lysis and generation of a luminescent signal proportional to the amount of ATP present. The amount of ATP is directly proportional to the number of cells present in culture. The CellTiter-Glo® 2.0 Assay generates a “glow- type” luminescent signal, which has a half-life generally greater than 3 hours, depending on cell type and medium used. The extended half-life eliminates the need to use reagent injectors and provides flexibility for continuous or batch-mode processing of multiple plates with excellent Z'-factor values for screening applications.

Assay features

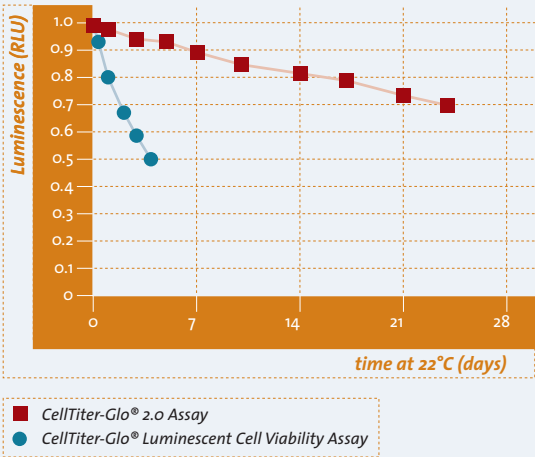
Assay type	Luminescent (glow-type; T _{1/2} > 3 h)
Markers	ATP
Applications	Cell viability, proliferation, cytotoxicity
Cell type	Cell lines and primary cells (adherent or in suspension)
Implementation	One-step assay with flexible storage capability
Time required	10 minutes
Sensitivity	15 living cells (384-well format)
Linearity	15–50,000 cells
Robustness	High Z' factor: 0.81 in 384-well format, scalable to 1536-well format

Stable for 1 week
at room temperature
Excellent for
primary Screening

CellTiter-Glo® 2.0 Reagent has increased stability at 22°C and 4°C for convenient every day use

Enhanced stability: Decrease of enzyme activity
< 20% at following storage conditions

	22°C	4°C
CellTiter-Glo®	12 hours	3.5 days
CellTiter-Glo® 2.0	1 week	4 month



Samples of reagent were placed at various temperatures for different lengths of time and then frozen at -80°C. Once all samples were collected, they were thawed and assayed by mixing 1:1 with 2 µM ATP in water. Luminescence was recorded after 10 minutes .

Performance with various cells & media

Medium	Cell type	Luminescence (RLU x 10 ⁴)		Signal Half-Life (hours)	
		CellTiter-Glo® Reagent	CellTiter-Glo® 2.0 Reagent	CellTiter-Glo® Reagent	CellTiter-Glo® 2.0 Reagent
Memα	MCF7	4.06	6.40	7.30	4.81
	DU145	8.42	12.45	7.00	5.13
McCoy's 5A	U20S	5.98	9.27	7.14	5.07
F12	CHO	5.86	8.76	6.97	4.99
RPMI	HCT116	6.75	10.86	7.53	4.95
	Jurkat	12.80	21.10	7.41	5.33
	U397	13.51	20.86	7.07	5.33
DMEM	HEK293	6.21	10.07	7.27	4.83
	HeLa	5.80	9.01	7.02	4.88
	HepG2	6.52	10.34	7.27	4.83

10,000 cells were plated for 24 hours, mixed 1:1 with reagent, and the luminescence was read over time.

* 100,000 cells were plated for suspension cells

CellTiter-Glo® 3D Viability Assay

Specifically developed for
3D-microtissues

Cell-based

Applications

Cell viability; proliferation; cytotoxicity in 3D microtissues.

Assay description

Cells assessed in 3D culture models frequently provide more physiologically relevant data than cells studied in standard 2D formats. Thus, there is a need for convenient and effective assays explicitly validated for 3D microtissues. **CellTiter-Glo® 3D** is a bioluminescent ATP detection assay for measuring cell viability with an optimized protocol and an improved formulation that has been designed to measure the viability of 3D microtissues. This single-component liquid reagent has significant lytic capacity, exhibits high ATP recovery, and can be used to measure the viability of microtissues grown in a variety of 3D culture models, including ECM-independent (e.g. hanging drop), ECM-dependent (e.g. Matrigel™), and synthetic scaffolds (e.g. Alvetex™).

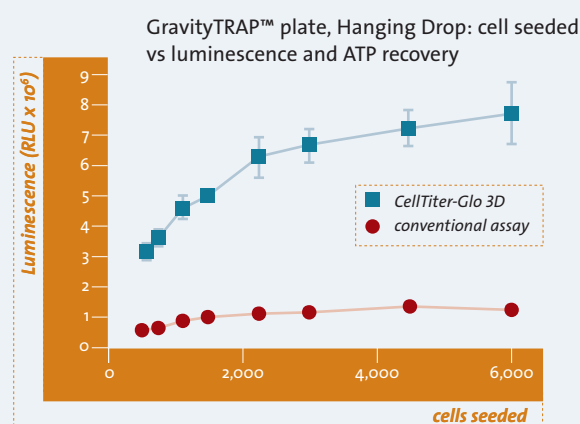
Assay principle

The CellTiter-Glo® 3D Assay is an improved reagent formulation of CellTiter-Glo® for bioluminescent detection of ATP with a more effective lysis. The “add-mix-read” protocol and single-component liquid format make this novel cell viability assay a simple and convenient reagent for assaying the viability of 3D microtissues. After adding the reagent directly to the cells and an incubation of 30 min, a stable luminescence signal can be measured with a half-life of > 4 h.

Assay features

Assay type	Luminescent (glow-type; $T_{1/2}$ > 4 h)
Markers	ATP
Applications	Cell viability, proliferation, cytotoxicity in 3D microtissues
3D microtissues	Hanging drop microtissues, Matrigel™, Alvetex™, collagen-matrix
Implementation	Homogeneous, one-step assay
Time required	30 minutes
Robustness	96- or 384-well plates, microtissues up to 700 microns (cell type dependent)

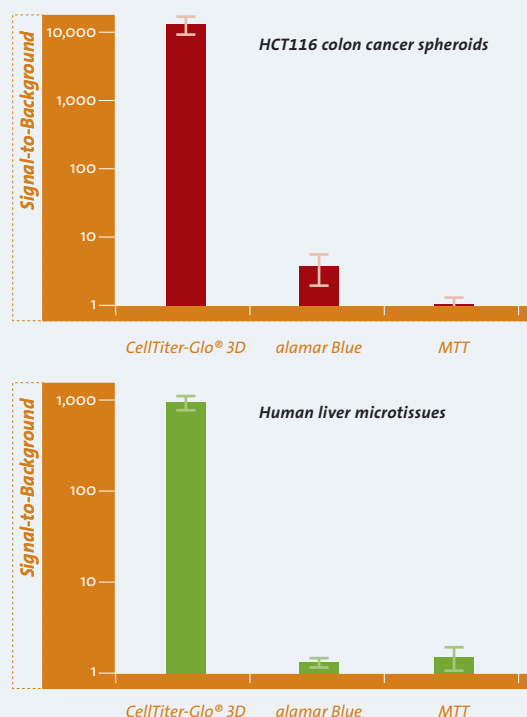
CellTiter-Glo® 3D Viability Assays applied on microtissues



cells seeded	diameter (µm)	ATP (nM)	
		CellTiter-Glo® 3D	conventional assay
6,000	716	1,327	290
2,250	533	1,079	262
1,125	468	799	206
563	355	539	134

HCT116 cells (RPMI +10% FBS) were grown by the hanging drop method for 4 days.

Sensitivity comparison of different viability assays applied to 3D microtissues



400 HCT116 colon cancer cells were seeded into a 96-well GravityPLUS™ hanging-drop plate (InSphero AG) and incubated for 4 days. Spheroids (~340 µm) and human liver microtissues (~200 µm) were assayed according to each of the assay manufacturer's protocols. The total assay times for the CellTiter-Glo® 3D, alamarBlue®, and MTT assays were 30 minutes, 3 hours, and 8 hours, respectively.

Single Reagent
addition

CellTiter 96® AQ_{ueous} One Solution Cell Proliferation Assay (MTS)

Cell-based

Applications

Cell viability; proliferation; cytotoxicity.

Assay description

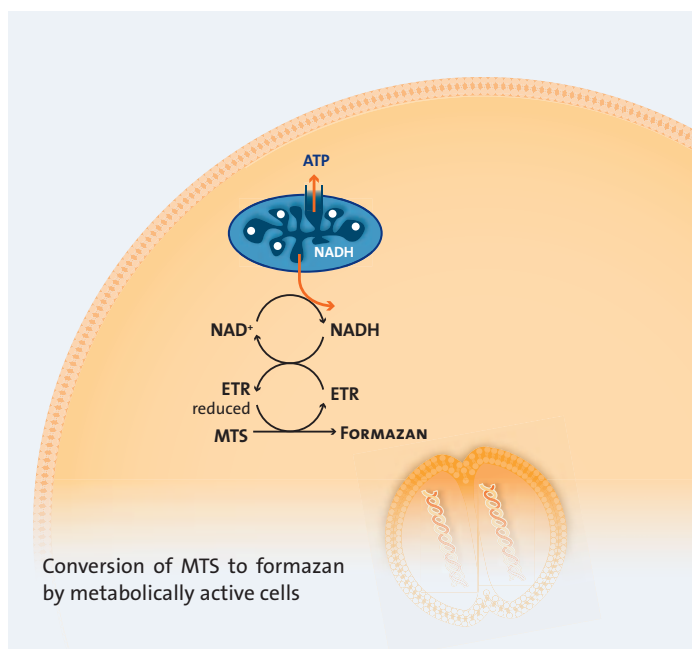
CellTiter 96® AQ_{ueous} One Solution Assay (MTS) is based on a colorimetric method for determining numbers of living cells and is suitable for measuring cell viability, proliferation and indirectly also cytotoxicity. One advantage that this one-step assay has over MTT assays is that the MTS formazan product is water-soluble. Consequently, no extraction steps using organic solvents are required. Unlike conventional colorimetric assays, the MTS assay can also be used for blood lymphocytes. Furthermore, the assay reagent has greater storage stability at 4°C than conventional reagents.

Assay principle

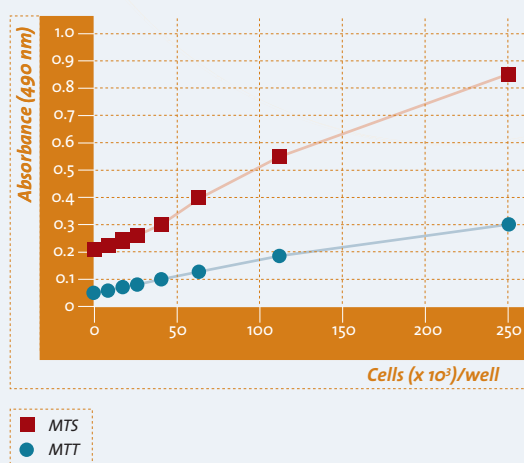
Detection of cell viability using MTS is based on the reduction of the tetrazolium salt MTS to water-soluble formazan dye by metabolically active cells. The assay reagent additionally contains an electron transfer reagent (ETR), phenazine ethosulfate (PES). PES is reduced intracellularly by reducing equivalents such as e.g. NADH or NADPH and outside the cell leads to the reduction of MTS to intensely-coloured formazan. The absorbance of the formazan at 490 nm can be measured in the 96-well plate directly, with no additional treatment steps required. The read-out is directly proportional to the number of living cells in culture.

Assay features

Assay type	Absorbance assay (Abs 490 nm +/- 40 nm)
Markers	Reducing equivalents such as e.g. NADH/NADPH
Applications	Cell viability, proliferation, cytotoxicity
Cell type	Cell lines, primary cells, plants, yeasts, blood lymphocytes
Implementation	Homogeneous, one-step assay
Time required	1–4 hours
Sensitivity	1,000 living cells (96-well format)



Comparison between MTT and MTS assays



Determination of the cell viability of PBMCs (Peripheral blood mononuclear cells) in a 96-well plate using MTT and MTS respectively. The MTS assay exhibits at an identical cell count significantly higher absorbance values than the MTT assay.

Abbreviations:

MTS: 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium

PES: phenazine ethosulfate

MTT: 3-(4,5-dimethylthiazol-2-yl)-5-diphenyltetrazolium bromide

CellTiter-Blue® Cell Viability Assay

Cell-based

Applications

Cell viability; proliferation; cytotoxicity; multiplexing.

Assay description

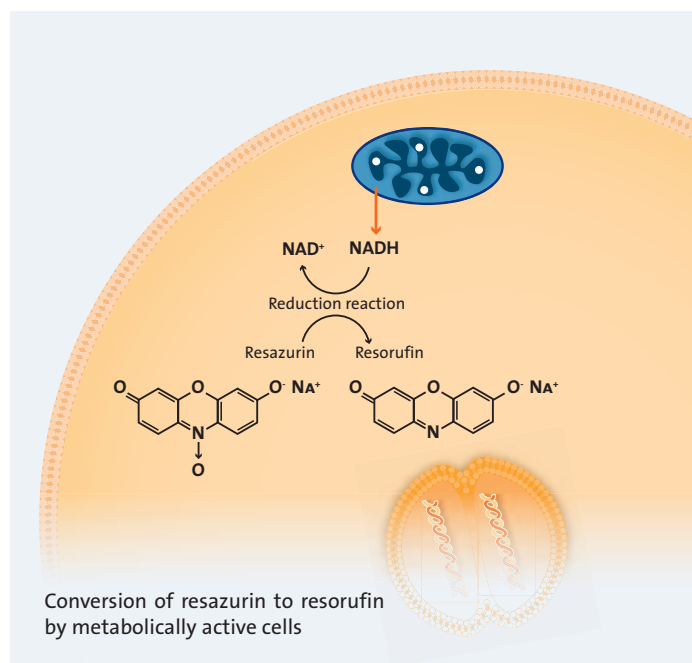
CellTiter-Blue® Assay is a fluorescent, cell-based assay for determining cell viability, proliferation and cytotoxicity. In contrast to comparable resazurin assays, the assay reagent resazurin is highly purified and is nontoxic to the cells, so flexible incubation times are possible. The assay can be performed in combination with other cell-based assays (e.g. Apo-ONE® Homogeneous Caspase-3/7 Assay) on the same cells (multiplexing).

Assay principle

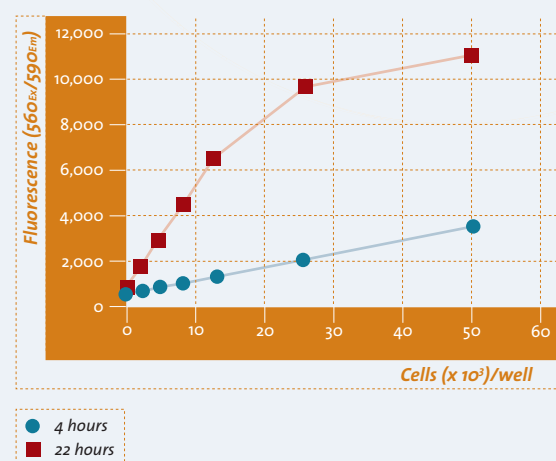
Detection of cell viability is based on the blue indicator dye resazurin, which is converted intracellularly by metabolically active cells into resorufin, which is pink and fluorescent. The assay reagent is added directly to the medium. The formation of the resorufin can be detected either in a fluorometer (recommended as the most sensitive method 560_{Ex}/590_{Em}) or in a spectrophotometer (ELISA reader, 570 nm).

Assay features

Assay type	Fluorescent (560 _{Ex} /590 _{Em})
Markers	Reducing equivalents such as e.g. NADH
Applications	Cell viability, proliferation, cytotoxicity, multiplexing
Cell type	Cell lines (adherent or in suspension), primary cells
Implementation	Homogeneous, one-step assay
Time required	1–4 hours (Incubation for up to 22 hours possible)
Sensitivity	400 living cells (96-well format)
Robustness	High Z' factor, 96- to 384-well formats



Flexible incubation times



Jurkat cells were seeded in a 96-well plate and incubated for 4 hours and 22 hours respectively using the CellTiter-Blue® Assay. After 4 hours, sensitivity is at 400 cells/well and the signal exhibits a linear correlation to the cell count over the entire measurement range. Where incubation is extended to 22 hours, the detection limit rises to about 50 cells/well; however, the assay no longer exhibits a linear correlation for cell counts in excess of 12,500 cells/well.

CellTiter-Fluor™ Cell Viability Assay

Cell-based

Applications

Cell viability; cytotoxicity; multiplexing with other cell-based assays.

Assay description

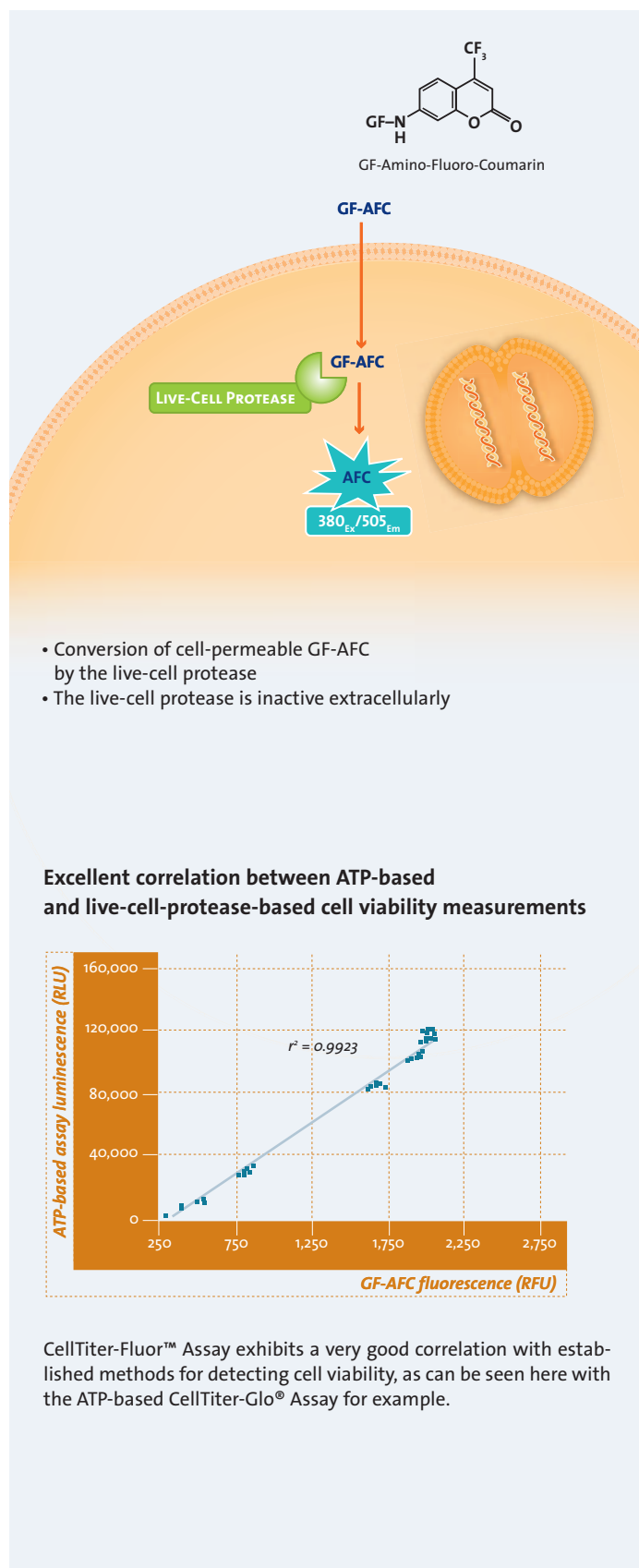
CellTiter-Fluor™ Assay is a fluorescent, cell-based assay for determining cell viability. The assay is particularly suitable for multiplexing, as the cells remain intact. The assay is frequently used for normalizing data, i.e. to compensate for differences between individual wells and plates. The **CellTiter-Fluor™ Assay** is also very well suited to automation. Because the marker detected is not affected when reducing capacity changes, the assay provides an orthogonal method to assess cell viability, often in the same well as other assays.

Assay principle

The assay is based on the measurement of a conserved and constitutive protease activity, known as live-cell protease, which is active only in living cells. Such activity is measured using the pro-fluorogenic, cell-permeable peptide substrate glycyphenylalanyl-aminofluorocoumarin (GF-AFC), which is converted intracellularly into the fluorescent product AFC. The fluorescence signal generated is proportional to cell viability and correlates with other cell-viability measurements, such as measurements of ATP or of reducing equivalents.

Assay features

Assay type	Fluorescent (380–400 _{Ex} /505 _{Em})
Markers	Live-cell protease
Applications	Cell viability, cytotoxicity, multiplexing with other cell-based assays
Cell type	Cell lines (adherent or in suspension), primary cells
Implementation	Homogeneous, one-step assay
Time required	0.5–3 hours
Sensitivity	40 living cells (96-well format)
Robustness	96- to 1536-well formats



BacTiter-Glo™ Microbial Cell Viability Assay

Cell-based

Applications

Viability of Gram-positive, Gram-negative bacteria and yeasts; simple determination of growth curves; activity determination and screening of antimicrobial substances.

Assay description

BacTiter-Glo™ Microbial Cell Viability Assay is a luminescent assay for determining the viability of bacteria in culture by measuring ATP levels. This unique one-step assay is suitable for high-throughput screening. It is characterized by exceptionally high sensitivity and a wide linear measuring range. The assay is compatible with commonly used media and solvents.

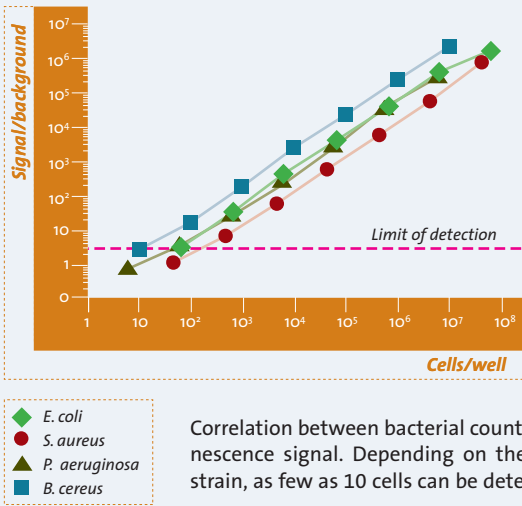
Assay principle

The assay is based on measurement of ATP levels and thus correlates with the number of metabolically active cells. The formulation of the assay reagent leads to lysis of the bacteria. ATP is released as a result, and the quantity is determined via an ATP-dependent luciferase reaction. Signal readings can be obtained after just a 5 minute incubation with the assay reagent.

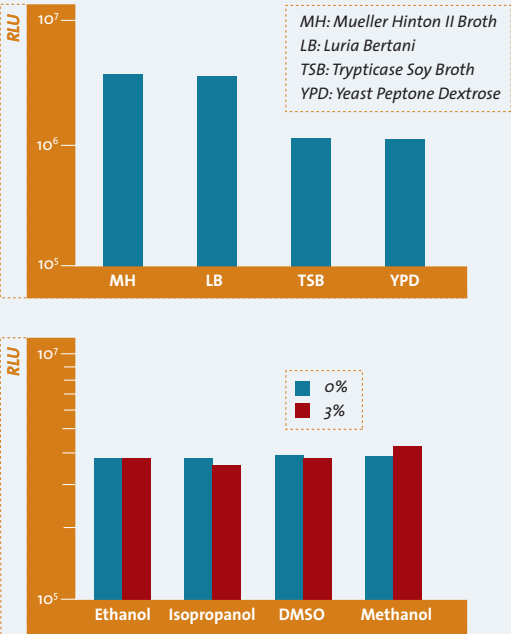
Assay features

Assay type	Luminescent (glow-type; $T_{1/2} > 0.5$ h)
Markers	ATP
Applications	Measurement of the viability of bacteria and yeast
Bacteria/Yeasts	Gram-positive bacteria, Gram-negative bacteria, yeast
Implementation	Homogeneous, one-step assay
Time required	5 minutes
Sensitivity	10 bacteria (1,000 times more sensitive than optical density measurement)
Robustness	High Z' factor, 96- to 1536-well formats

Excellent sensitivity and linearity



The BacTiter-Glo™-Assay is compatible with commonly used media and solvents



The compatibility of the BacTiter-Glo™ Assay was tested in different media and in relation to various solvent additives, using $\sim 1 \times 10^{-12}$ moles of ATP.

Iib Cytotoxicity

The term “cytotoxicity” stands for the potential to damage cells and initiate cell death. It is applied, for instance, to both chemical and biological compounds as well as to immune cells (e.g. cytotoxic T cells). Cytotoxic activity leads to a reduction in cell viability and initiates cell death through necrosis and/or apoptosis. Cell viability assays are frequently used in order to detect the cytotoxic potential of a substance, for example. If, however, you want to differentiate between necrotic and apoptotic processes, further assays that are based on the detection of other markers are required.

Necrosis is measured by means of cell membrane integrity tests. Typical characteristics of necrosis include the rapid loss of cell membrane integrity and the release of cytoplasmic content. In the case of apoptosis, by contrast, membrane integrity is retained and these cells are cleared *in vivo* by phagocytes. When interpreting data sets, one should keep in mind that phagocytes are absent in the cell culture, and that apoptotic cells also lose their membrane integrity. The loss of membrane integrity in apoptotic cells is termed secondary necrosis and

takes place at a later point in time compared to primary necrosis, which proceeds rapidly. The choice of an optimum treatment period is therefore crucial and should be empirically determined by performing a time curve. Depending on the compound and its concentration, various phenomena can be observed, i.e. cell death will be induced via necrotic and apoptotic pathways.

The cytotoxicity assays described below are based on the detection of cytosolic enzymes (lactate dehydrogenase (LDH); dead-cell protease) which have been released into the culture medium following membrane damage, a DNA staining method for real-time monitoring of cytotoxicity and an ATP-based method.

LDH-Glo™ Cytotoxicity Assay

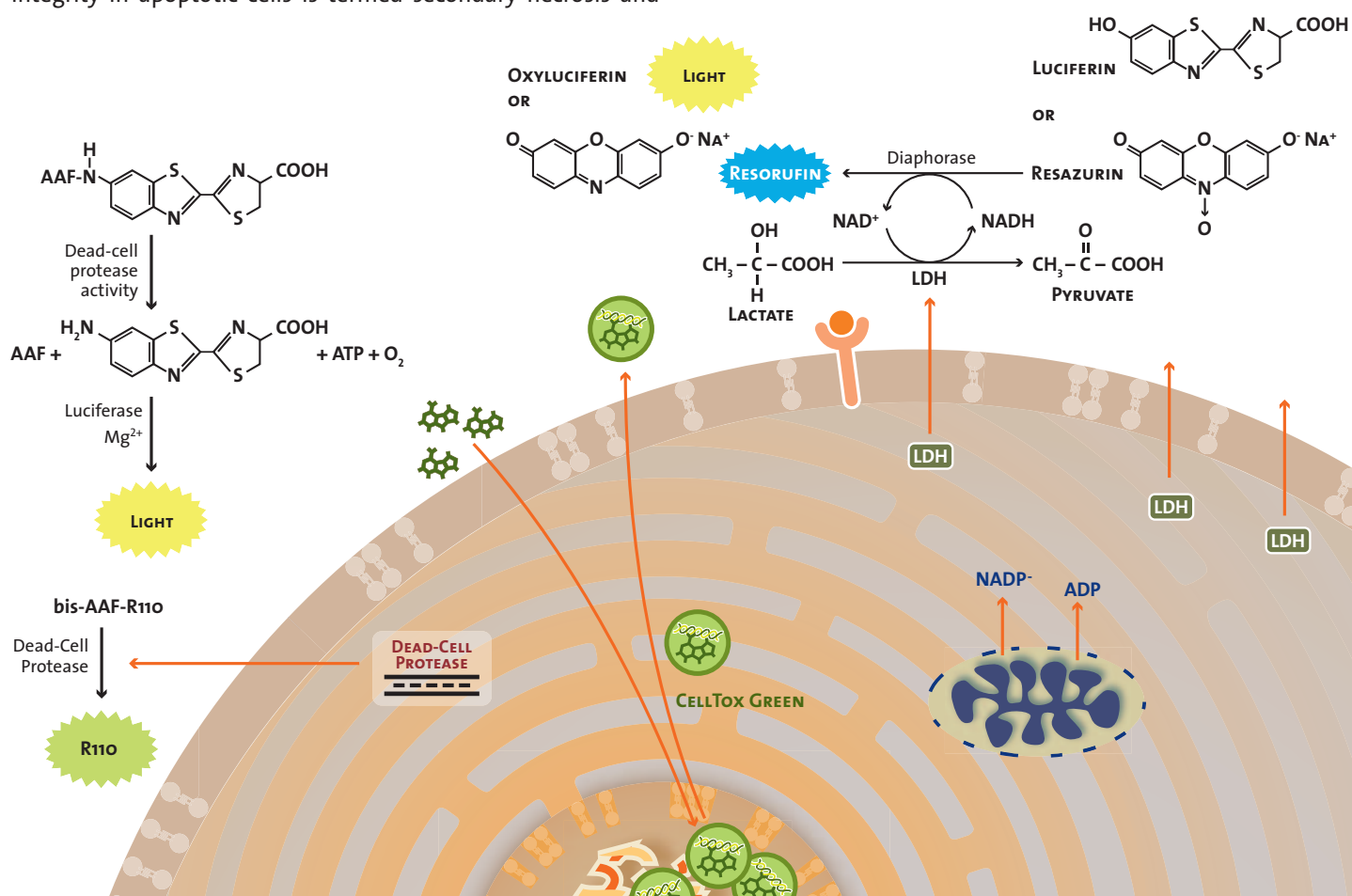
CellTox™ Green Cytotoxicity Assay

CytoTox-Glo™ Cytotoxicity Assay

CytoTox-Fluor™ Cytotoxicity Assay

CytoTox-ONE™ Homogeneous Membrane Integrity Assay (LDH)

ViralTox-Glo™ Assay



LDH-Glo™ Cytotoxicity Assay

Cell-based

Sample over time
Suitable for
3D-microtissues

Applications

Monitoring cytotoxicity from small numbers of cells and from the same sample over time.

Assay description

The **LDH-Glo™ Cytotoxicity Assay** is a bioluminescent plate-based assay for quantifying lactate dehydrogenase (LDH) release into the culture medium upon plasma membrane damage. The bright luminescent signal provides the sensitivity to determine cytotoxicity in samples low in cell number such as 3D micro-tissue spheroids, microfluidic cell culture chips, primary cells and stem cells. This LDH assay involves removing only a small amount of cell media (2–5 µl) from each treated well, allowing to get more data by sampling the same well over time, and by using the remaining media and cells for other cell-based assays.

Assay principle

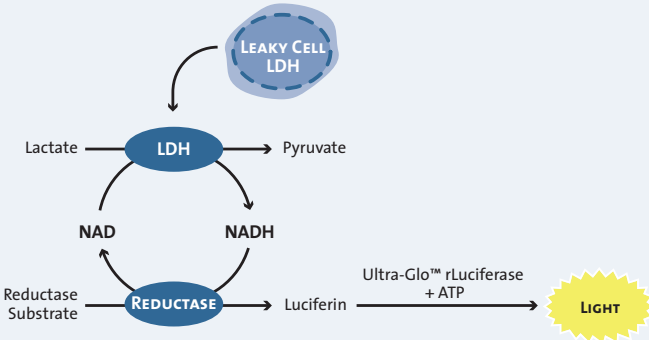
Samples are collected by removing 2–5 µl culture medium at the desired experimental time points and directly diluted in LDH Storage Buffer. After collecting and diluting all samples, LDH can be measured immediately, or samples can be stored at or below –20°C up to four weeks.

The LDH Detection Reagent (containing Lactate, NAD⁺, Reductase, Reductase Substrate and Ultra-Glo™ rLuciferase) is added to a sample of diluted cell culture media. LDH catalyzes the oxidation of lactate with concomitant reduction of NAD⁺ to NADH. Reductase uses NADH and Reductase Substrate to generate luciferin, which is converted to a bioluminescent signal by Ultra-Glo™ rLuciferase. The light signal generated is proportional to the amount of LDH present.

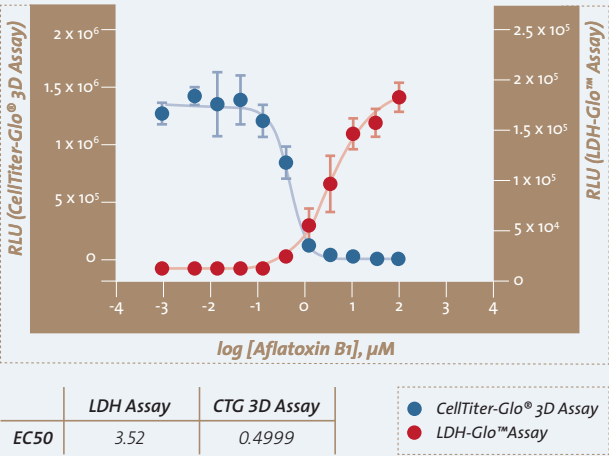
Assay features

Assay type	Luminescent
Markers	LDH
Applications	Cytotoxicity; multiplexing
Cell type	Cell lines, 3D-microtissues, primary cells, stem cells
Implementation	Non-lytic, one-step assay; take samples from the same well over time
Time required	30–60 minutes
Sensitivity	< 10 dead cells
Robustness	Scalable to 384- and 1536-well plates

LDH-Glo™ Cytotoxicity Assay principle



Multiplexing with CellTiter-Glo® 3D Cell Viability Assay



Human liver microtissues were treated with Aflatoxin B1 for 48 hours. Samples (10 µl) were collected in PBS at a 1:2.5 dilution, then further diluted 10-fold. Change in toxicity during treatment was determined using the LDH-Glo™ Cytotoxicity Assay by combining 15 µl diluted samples with 15 µl LDH Detection Reagent and recording luminescence after a 60-minute, room-temperature incubation. After samples were removed for LDH determination, an equal volume of CellTiter-Glo® 3D Reagent was added to the remaining microtissue sample to assess viability.

CellTox™ Green Cytotoxicity Assay

Cell-based

Applications

Real-time cytotoxicity measurements; membrane damage; multiplexing with other assays.

Assay description

The **CellTox™ Green Cytotoxicity Assay** measures changes in membrane integrity that occur as a result of cell death. The assay system uses a proprietary asymmetric cyanine dye that is excluded from viable cells but preferentially stains the DNA from dead cells. The CellTox™ Green Dye is non-toxic to cells, and the signal remains constant after exposure of 72 hours, making it ideal for determining toxic effects of treatments throughout an extended exposure or as an endpoint determination. CellTox™ Green Assay can be multiplexed with other spectrally distinct measures of cell health to provide mechanistic information relating to cytotoxicity.

Assay principle

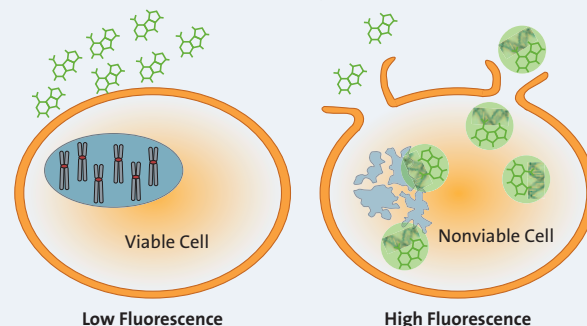
When the dye binds DNA, released from membrane-compromised cells, its fluorescence properties are substantially enhanced. Viable cells produce no appreciable increases in fluorescence. Therefore, the fluorescence signal produced by the binding interaction with dead cell DNA is proportional to cytotoxicity. The CellTox™ Green Dye is well tolerated by a wide variety of cell types and is essentially nontoxic. The dye can be diluted in culture medium and delivered directly to cells at seeding or at dosing, allowing “no-step” real-time measures of cytotoxicity. The dye also can be diluted in assay buffer and delivered to cells as a conventional endpoint measure after an exposure. The dye is photostable and can be used for imaging applications.

Assay features

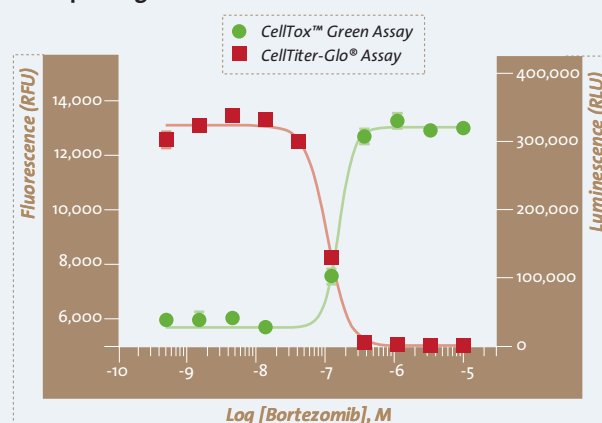
Assay type	Stable fluorescence signal over 72 h (485–500 _{Ex} /520–530 _{Em})
Markers	DNA
Applications	Real-time cytotoxicity measurements; multiplexing with other assays
Cell type	Cells, 3D cultures
Implementation	Add assay reagent directly to cells or with dosing media
Time required	15 minutes incubation
Robustness	96- to 1536-well formats



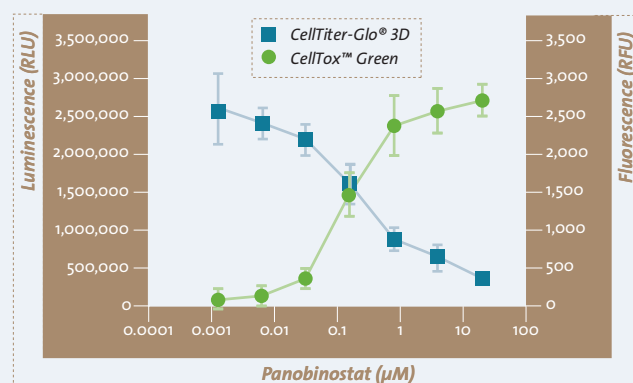
Assay Principle: CellTox™ Green binds DNA of cells with impaired membrane integrity



Multiplex for more informative data per well Multiplexing with CellTiter-Glo®



Multiplexing with CellTiter-Glo® 3D



HCT116 cells were cultured in InSphero GravityPLUS™ 3D Cell Culture system for 4 days to form ~350 μm microtissues. Samples were treated with CellTox™ Green and panobinostat for 48 hr. After recording fluorescence, an equal volume of CellTiter-Glo® 3D was added, the plate was shaken for 5', and the luminescence was recorded after 30 min incubation.

CytoTox-Glo™ Cytotoxicity Assay

Cell-based

Applications

Cytotoxicity; membrane damage; multiplexing with other assays.

Assay description

CytoTox-Glo™ Assay is a luminescent cell-based assay for determining cytotoxicity. The assay is particularly suitable for multiplexing, as the cells remain intact, and is a component of the MultiTox-Glo Multiplex Cytotoxicity Assay. It is frequently used for normalizing data, i.e. to compensate for differences between individual wells and plates. The CytoTox-Glo™ Assay correlates very well with other cytotoxicity measurements such as LDH detection and DNA staining.

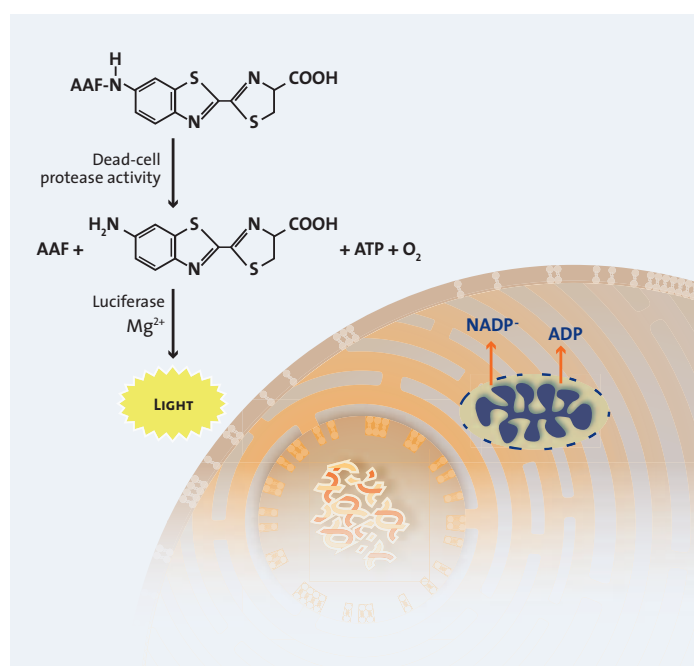
Assay principle

The principle of the **CytoTox-Glo™ Assay** is based on measurement of the activity of dead-cell protease, which is released into the medium following cell membrane damage. Using a luminogenic peptide substrate Ala-Ala-Phe-aminoluciferin (AAF-aminoluciferin), which cannot pass through the cell membrane, dead-cell protease activity is measured indirectly via a downstream luciferase reaction. The reagent is added directly to the cells. A readout for the assay can be obtained after just 15 minutes. The luminescent signal is a measure of the number of damaged cells. If in a further step a lytic reagent is then added, a value can also be determined for the total cell count (total lysis). This value is suitable for normalization.

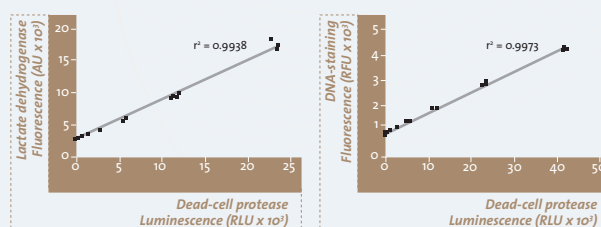
Assay features

Assay type	Luminescent (glow-type)
Markers	Dead-cell protease
Applications	Cytotoxicity, membrane integrity, Complement dependent cytotoxicity (CDC), multiplexing with other cell-based assays
Cell type	Cell lines, primary cells
Implementation	Homogeneous, one-step assay
Time required	15 minutes
Sensitivity	10 dead cells (96-well format)
Robustness	96- to 1536-well formats

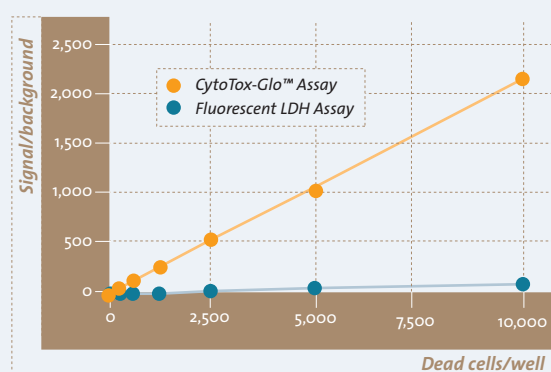
Most sensitive
homogeneous
Cytotoxicity Assay



The CytoTox-Glo™ Assay correlates very well with established methods for determining membrane integrity



Comparison between LDH Assay and CytoTox-Glo™ Assay



Increased sensitivity and larger measurement range with CytoTox-Glo™

CytoTox-Fluor™ Cytotoxicity Assay

Cell-based

Applications

Cytotoxicity; membrane damage; multiplexing with other assays.

Assay description

CytoTox-Fluor™ Assay is a fluorescent assay for determining cytotoxicity. It measures the proportion of dead cells in a cell culture sample. This assay is frequently used for multiplexing and is a component of the following multiplexing assays: MultiTox-FluorAssay, ApoTox-Glo™-Triplex Assay, Mitochondrial ToxGlo™ assay.

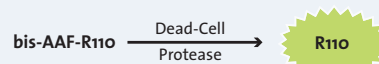
Assay principle

The assay is based on the detection of dead-cell protease, which is released following membrane damage. The fluorogenic cell-impermeable dye bis-Ala-Ala-Phe-rhodamine-110 (bis-AAF-R110) is specifically recognized by the dead-cell protease and converted to fluorescent rhodamine-110. The measured fluorescent signal is a measure of the number of damaged cells in culture. A readout of the fluorescent signal can be obtained 0.5–3 hours after the assay reagent has been added.

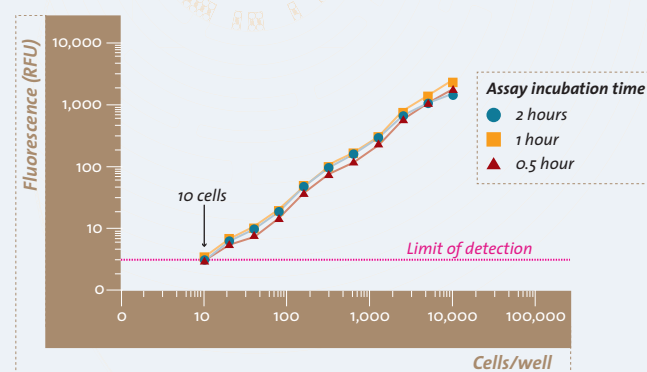
Assay features

Assay type	Fluorescent (485 _{Ex} /520 _{Em})
Markers	Dead-cell protease
Applications	Cytotoxicity, multiplexing with other cell-based assays
Cell type	Cell lines, primary cells
Implementation	Homogeneous, one-step assay
Time required	0.5–3 hours
Sensitivity	10 dead cells (96-well format)
Robustness	96- to 1536-well plates

Excellent Multiplexing partner
with
Luminescent Assays

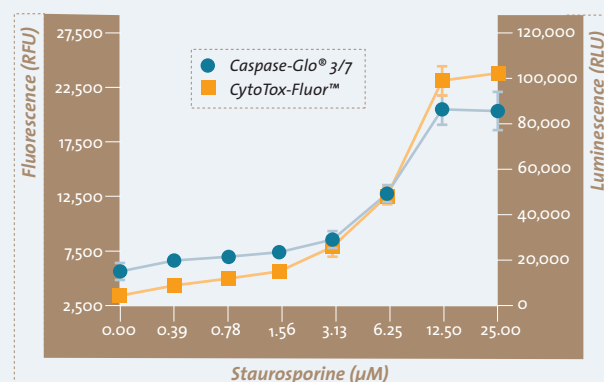


Excellent sensitivity



Sonicated Jurkat cells were seeded in a 96-well plate and dead-cell protease activity determined using the CytoTox-Fluor™ Assay. The assay is linear up to 100,000 cells. The detection limit lies at just 10 cells.

Multiplexing of the CytoTox-Fluor™ Assay and the Caspase-Glo® 3/7 Assay



Besides damaging the cell membrane, staurosporine induces activation of caspase-3/7 and thus activation of apoptotic processes.

CytoTox-ONE™ Homogeneous Membrane Integrity Assay (LDH)

Cell-based

Applications

Cytotoxicity; membrane damage; multiplexing with other assays.

Assay description

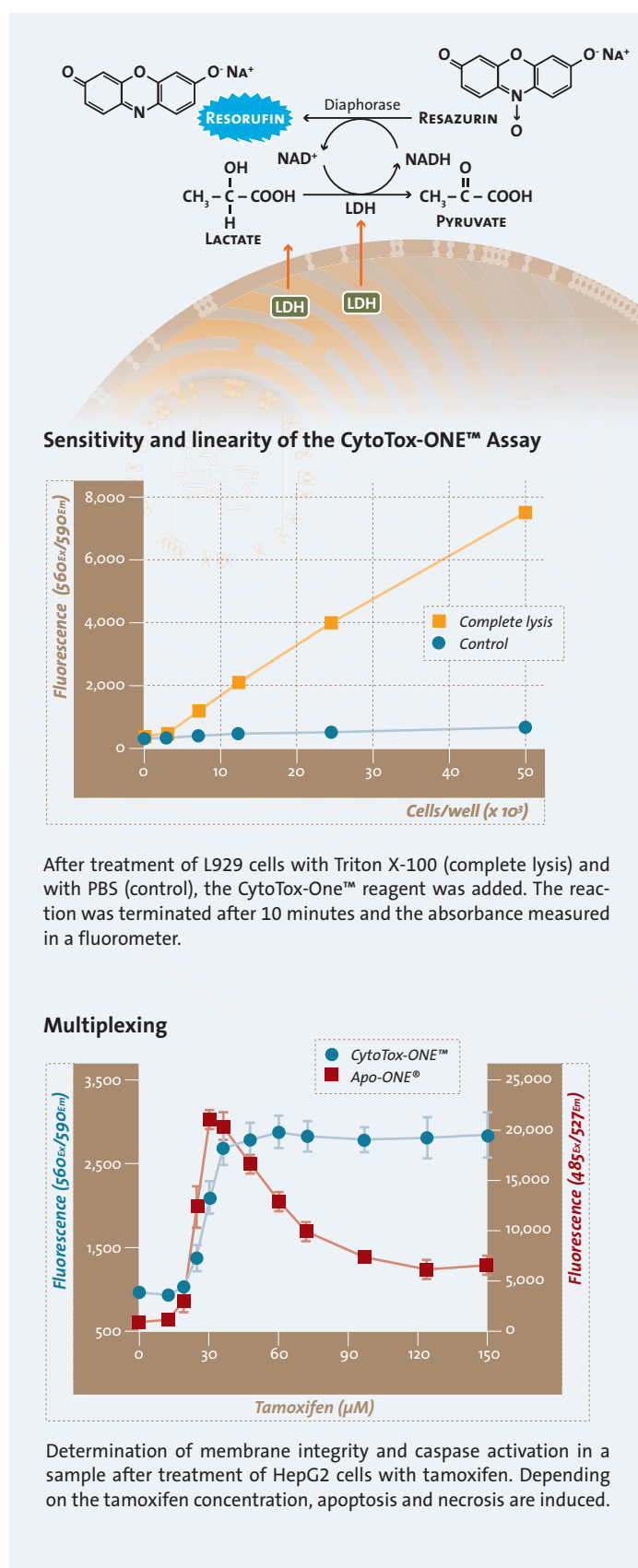
CytoTox-ONE™ assay can be used to measure both the cytotoxic effect of substances on cellular systems and cell-mediated cytotoxicity (e.g. immune response). Where cells are damaged by these processes, the cell membrane loses its integrity and cytosolic proteins, for example, such as lactate dehydrogenase (LDH), pass into the extracellular space. This assay can be combined with other assays, such as CellTiter-Glo®-Assay or Apo-ONE® Caspase-3/7-Assay, in the same experimental setup.

Assay principle

LDH activity in a coupled enzymatic reaction is measured as a gauge of cell damage. The assay reagent contains lactate, NAD^+ , resazurin and the enzyme diaphorase. In the first step, lactate is oxidized to pyruvate by the LDH. Here, the reducing equivalents are initially transferred to NAD^+ and then in the second step, with the aid of the diaphorase, to the fluorogenic dye resazurin. The fluorescent signal of the resorufin that is produced is proportional to the quantity of LDH released and thus to the number of damaged cells.

Assay features

Assay type	Fluorescent ($560_{\text{Ex}}/590_{\text{Em}}$)
Markers	LDH
Applications	Cytotoxicity; multiplexing
Cell type	Cell lines, primary cells
Implementation	Homogeneous, two-step assay
Time required	10 minutes
Sensitivity	200 dead cells (96-well format)
Robustness	96- to 384-well formats



Viral ToxGlo™ Assay

Cell-based

Easily monitor
cytopathic effects

Applications

Monitoring viral-induced cytopathic effect (CPE) and the corresponding tissue culture infective dose (TCID₅₀); determination of potential antiviral potency or off-target toxicity of test compounds.

Assay description

The **Viral ToxGlo™ Assay** is a simple, quantifiable method of determining viral-induced cytopathic effects (CPE) in host cells caused by lytic virions. The assay measures cellular ATP as a surrogate measure of host cell viability. When CPE occurs due to viral infection, ATP depletion can be measured and correlated with viral burden. The amount of ATP detected is directly proportional to the number of viable host cells in culture and can be used as a simple method to quantify viral-induced CPE. The system detects as few as 15 cells/well in a 384-well format in 10 minutes after reagent addition and mixing and is designed for use in multiwell formats, making it ideal for automated high-throughput screening.

Assay principle

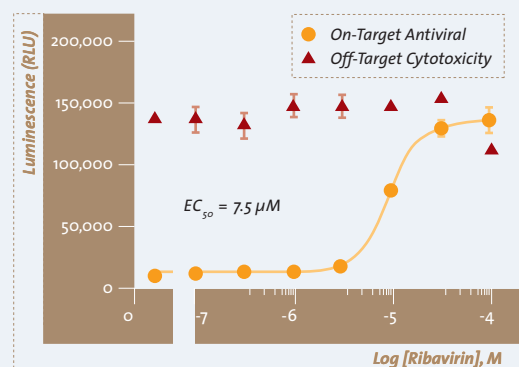
The homogeneous “add-mix-read” assay consists of a single reagent that is added directly to host cells following viral treatment. A “glow-type” luminescent signal is generated that is proportional to the amount of ATP present and to number of cells. Cell washing, multiple pipetting steps and visual assessment are not required to detect CPE. Luminescent signal is very stable with a half-life generally > 5 hours dependent on cell type and medium used. No fluorescence interference results in high signal to background and delivers excellent Z' values in screening applications.

Assay features

Assay type	Luminescent (glow-type; $T_{1/2}$ > 5 h)
Markers	ATP
Applications	Monitoring CPE and the corresponding TCID ₅₀ ; determination of potential antiviral potency
Virus	lytic virus, that induce cytopathic effects
Implementation	Homogeneous, one-step assay
Time required	10 minutes
Sensitivity	15 living cells (384-well format)
Robustness	High Z' factor; scalable from 96- to 1536-well formats

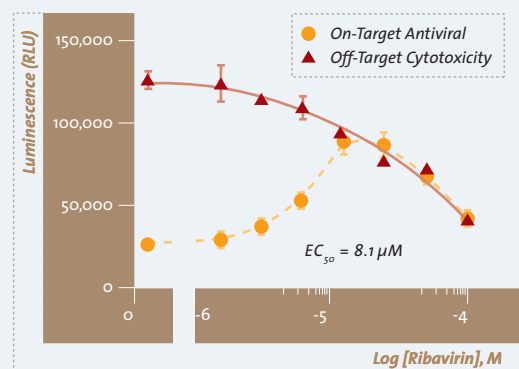
Calculation of antiviral potency

A.



Enhanced Luminescence implicates antiviral potency of test compound

B.



A. Half-log (3.16-fold) dilutions of Ribavirin were added to either MDCK cells with 100 TCID₅₀ of H1N1 (on-target) or MDCK cells only (off-target) for 72 hours.

B. Twofold serial dilutions of Ribavirin were added to replicate wells of a 96-well plate containing BHK-21 cell monolayers. Either 100 TCID₅₀ of Dengue virus (Serotype 2) or medium alone were immediately added to two series of replicates to determine antiviral efficacy (on-target) and cytotoxicity (off-target), respectively, for 96 hours. For both panels, after incubation, ATP Detection Reagent was added and luminescence measured.

Experimental data was provided by Southern Research Institute, Birmingham, AL, and is used with permission.

IIc Apoptosis

Morphologically, apoptosis is first characterized by a change in the refractive index of the cell followed by cytoplasmic shrinkage and nuclear condensation. The cell membrane begins to show blebs or spikes, and eventually separates from the dying cell to form so-called "apoptotic bodies". Apoptotic cells also cease to maintain phospholipid asymmetry between the leaflets of the cell membrane, and phosphatidylserine (PS) appears on the outer leaflet. Flipped-out PS serves as a recruitment signal for macrophages or adjacent cells to remove apoptotic bodies and the dying cell by phagocytosis. As membrane integrity is maintained throughout the whole process, a key characteristic of apoptosis is that it does not provoke an inflammatory response and only individual cells are affected by apoptosis *in vivo*. In contrast, necrosis goes along with a loss of cell membrane integrity, release of cellular constituents, and conse-

quently inflammation. The caspase family of cysteine proteases are the central mediators of the proteolytic cascade leading to cell death and elimination of compromised cells. As such, the caspases are tightly regulated both transcriptionally and by endogenous anti-apoptotic polypeptides, which block their catalytic activity. Assays that directly measure caspase activity can provide valuable information for researchers about the underlying mechanism of cell death. Phosphatidylserine (PS) and Caspase activity are the common markers of apoptosis.

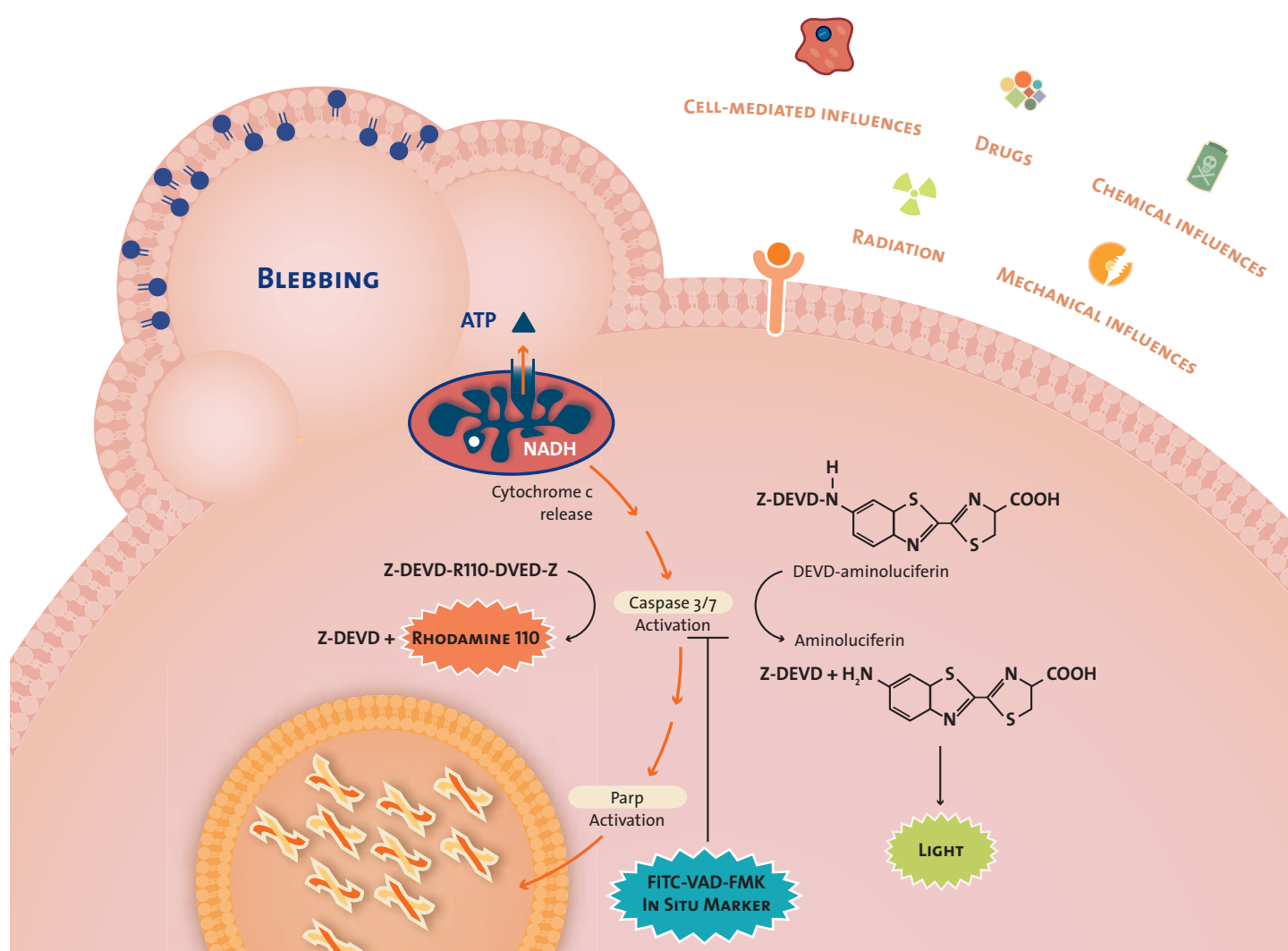
RealTime-Glo™ Annexin V Apoptosis and Necrosis Assay

Overview of caspase assays

Caspase-Glo® 3/7 Assay

Apo-One® Homogeneous Caspase-3/7 Assay

CaspACE™ FITC-VAD-FMK *In Situ* Marker



RealTime-Glo™ Annexin V Apoptosis and Necrosis Assay

Cell-based

Applications

Monitoring of apoptosis progression in real-time up to 48 hrs.

Assay description

The **RealTime-Glo™ Annexin V Apoptosis and Necrosis Assay** is a live-cell (non-lytic) assay that measures the real-time exposure of phosphatidylserine (PS) on the outer leaflet of cell membranes during the progression of apoptosis. Annexin V-NanoBiT® luciferase fusion proteins supplied in the assay reagent bind to flipped-out PS during early apoptosis leading to the emission of a luminescence signal. The assay also includes a cell-imper-

meant, profluorescent DNA dye, which detects necrosis. This simple “add-read-assay” allows to acquire endpoint data or to perform real-time kinetics of up to 48 hours without the need for multiple plates or complicated processing.

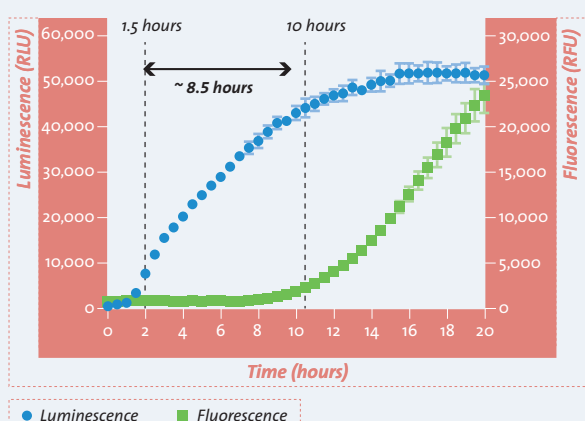
Assay principle

The Detection Reagent used in the assay contains near-equimolar ratios of two Annexin V fusion proteins (Annexin V-LgBiT and Annexin V-SmBiT) containing complementary subunits of NanoBiT® Luciferase. The LgBiT and SmBiT luciferase subunits have only modest affinity for each other. Luminescence remains low until PS exposure brings the subunits into complementing proximity to form a functional luciferase. The reagent also contains a time-released luciferase substrate that provides a constant source of substrate over experimental exposure periods up to 48 hrs. The Annexin V Apoptosis and Necrosis Assay also includes a cell-impermeable dye that yields a fluorescent signal upon binding to DNA when membrane integrity is lost during necrosis. The combination and timing of luminescent and fluorescent signals is used to differentiate secondary necrosis occurring during late apoptosis from necrosis caused by other cytotoxic events.

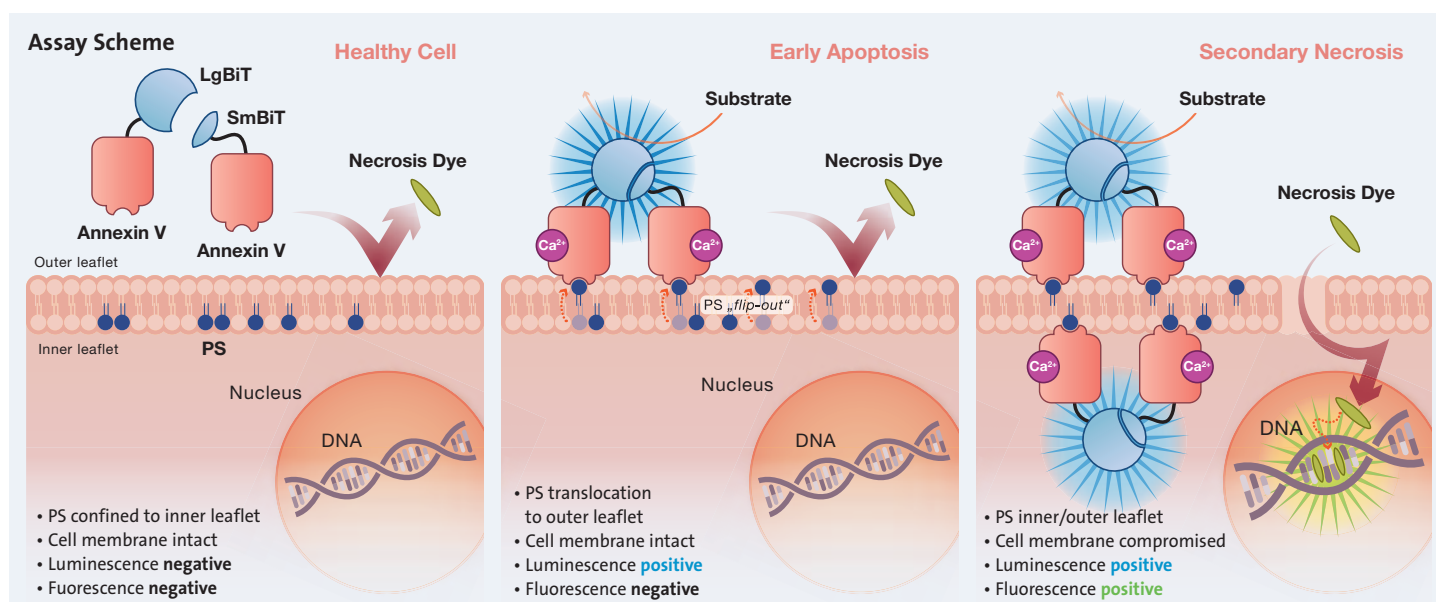
Assay features

Assay type	Luminescent (Apoptosis), Fluorescent (Necrosis), (485– 500 _{Ex} /520– 530 _{Em})
Markers	PS, Membran integrity
Cell type/Sample	Cell lines, primary cells, 3D cultures
Implementation	Non-lytic, one-step assay, Same-well multiplex compatibility
Robustness	96- to 384-well formats

Real-time kinetics of apoptosis (RLU) and necrosis (RFU)



DLD-1 Cells: 400 ng/mL TRAIL Extrinsic Inducer of Apoptosis A significant time delay between the emergence of PS:Annexin V binding and the loss of membrane integrity of 8.5 hrs can be detected indicating an apoptotic phenotype followed by secondary necrosis.



Overview of caspase assays

Caspases play a key role in apoptosis. They are activated via a variety of signaling pathways, some of which are currently still under research. It is generally accepted that activation of the initiator caspase, caspase-8, is induced via the superfamily of death receptors. Caspase-8 subsequently activates the effector caspases -3, -6 and -7, and these in turn cleave numerous cellular protein substrates, which ultimately cause the death of the cell.

The “intrinsic” apoptosis pathway is triggered e.g. by ultraviolet light, viral infection or damage to the cell membrane. As a consequence, cytochrome c is released into the cytosol. These events activate the initiator caspase, caspase-9, and this in turn in the next step activates the effector caspases -3, -6 and -7.

	Caspase	Biological relevance	Assay name	Assay substrate	Assay optimization through the addition of inhibitors
Initiator caspases	Caspase-8	Receptor-mediated apoptosis (activated e.g. by Fas-Ligand, TNF- α).	Caspase-Glo® 8 Assay	Z-LETD-aminoluciferin	• Proteasome inhibitor (MG-132)
	Caspase-9	The mitochondria are damaged by oxidative stress, viral infection, etc. and cytochrome c is released. Caspase-9 is activated by binding to cytochrome c.	Caspase-Glo® 9 Assay	Z-LEHD-aminoluciferin	• Proteasome inhibitor (MG-132)
Effector caspases	Caspase-3/7	Primary effector caspases in the apoptosis pathway. They can be activated by caspase-8 or caspase-9. This triggers apoptosis through proteolysis of anti-apoptotic proteins (ICAD, Bcl-2 proteins, PARP, etc.).	Caspase-Glo® 3/7 Assay Apo-One® Homogeneous Caspase-3/7 Assay	Z-DEVD-aminoluciferin Z-DEVD-R110-DVED-Z	

Note:

The Caspase-Glo® 3/7, 8, 9 Assays can be used directly in cell culture or with purified enzyme preparations.

The functioning of some assays can be optimized by adding inhibitors.

You will find more detailed information on this in the relevant technical guides at www.promega.com.

Caspase-Glo® 3/7 Assay

Cell-based/Biochemical

Suitable for
3D-microtissues
High Sensitivity
for HTS Screening

Applications

Apoptosis; multiplexing with other cell-based assays; inhibitor screening.

Assay description

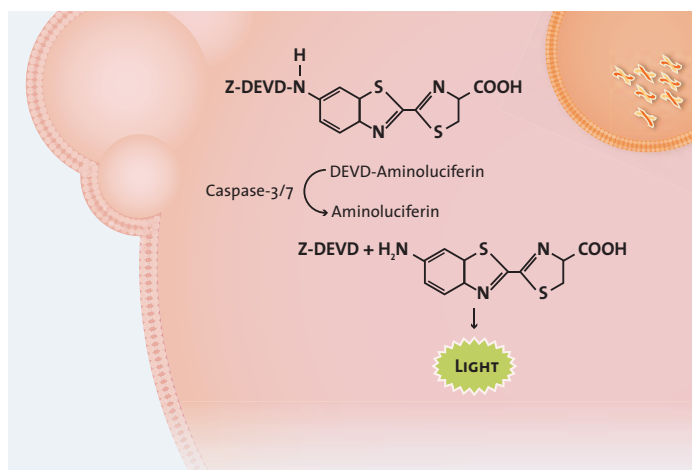
Caspase-Glo® 3/7 Assay is an extremely sensitive test system for determining apoptosis in cell culture or in enzyme preparations. The activity of the effector caspases, caspases -3 and -7, is measured through cleavage of a proluminescent substrate. This contains the tetrapeptide DEVD as a recognition sequence. Caspase-3 and caspase-7 activity are detected as a result, with an excellent signal-to-background ratio. Caspase-Glo® 3/7 Assay is quick and easy to perform. It can be flexibly configured for cell cultures or enzyme preparations in the desired format (cuvette, 6- to 1536-well plates).

Assay principle

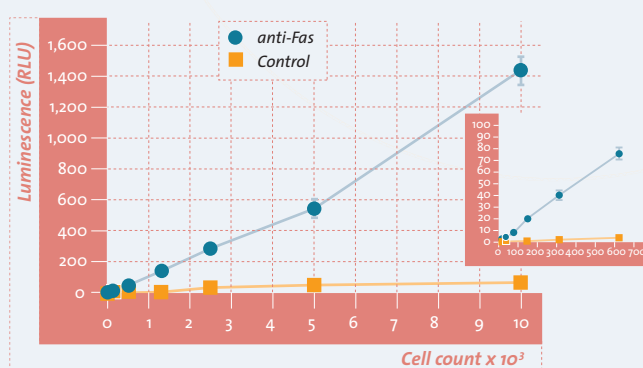
The assay is based on measurement of caspase-3/7 activity via the tetrapeptide DEVD. In this detection process, two enzymatic steps occur in immediate succession: the Caspase-Glo® 3/7 reagent contains a substrate for caspase-3 and -7 (Z-DEVD-aminoluciferin), which is cleaved and releases aminoluciferin. The aminoluciferin serves as a substrate for the thermostable and particularly robust Ultra-Glo™ Luciferase, which catalyses a luciferase reaction with an extended half-life (glow-type luciferase reaction).

Assay features

Assay type	Luminescent (glow-type)
Markers	Caspase-3 and -7
Applications	Apoptosis, multiplexing with other cell-based assays
Cell type/Sample	Cell lines, primary cells, 3D culture models, enzyme preparations
Implementation	Homogeneous, one-step assay
Time required	0.5–3 hours
Sensitivity	100 apoptotic cells (96-well format), excellent signal-to-background ratio
Robustness	High Z' factor, 96- to 1536-well formats

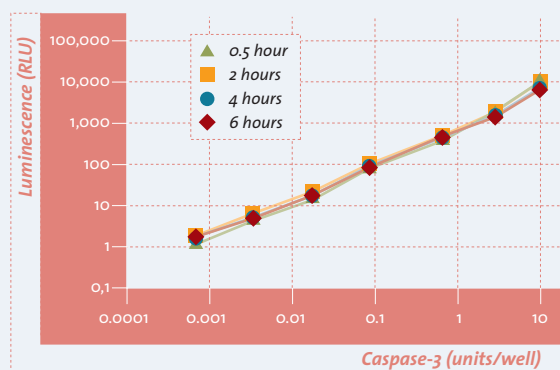


Wide linear measurement range from 20 to 10,000 cells



Jurkat cells were treated for 4.5 hours with anti-Fas mAb in order to induce apoptosis. After the Caspase-Glo® 3/7 Assay reagent was added to the 96-well plate, the signal reading was taken after an incubation time of 1 hour. The values for 0–625 cells are shown in the detail enlargement.

High stability of the luminescence signal



Recombinant caspase-3 was incubated in a 96-well format for 0.5–6 hours with Caspase-Glo® 3/7 Assay reagent. The luminescent signal is stable for several hours. The assay is linear over at least five orders of magnitude.

Apo-ONE® Homogeneous Caspase-3/7 Assay

Cell-based

Suitable for
3D-microtissues

Applications

Apoptosis; multiplexing with other cell-based assays; inhibitor screening.

Assay description

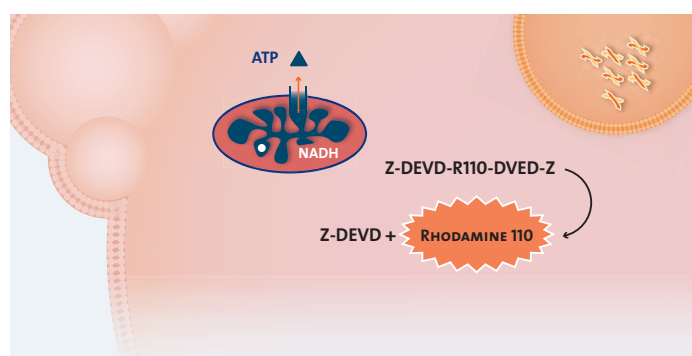
Apo-ONE® Homogeneous Caspase-3/7 Assay is a fluorescent cell-based assay for determining caspase-3 and caspase-7 activity. This assay is also suitable for multiplexing.

Assay principle

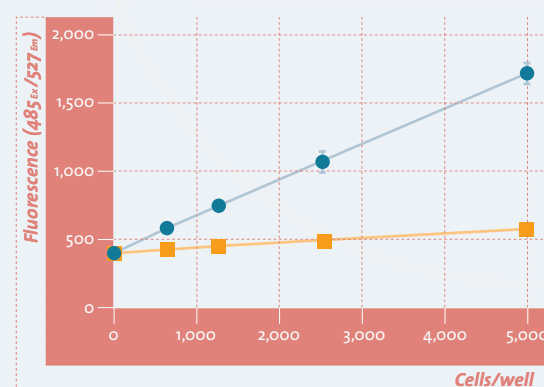
Apo-ONE® Homogeneous Caspase-3/7 Assay uses a fluorescent marker substrate (Z-DEVD-R110-DVED-Z) for detecting active caspase-3 and -7 in a wide variety of starting materials. Coupling rhodamine 110 to the caspase substrate allows a substantially higher degree of sensitivity to be achieved than in comparable fluorescent assays (approx. 600 apoptotic cells in a 96-well format). The fluorescence signal is proportional to the quantity of activated caspases.

Assay features

Assay type	Fluorescent (499 _{Ex} /521 _{Em})
Markers	Caspase-3 and -7
Applications	Apoptosis, multiplexing with other cell-based assays
Cell type/Sample	Cell lines, primary cells, enzyme preparations
Implementation	Homogeneous, one-step assay
Time required	1–18 hours
Sensitivity	~ 600 apoptotic cells (96-well format), excellent signal-to-background ratio
Robustness	High Z' factor, 96- to 384-well formats



Sensitivity

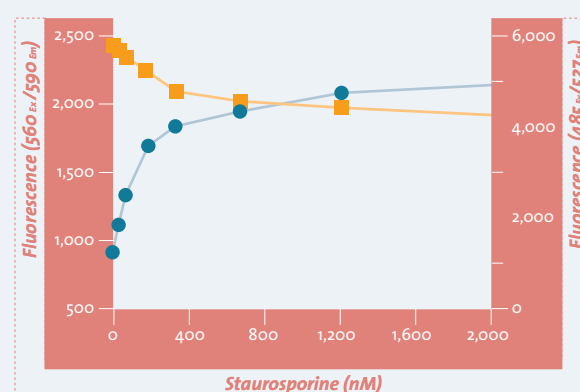


● anti-Fas
■ Control

Jurkat cells were treated for 4.5 hours with anti-Fas mAb in order to induce apoptosis. Following addition of the Apo-One®-Assay reagent to the 96-well plate, the signal reading was taken after an incubation time of 1 hour.

Multiplexing:

Determination of two end points in a sample



● Apo-ONE®
■ CellTiter-Blue®

Determination of cell viability and apoptosis in a sample. The cell viability of Jurkat cells treated with staurosporine was determined using CellTiter-Blue® reagent (5 hours) Caspase activity was subsequently determined using Apo-One® Assay reagent (1 hour)

CaspACE™ FITC-VAD-FMK *In Situ* Marker

Cell-based

Applications

In situ detection of apoptotic cells in a flow cytometer or under a fluorescence microscope.

Assay description

CaspACE™ FITC-VAD-FMK *In Situ* Marker is designed for fast and direct detection of caspase activity for flow and imaging based applications. It is a fluorescent analog of the pan-caspase inhibitor Z-VAD-FMK (carbobenzoxy-valyl-alanyl-aspartyl-[O-methyl]-fluoromethylketone). The carbobenzoxy group is replaced by the fluorescent dye fluorescein isothiocyanate (FITC). This marker is cell-permeable and is delivered direct to the cells.

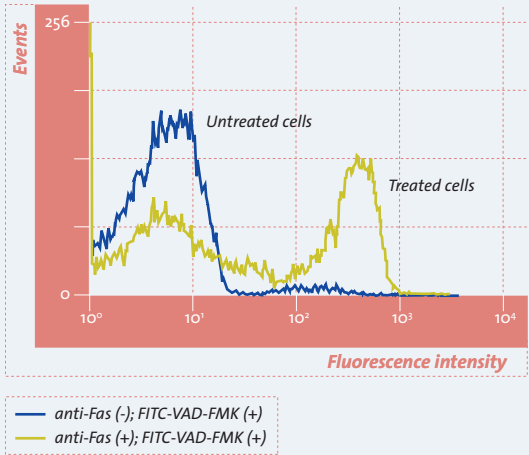
Assay principle

The cell-permeable FITC-VAD-FMK marker binds irreversibly in a stoichiometric ratio to all activated pan-caspases. The marker is supplied as a 5 mM stock solution in DMSO. The reagent is added to the cells directly and incubated for 20 minutes. The cells can then be analyzed in a flow cytometer. Where the analysis is to be carried out using a fluorescence microscope, however, subsequent fixing with formalin will be required.

Assay features

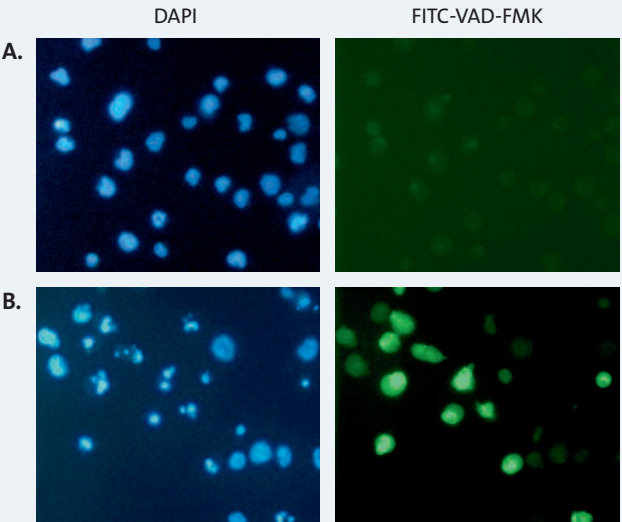
Assay type	Fluorescent
Markers	Pan-caspases
Applications	Apoptosis, can be combined with antibody labeling
Cell type	Cell lines, primary cells
Implementation	Add directly to the medium
Time required	0.5–1 hours
Sensitivity	4,000 apoptotic cells

Flow cytometric analysis of apoptotic Jurkat cells



Jurkat cells were treated with anti-Fas mAb for 4 hours in order to induce apoptosis and then stained with CaspACE™ FITC-VAD-FMK *In Situ* Marker (final concentration 10 μM). The fluorescence profiles were determined by flow cytometry.

Fluorescence microscopic analysis of apoptotic Jurkat cells



In situ marking with CaspACE™ FITC-VAD-FMK corresponds to the flow cytometric analysis shown above. Jurkat cells were analyzed under a fluorescence microscope. **A:** untreated cells and **B:** cells treated with anti-Fas. Nuclear staining with the DAPI stain confirms activation of apoptosis (condensed chromatin and fragmented nucleus) of the FITC-VAD-FMK-marked cells.

Ild Autophagy

Autophagy is a mechanism used by cells to respond to a shortage in nutrients or to eliminate damaged and harmful subcellular material. Its execution is strongly regulated via the activity of the protein kinase mTOR (mechanistic target of rapamycin) that inhibits autophagy by phosphorylation of autophagy-initiating components. The microtubule-associated 1A/1B-light chain 3 (LC3) protein is a widely-used marker to study autophagy in mammalian cell culture. Shortly after synthesis, proLC3 is constitutively converted to LC3-I by proteolytic removal of five, C-terminal amino acids. Following initiation of autophagy, cytosolic LC3-I is conjugated to phosphatidylethanolamine (PE) and targeted to the membranes of emerging autophagosomes (①). Subsequent formation of autolysosomes through fusion of autophagosomes with acidified lysosomes leads to hydrolytic degradation of engulfed subcellular components, including captured LC3-II (②). Thus, the total level of LC3 protein decreases

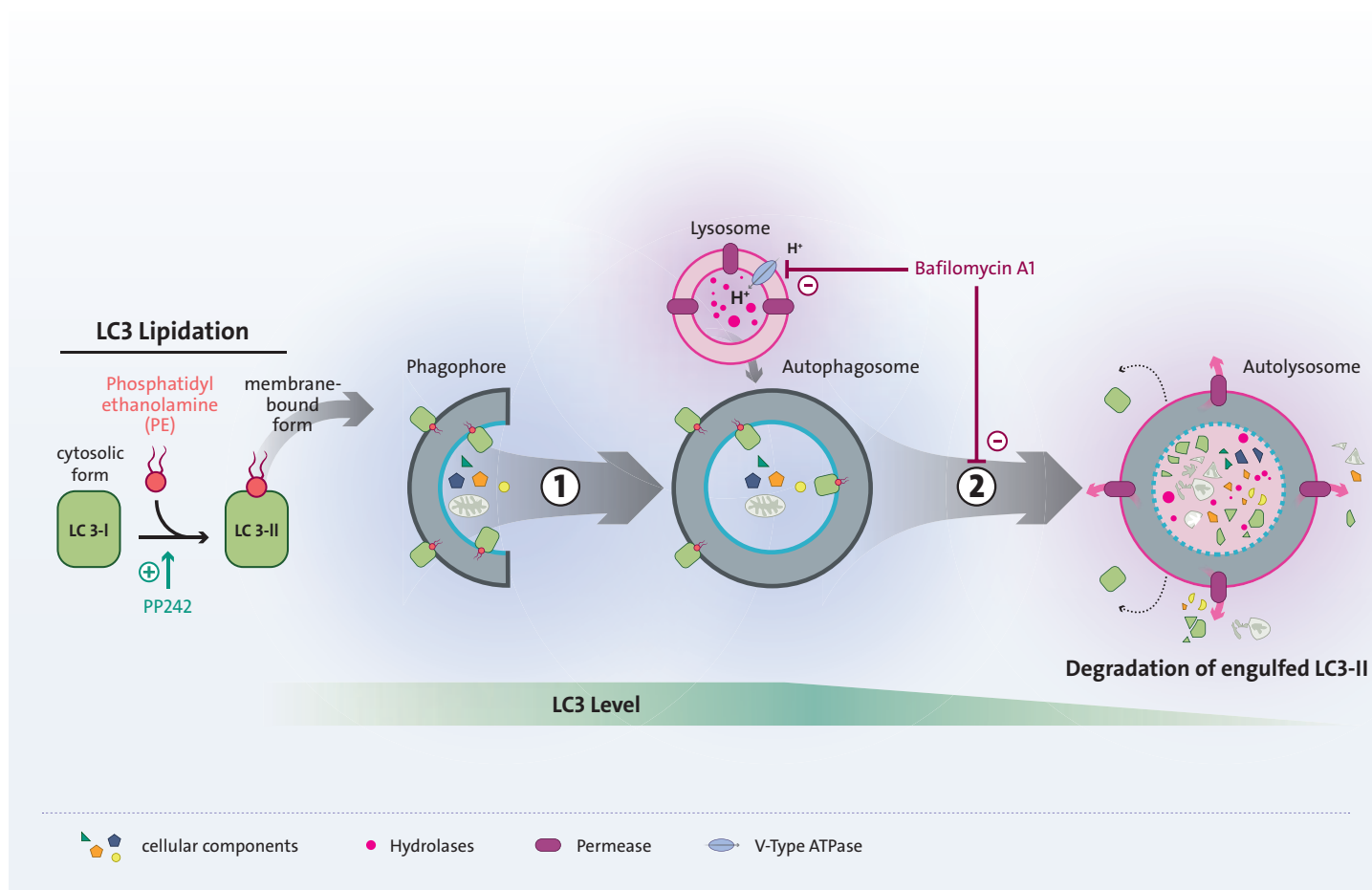
as a consequence of autophagic flux. Current methodologies to measure autophagic flux are labor-intensive and not always easy to interpret. Promega has developed a homogeneous plate reader-based assay to quantify autophagic flux based on the HiBiT technology. Data is acquired with a standard luminescence-compatible microplate reader. The assay is designed to measure changes in the total cellular level of LC3 protein that is subject to autophagic degradation period.

Autophagy LC3 HiBiT Reporter Assay Systems

U2OS Autophagy LC3 HiBiT Reporter Cell Line and Detection System

HEK293 Autophagy LC3 HiBiT Reporter Cell Line and Detection System

Autophagy LC3 HiBiT Reporter Vector and Detection System



Autophagy LC3 HiBiT Reporter Assay System

Cell-based

Multiplexing with
CellTox™ Green

Applications

Quantitative assessment of autophagic flux via detection of LC3-II degradation using the HiBiT technology.

Assay description

The **Autophagy LC3 HiBiT Reporter Assay System** provides a homogeneous, bioluminescent, plate reader-based method to assess autophagic flux. The method is based on the well-established role of the autophagy protein LC3 (Atg8) as an important marker for investigation of autophagic activity in cell culture models, and the effectiveness of the Nano-Glo® HiBiT Lytic Detection System to sensitively quantify HiBiT-tagged LC3 proteins in cell lysates using a simple add-mix-read assay protocol. The Autophagy System enables simple and reliable discrimination between stimulatory and inhibitory effects on autophagic flux.

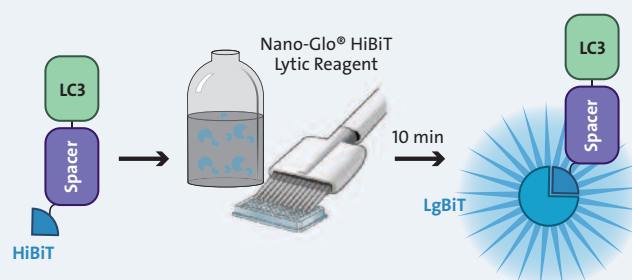
Assay principle

The Autophagy LC3 HiBiT Reporter has been engineered by tagging human LC3B with HiBiT. The amount of Autophagy LC3 HiBiT reporter subsequently expressed in cells is determined by adding a lytic detection reagent containing the luciferase substrate furimazine as well as Large BiT (LgBiT), the HiBiT-complementary subunit. Upon cell lysis, HiBiT spontaneously complements with LgBiT ($K_D = 0.7\text{nM}$) to generate a bright, luminescent enzyme. Thereby the amount of luminescence is proportional to the amount of Autophagy LC3 HiBiT reporter with a dynamic range of more than seven orders of magnitude. Hence, the accelerated degradation of the autophagy reporter due to an increase in autophagic flux negatively correlates with the assay signal. Cytotoxic events can be determined by multiplexing with the CellTox™ Green Cytotoxicity Assay in the same-well.

Assay features

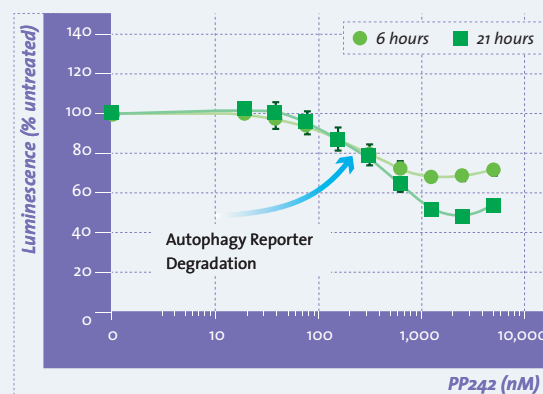
Assay type	Luminescent (Glow type, $T_{1/2} > 3\text{ h}$)
Markers	Human LC3
Applications	Quantitative assessment of autophagic flux in 2D and 3D cell culture models
Cell type	Available as propagatable cell lines (U2OS, HEK293) or Vector System
Implementation	Homogeneous, plate-based assay
Time required	10 min–3 hrs
Sensitivity	Strong signal-to-background values (>100)
Robustness	Z' factor: 0.67 in 384-well format

Add-mix-measure assay to detect autophagic flux

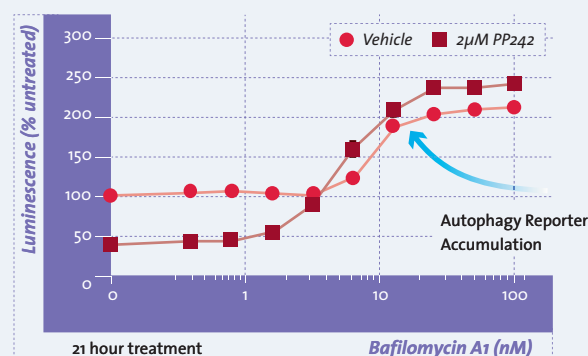


By expression of a HiBiT-tagged, LC3-based reporter (in the Autophagy LC3 HiBiT Reporter), changes in autophagic flux can be easily quantified in a simple add-mix-measure assay which detects changes in reporter abundance using the Large BiT (LgBiT)-containing Nano-Glo® HiBiT Lytic Detection System. LC3-based autophagy reporter contains an N-terminal, small peptide tag, HiBiT, that exhibits very high affinity ($K_D = 0.7\text{nM}$) for LgBiT.

U2OS Autophagy LC3 HiBiT Reporter Cell Line



Induction of autophagy upon treatment of U2OS Autophagy LC3 HiBiT Reporter Cells with PP242, an mTOR-specific inhibitor, results in a concentration-dependent decline in the luminescent assay signal.



Reporter cells were treated with increasing concentrations of Baf A1 (lysosomal inhibitor) without or with PP242. Co-treatment with PP242 significantly increase the assay window.

Ile Inflammasom/Immunogenic cell death

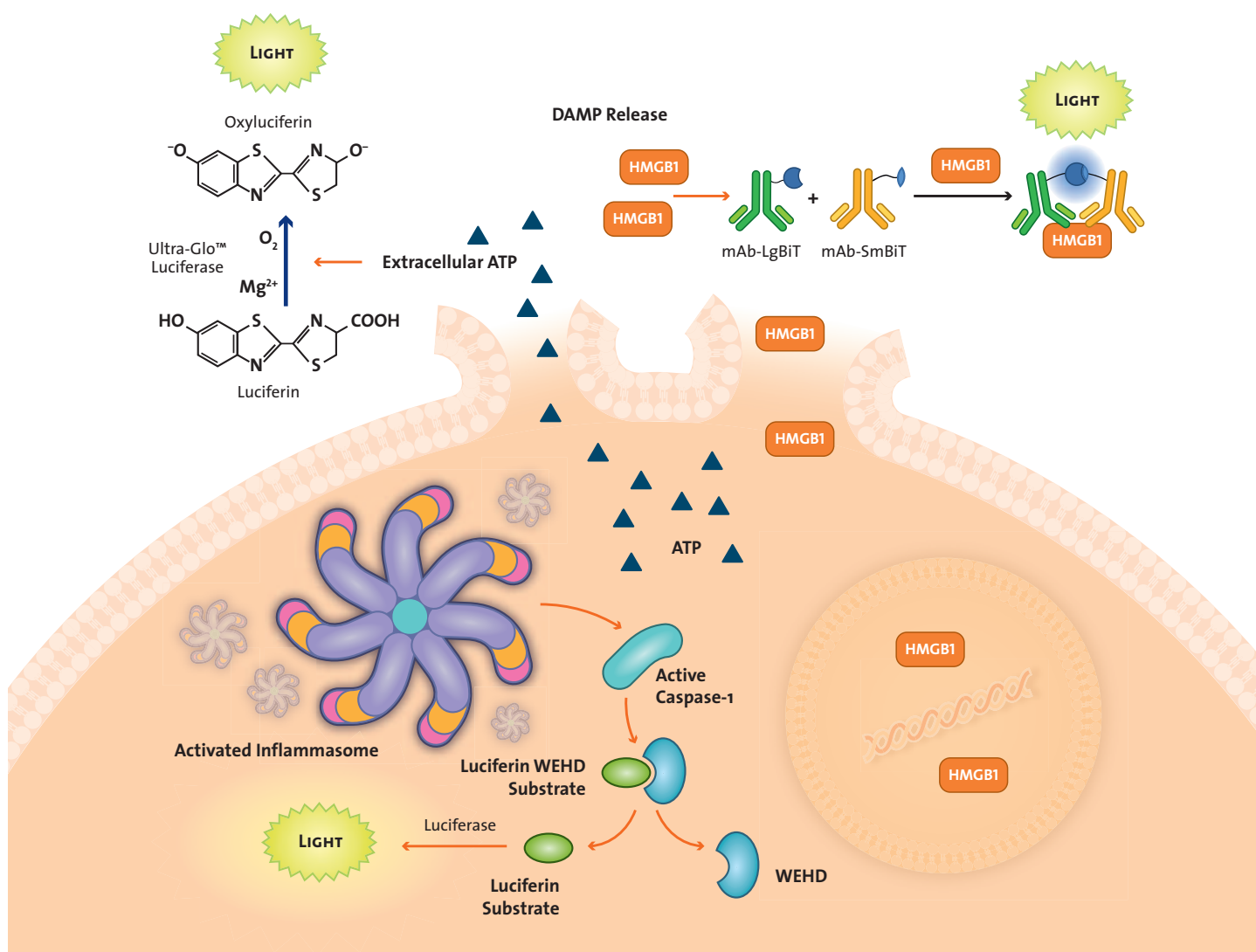
Inflammasomes are protein complexes induced by diverse inflammatory stimuli. Innate immune cells respond to pathogens and other danger signals with inflammasome formation and conversion of procaspase-1 zymogen into catalytically active caspase-1. Caspase-1 activation results in: 1) the processing and release of cytokines IL-1 β and IL-18 and 2) pyroptosis. Pyroptosis is a possible mechanism of immunogenic cell death (ICD), a form of regulated cell death that activates adaptive immunity. The release of endogenous factors, damage-associated molecular patterns (DAMPs), plays a central role in the induction of immunogenic cell death. These include the release of ATP and the high-mobility group box 1 protein (HMGB1).

An increasing trend in immuno-oncology is the identification of therapies, including certain small-molecule chemotherapeutic agents, antibody-drug conjugates, or oncolytic viruses that induce ICD. Assays that measure the release of DAMPs *in vitro* are an effective tool to identify novel immunotherapies.

Caspase-Glo® 1 Inflammasome Assay

RealTime-Glo™ Extracellular ATP Assay

Lumit™ HMGB1 Human/Mouse Immunoassay



Caspase-Glo® 1 Inflammasome Assay

Cell-based/Biochemical

Applications

Determination of inflammasome activity by inducers or inhibitors in cells or cell culture media; assay purified caspase-1 activity; multiplexing with other assays.

Assay description

The **Caspase-Glo® 1 Inflammasome Assay** is a homogeneous, bioluminescent method to selectively measure the activity of caspase-1, a member of the cysteine aspartic acid-specific protease (caspase) family and an essential component of the inflammasome.

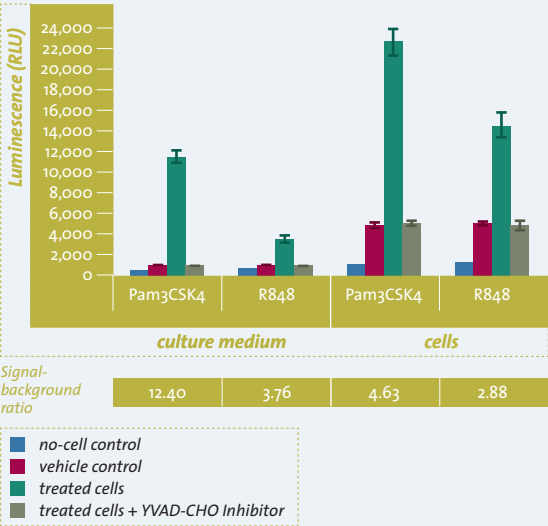
Assay principle

The **Caspase-Glo® 1 Inflammasome Assay** provides a luminogenic caspase-1 substrate, Z-WEHD-aminoluciferin, in a lytic reagent optimized for caspase-1 activity. A single addition of this reagent results in cell lysis, substrate cleavage by caspase-1 and generation of light. The coupled-enzyme system reaches a steady-state between caspase cleavage of the substrate and luciferase conversion of aminoluciferin. These simultaneous reactions generate a stable luminescent signal, which is proportional to caspase activity. Inclusion of the proteasome inhibitor MG-132 in the reagent eliminates nonspecific proteasome-mediated cleavage of the substrate, enabling sensitive measurement of caspase-1 activity.

Assay features

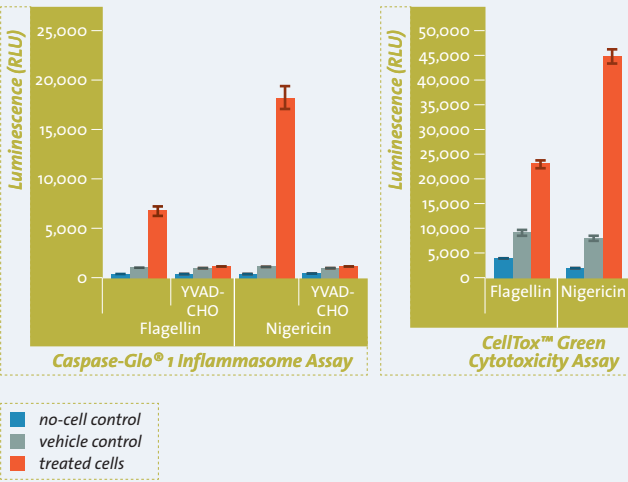
Assay type	Luminescent (glo-type; $T_{1/2} > 3\text{ h}$)
Markers	Caspase-1 activity
Applications	Determination of inflammasome activity or purified caspase-1 activity.
Cell type	Cells or medium from cultured cells in multiwell plates. No lysate preparation or multiple pipetting steps required.
Implementation	Homogeneous, one-step assay
Time required	1 hour (after adding the reagent)
Specific Activity	The selective caspase-1 substrate (Z-WEHD) and Inhibitor (MG-132) enable direct detection of caspase-1 activity. The kit also includes a caspase-1-specific inhibitor (Ac-YVAD-CHO inhibitor) to confirm specific activity in parallel samples.
Robustness	Reactions are scalable in 96- and 384-plates

Caspase-Glo® 1 Inflammasome Assay can monitor released caspase-1 in culture medium



THP-1 cells were differentiated for 2 days with 20nM phorbol-12-myristate-13-acetate (PMA), followed by treatment with either Pam3CSK4 (2 µg/ml) or resiquimod (R848, 20 µM) for 2 hours. Half of the culture medium (50 µl/well) was transferred to a second plate, 50 µl/well of Caspase-Glo® 1 Reagent or Caspase-Glo® 1 YVAD-CHO Reagent was added and luminescence was measured.

Caspase-Glo® 1 can be multiplexed with CellTox™ Green



THP-1 cells were added to plates at 5×10^5 cells/ml in 100 µl of medium and differentiated with PMA (20nM, 3 days) in 96-well plates followed by treatment with flagellin (1 µg/ml, 1 hour) or nigericin (20 µM, 2 hours). Half of the culture was transferred to a separate plate, and Caspase-Glo® 1 Reagent or Caspase-Glo® 1 YVAD-CHO Reagent was added to each well. Luminescence was recorded after 30 minutes. The original plate with the cells and half of the culture medium was then assayed using the CellTox™ Green Cytotoxicity Assay.

RealTime-Glo™ Extracellular ATP Assay

Cell-based

Applications

Live-cell kinetic assay for measuring extracellular ATP.

Assay description

The **RealTime-Glo™ Extracellular ATP Assay** is a non-lytic, bioluminescent assay designed for kinetic monitoring of ATP released from dying, stressed, or activated cells. Extracellular ATP can function as a damage-associated molecular pattern (DAMP) and is a key biomarker for determining whether a treatment induces immunogenic cell death, a specialized form of cell death that results in an immune response.

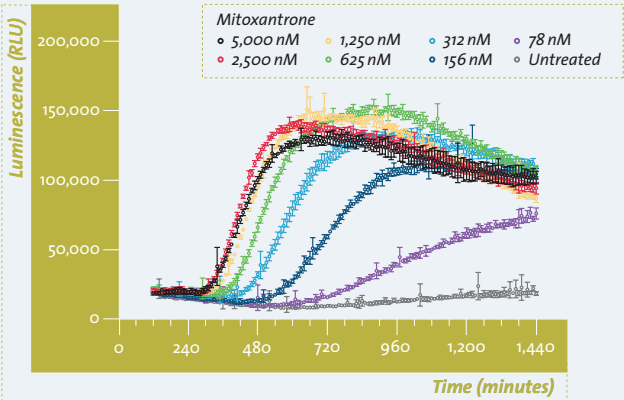
Assay principle

The **RealTime-Glo™ Extracellular ATP Assay** is based on the measurement of ATP content in an ATP-dependent luciferase reaction. The non-lytic reagent (Ultra-Glo™ Recombinant Luciferase, luciferin) is prepared by adding cell culture medium to the lyophilized substrate and directly applied to the cells at the time of dosing or treatment. The luciferase generates a nearly simultaneous luminescent response proportional to the current extracellular ATP levels present in the cell culture for up to 24 hours.

Assay features

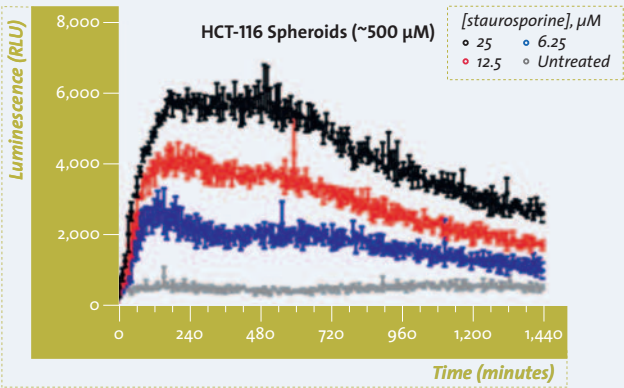
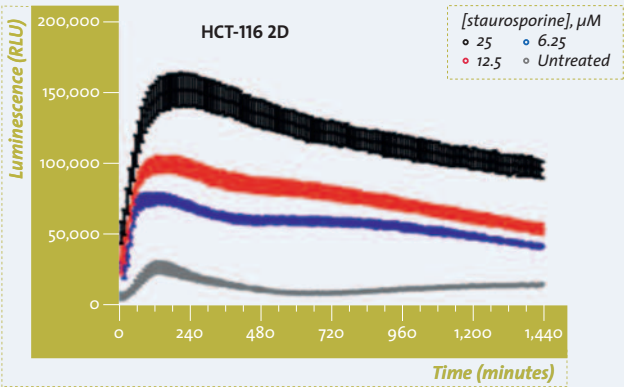
Assay type	Luminescent
Markers	Extracellular ATP
Applications	Kinetic monitoring of immunogenic cell death
Cell type	Cell lines, 2D and 3D cell models
Implementation	Kinetic, non-lytic, homogeneous two-step assay
Time required	5 minutes to add reagent and collect first data point, measures kinetics for up to 24 hours
Robustness	Scalable from 96 to 384 well formats, high throughput suitability: Z' factor 0.77 in 384-well format

Kinetic Monitoring of Extracellular ATP



U937 cells were treated with a dilution of mitoxantrone, an anthracycline shown to induce immunogenic cell death. RealTime-Glo™ Extracellular ATP Assay Reagent was added, and the assay plate was placed in a plate reader set at 37°C. Luminescence was collected every 10 minutes for 24 hours.

Compatible with 2D and 3D Cell Models



HCT-116 cells were grown in monolayer (top) and spheroids (bottom) using ultra-low attachment plates. Cells were treated with dilutions of staurosporine, and the RealTime-Glo™ Extracellular ATP Assay Reagent was added. The timing for ATP release differs between monolayer and spheroid cell culture, and the reagent is compatible with both cell culture models.

Lumit™ HMGB1 Human/Mouse Immunoassay

Cell-based

Applications

Monitoring HMGB1 release from cell culture samples.

Assay description

High-mobility group box 1 (HMGB1) functions as a damage-associated molecular pattern (DAMP) released from cells in inflammatory response. In addition, HMGB1 serves as a bio-marker for immunogenic cell death and may act as a proinflammatory cytokine. Current methods to detect HMGB1, such as ELISA, require lengthy protocols and tedious wash steps. **Lumit™ HMGB1 Immunoassay** simplifies HMGB1 quantitation with an add-mix-measure protocol performed directly on cells or transferred supernatant and is completed in less than 90 minutes. The immunoassay is cross-reactive with mouse HMGB1 with approximately 2-fold less sensitivity as compared to detection of human HMGB1.

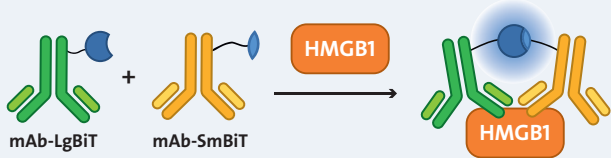
Assay principle

Lumit™ Immunoassays are based on NanoLuc® Binary Technology (NanoBiT®). A pair of anti-human HMGB1 antibodies covalently labeled with the small and large subunits of the NanoBiT® Luciferase, known as SmBiT and LgBiT, respectively, are incubated with the sample. When the labeled antibodies recognize and bind to HMGB1, the two antibodies come into spatial proximity, allowing SmBiT and LgBiT to form an active luciferase and generate a bright luminescence signal in the presence of the Lumit™ Substrate. The light generated is directly proportional to the amount of HMGB1 present in the sample.

Assay features

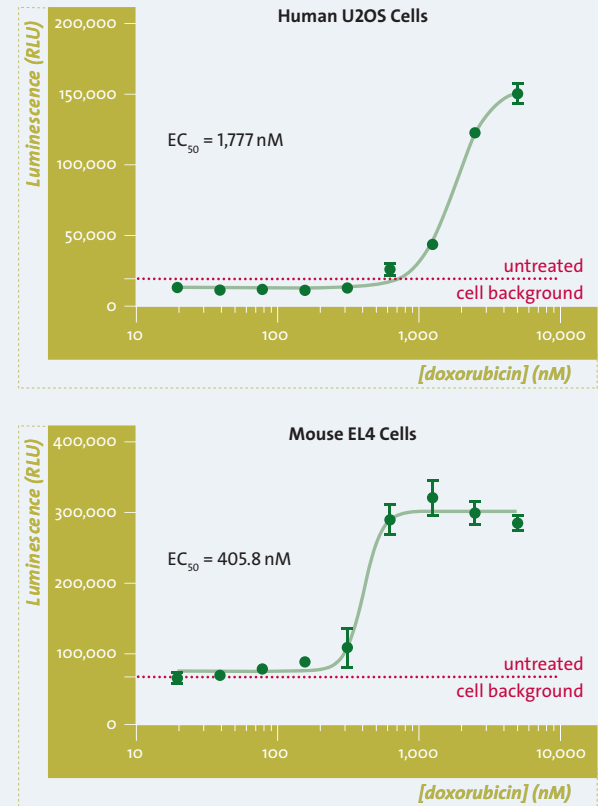
Assay type	Luminescent
Markers	High-mobility group box 1 (HMGB1)
Applications	Quantitative detection of human or mouse HMGB1
Cell type/Sample	Cell culture samples of various cell culture models or transferred supernatant
Implementation	Homogeneous, no-wash assay
Time required	60 – 90 minutes
Sensitivity	Limit of detection (LOD) 1 ng/ml (human) or 3 ng/ml (mouse) with broad dynamic range 1 – 729 ng/ml or 3 – 2187 ng/ml, respectively

Principle of Lumit™ HMGB1 Immunoassay



Primary antibodies to human HMGB1 are labeled with subunits of the NanoBiT® Luciferase. Upon binding of the antibodies to HMGB1 the subunits are brought together and produce light proportional to the amount of HMGB1 with the addition of the Lumit™ Substrate.

Detect Drug-Induced Immunogenic Cell Death in Various Cell Culture Models



Human U2OS cells and mouse EL4 cells were treated with doxorubicin for 24 hours. After 24 hours, the Lumit™ HMGB1 Immunoassay was performed directly on cell culture samples to measure HMGB1 released from treated cells.

Ilf Multiplexing

The demand for higher throughput in current biomedical research has had a major impact on the use of cell-based assays. It is becoming increasingly important to be able to combine assays with one another. Such multiplexing of cell-based assays, as it is known, allows the efficient analysis of more than one parameter in an experimental setup. Various combinations of assays are feasible here, provided the assay chemistry is compatible and the detection signals can be distinguished from one another. The measurement and linking of multiple parameters renders data meaningful and reproducible. Depending on the combination, one assay can serve as an inter-

nal control for another assay. This approach saves time, sample materials and expensive test compounds. Multiplexing consequently makes it possible to gain an improved understanding of complex cellular processes.

MultiTox-Fluor Multiplex Cytotoxicity Assay

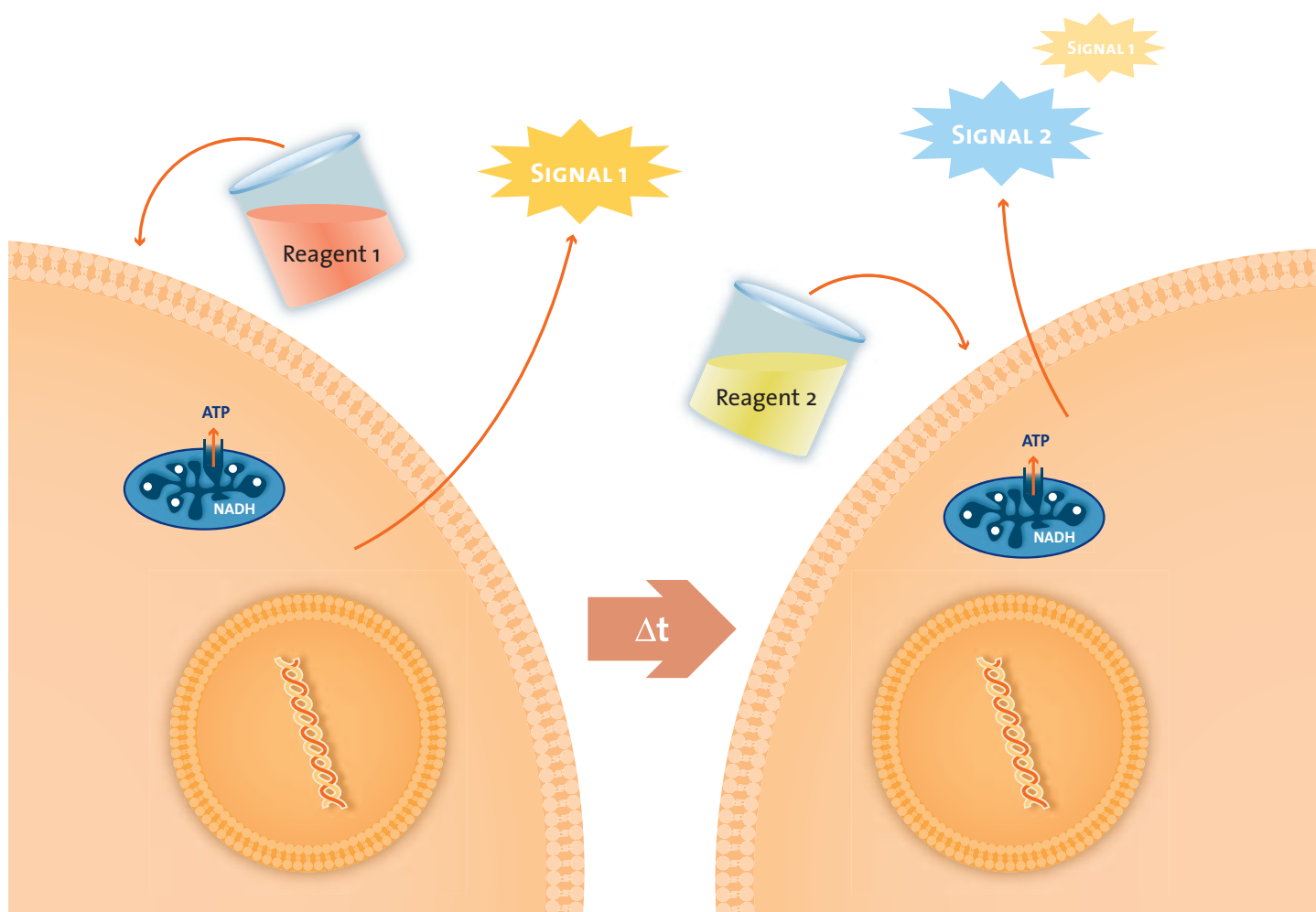
MultiTox-Glo Multiplex Cytotoxicity Assay

ApoLive-Glo™ Multiplex Assay

ApoTox-Glo™ Triplex Assay

ONE-Glo™ + Tox Luciferase Reporter and Cell Viability Assay

Mitochondrial ToxGlo™ Assay



Overview of cell-based assays that can be combined

To multiplex two or more assays, the assays must meet certain criteria: the signals must be spectrally or temporally distinct, the assay chemistries must be compatible, and the assays must

fit into the same well or be easily separated. Furthermore, some of the multiplexing examples in the table are using modified protocols. Please contact our technical service for guidance!

Cell Viability Assay

1st Assay	2nd Assay	Information obtained by multiplexing
RealTime-Glo™ MT Cell Viability Assay Luminescence	CellTox™ Green Cytotoxicity Assay	Cell viability and cytotoxicity (membrane integrity)
	CytoTox-Fluor™ Cytotoxicity Assay	Cell viability and cytotoxicity (protease release)
	NAD ⁺ /NADH-Glo™ Assay, NADP ⁺ /NADPH-Glo™ Assay	Cell viability and measurement of NAD(P) ⁺ and NAD(P)H
	Reporter assays	Cell viability and reporter gene activity
	RNA isolation	Cell viability and RNA analysis
CellTiter-Fluor™ Assay Fluorescence Live-Cell-Protease-Activity (Gly-Phe-AFC, 400 _{ex} /505 _{em})	CellTiter-Glo® Assay, CellTiter-Glo® 2.0 Assay	Viability
	CytoTox-Glo™ Assay (available as MultiTox-Glo™ Multiplex Assay)	Cytotoxicity
	CytoTox-Fluor™ Assay (available as MultiTox-Fluor™ Multiplex Assay)	
	Caspase-Glo® 3/7 Assay (available as ApoLive-Glo™ Multiplex Assay)	Apoptosis
	GSH-Glo™ Assay	Oxidative Stress
CellTiter-Blue® Assay Fluorescence (Resazurin to Resorufin, 560 _{ex} /590 _{em})	P450-Glo™ Assay	Cytochrom P450
	CytoTox-ONE™ Assay	Cytotoxicity
	Apo-ONE® Caspase 3/7 Assay	Apoptosis

Cytotoxicity Assay

1st Assay	2nd Assay	Information obtained by multiplexing
LDH-Glo™ Assay Luminescence	LDH-Glo™ Assay uses supernatant of cell culture medium and can be combined with all available cell-based assays	
CellTox™ Green Assay Fluorescence (DNA-dye, 485–500 _{ex} /520–530 _{em})	CellTox™ Green Assay can be combined with all available Glo-Assays	
CytoTox-ONE™ Assay LDH-Release, Fluorescence (Resazurin to Resorufin, 560 _{ex} /590 _{em})	CellTiter-Glo® Assay, CellTiter-Glo® 2.0 Assay	Viability
	CellTiter-Blue® Assay	Apoptosis
	Caspase-Glo® 3/7 Assay	
CytoTox-Fluor™ Assay Fluorescence Dead-Cell Protease-Activity (bis-AAF-R110, 485 _{ex} /520 _{em})	Apo-ONE® Caspase 3/7 Assay	
	CellTiter-Glo® Assay, CellTiter-Glo® 2.0 Assay	Viability
	Caspase-Glo® 3/7 Assay	Apoptosis
	ONE-Glo™ Assay	Single Reporter System
	GSH-Glo™ Assay	Oxidative Stress

Apoptosis Assay

1st Assay	2nd Assay	Information obtained by multiplexing
RealTime-Glo™ Annexin V Apoptosis and Necrosis Assay Luminescence	CellTiter-Blue® Assay	Viability
	CellTiter-Glo® Assay, CellTiter-Glo® 2.0 Assay	Viability
	Caspase-Glo® 3/7 Assay	Apoptosis
Apo-ONE® Caspase 3/7 Assay Fluorescence Caspase-Activity (Z-DEVD-R110, 499 _{ex} /521 _{em})	CellTiter-Blue® Assay	Viability
	CytoTox-ONE™ Assay	Cytotoxicity
	Caspase-Glo® 8 and 9 Assay	Apoptosis
	EnduRen™ Live Cell Substrate	Live Cell Substrate

Autophagy Assay

1st Assay	2nd Assay	Information obtained by multiplexing
CellTox™ Green Assay Luminescence	Autophagy LC3 HiBiT Reporter Assay System	Autophagy flux and Cytotoxicity

MultiTox-Fluor Multiplex Cytotoxicity Assay

Cell-based

Applications

Simultaneous determination of cell viability and cytotoxicity in the same well.

Assay description

Multitox-Fluor Multiplex Cytotoxicity Assay is a fluorescent cell-based assay for simultaneously determining cell viability and cytotoxicity in the same well. The assay is a combination of the CellTiter-Fluor™ and the CytoTox-Fluor™ Assays.

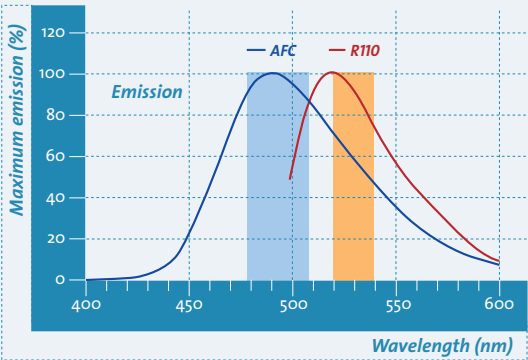
Assay principle

The assay is based on the simultaneous determination of live-cell protease activity and dead-cell protease activity. Both are forms of protease activity that deliver significant information about cell viability and cytotoxicity. The assay reagent contains two different fluorogenic peptide substrates: the cell-permeable GF-AFC for determining cell viability and the cell-impermeable bis-AAF-R110 for determining cytotoxicity. For further details, see CellTiter-Fluor™ Assay and CytoTox-Fluor™ Cytotoxicity Assay.

Assay features

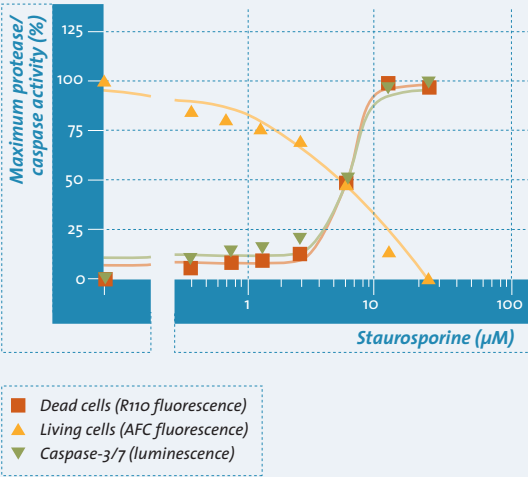
Assay type	Fluorescent (AFC 400 _{Ex} /505 _{Em} , R110 485 _{Ex} /520 _{Em})
Markers	Live-cell protease; dead-cell protease
Applications	Simultaneous measurement of cell viability and cytotoxicity in the same well
Cell type	Cell lines (adherent or in suspension), primary cells
Implementation	Homogeneous, one-step assay
Time required	0.5–3 hours
Sensitivity	40 living cells; 10 dead cells (96-well format)
Robustness	96- to 1536-well formats

Measurement signals can be differentiated by their distinct fluorescence spectra.



Optimal results are achieved through the use of filters for excitations at 400 nm (AFC) and 485 nm (rhodamine 110).

Multiplexing with other assays



Detection of the cell viability and cytotoxicity of LN-18 cells treated with staurosporine, using MultiTox-Fluor reagent and Caspase-Glo® 3/7 reagent. With rising staurosporine concentration, cell viability decreases while caspase activity increases.

MultiTox-Glo Multiplex Cytotoxicity Assay

Cell-based

Applications

Sequential determination of cell viability and cytotoxicity in the same well.

Assay description

MultiTox-Glo Multiplex Cytotoxicity Assay is a cell-based assay for sequentially determining cell viability and cytotoxicity in the same well. The assay is a combination of the CellTiter-Fluor™ and CytoTox-Glo™ Assays.

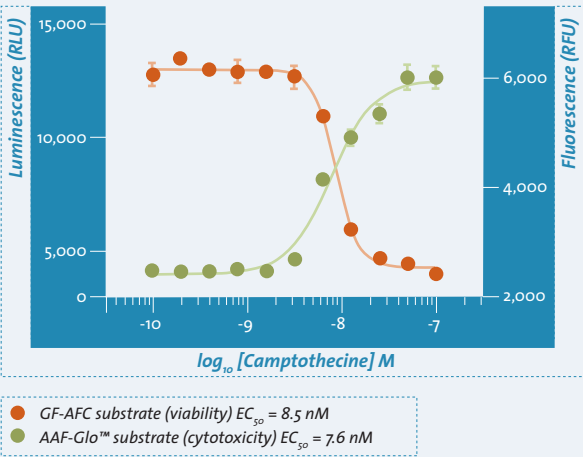
Assay principle

The assay is based on the sequential determination of live-cell protease activity and dead-cell protease activity. Both are forms of protease activity that deliver significant results about cell viability and cytotoxicity. The assay reagent contains two different peptide substrates: the fluorogenic cell-permeable GF-AFC for determining cell viability and the luminogenic cell-impermeable AAF-aminoluciferin for determining cytotoxicity. For further details, see CellTiter-Fluor™ and CytoTox-Glo™ Assays.

Assay features

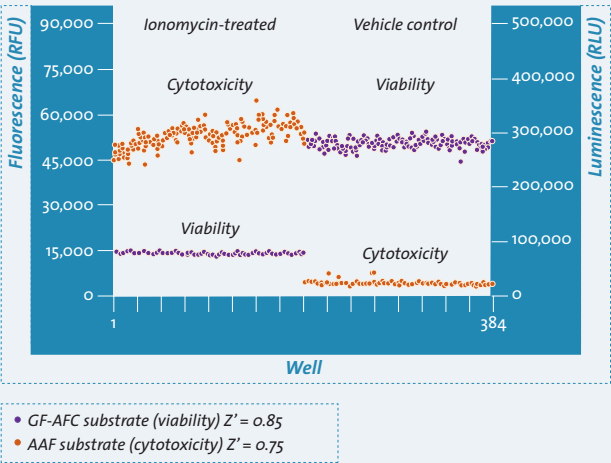
Assay type	Fluorescent (AFC 400 _{Ex} /505 _{Em}); luminescent (glow-type)
Markers	Live-cell protease; dead-cell protease
Applications	Sequential measurement of cell viability and cytotoxicity in the same well
Cell type	Cell lines (adherent or in suspension), primary cells
Implementation	Homogeneous, two-step assay
Time required	0.5 hours
Sensitivity	40 living cells; 10 dead cells (96-well format)
Robustness	96- to 1536-well formats

MultiTox-Glo Multiplex Cytotoxicity Assay



Comparable EC₅₀ values through parallel measurement of live-cell and dead-cell protease. Jurkat cells were treated with the alkaloid camptothecin for 24 hours. Cell viability and cytotoxicity were determined using MultiTox-Glo. The EC₅₀ values determined are comparable.

Low variation in measurement results & a high signal-to-noise ratio lead to excellent Z' factors



Low dispersion of individual measurements using the MultiTox-Glo reagent. In this trial, 5x10³ cells in a 384-well plate were treated for 2 hours with the cytotoxic agent ionomycin (50 μM) and the vehicle control, respectively. The MultiTox-Glo reagent was added as described in Technical Bulletin #TB358. The results show a low variation in the individual measurement results and a very good signal-to-noise ratio. This is also expressed in the excellent Z' factors of >0.5.

ApoLive-Glo™ Multiplex Assay

Cell-based

Applications

Sequential determination of cell viability and apoptosis in the same well.

Assay description

ApoLive-Glo™ Multiplex Assay enables the measurement of cell viability and apoptosis in the same well. In this way, the quality of the data is significantly improved compared with that of a single-parameter assay. At the same time, it reduces the outlay in terms of time and cost.

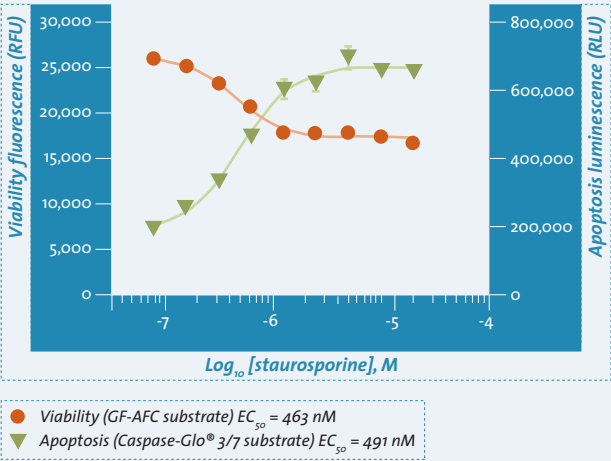
Assay principle

The assay is a combination of the CellTiter-Fluor™ Cell Viability Assay and the Caspase-Glo® 3/7 Assay. In the first step, cell viability is determined through live-cell protease activity using the peptide substrate GF-AFC. In the second step, apoptosis is determined by measuring caspase-3/7 activity.

Assay features

Assay type	Fluorescent (AFC 400 _{Ex} /505 _{Em}); luminescent (glow-type)
Markers	Live-cell protease + caspase-3/7
Applications	Sequential measurement of cell viability and apoptosis in the same well
Cell type	Cell lines (adherent or in suspension), primary cells
Implementation	Homogeneous, two-step assay
Time required	1–3 hours
Sensitivity	40 living cells; 100 apoptotic cells (96-well format)
Robustness	96- to 384-well plates

Induction of apoptosis by staurosporine



Jurkat cells were treated with staurosporine for 6 hours, and cell viability and apoptosis were determined using the ApoLive-Glo™ Multiplex Assay. With rising staurosporine concentration, cell viability decreases while caspase 3/7 activity increases.

ApoTox-Glo™ Triplex Assay

Cell-based

Applications

Sequential determination of cell viability, cytotoxicity and apoptosis in the same well; cell death research.

Assay description

ApoTox-Glo™ Triplex Assay is a novel multiplex analysis system for determining viability, cytotoxicity and apoptosis of cell lines in a single well. The triplex assay measures the three parameters in two steps by means of fluorescence and luminescence.

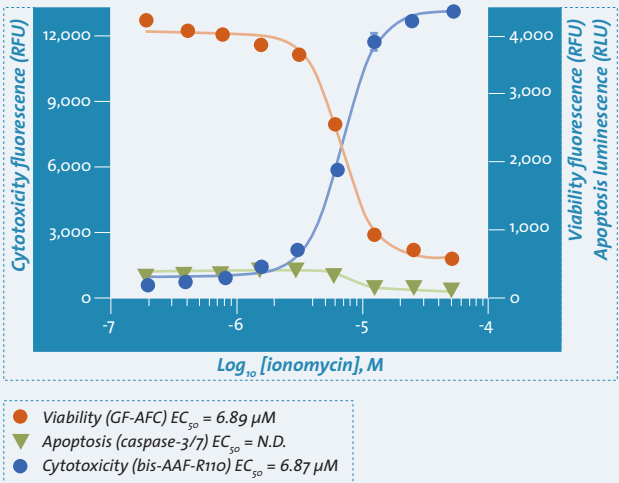
Assay principle

The assay is a combination of the Multitox-Fluor™ Multiplex Cytotoxicity Assay and the Caspase-Glo® 3/7 Assay. In the first part of the assay, cell viability and cytotoxicity are determined by measuring two protease activities: live-cell protease and dead-cell protease. In the second step, apoptosis is determined by measuring caspase-3/7 activity.

Assay features

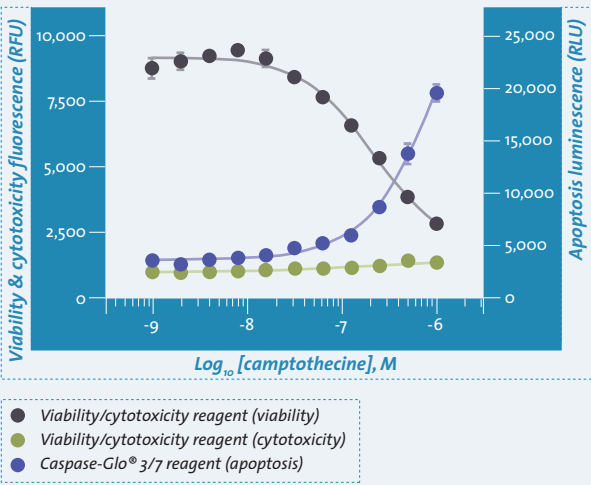
Assay type	Fluorescent (AFC 400 _{Ex} /505 _{Em} , R110 485 _{Ex} /520 _{Em}); Luminescent (glow-type)
Markers	Live-cell and dead-cell protease; caspase-3/7
Applications	Sequential measurement of cell viability, cytotoxicity and apoptosis
Cell type	Cell lines (adherent or in suspension), primary cells
Implementation	Homogeneous, two-step assay
Time required	1–3 hours
Sensitivity	40 living cells; 10 dead cells; 100 apoptotic cells (96-well format)
Robustness	96- to 384-well plates

Induction of primary necrosis by ionomycin



Treatment of Jurkat cells with ionomycin for 6 hours leads to a reduction in cell viability with no caspase-3/7 activation. However, cell-membrane damage due to the release of dead-cell protease is observed (rise in the cytotoxicity curve). The data supports the assumption that ionomycin induces primary necrosis.

Induction of apoptosis by camptothecin



Treatment of K562 cells with camptothecin for 48 hours. Camptothecin leads to a dose-dependent reduction in cell viability without damaging the cell membrane. A dose-dependent induction of apoptosis is observed.

ONE-Glo™ + Tox Luciferase Reporter and Cell Viability Assay

Cell-based

Applications

Determination of firefly luciferase reporter gene activity and cell viability in the same well; normalize expression to viability.

Assay description

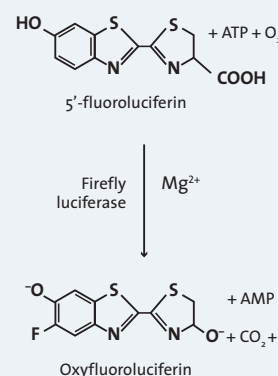
ONE-Glo™ + Tox Luciferase Reporter and Cell Viability Assay is an assay with 2 pipetting steps for simultaneously determining firefly luciferase reporter gene expression and cell viability in the same well. Using this assay significantly simplifies and improves the interpretation of reporter gene expression data.

Assay principle

The assay is a combination of the CellTiter-Fluor™ Cell Viability Assay and the One-Glo™ Luciferase Assay System. In the first step, cell viability is detected by measuring live-cell protease activity. Here, conversion of the pro-fluorogenic substrate GF-AFC into fluorescent AFC is measured. In the second step, luciferase reporter gene activity is determined using 5'-fluoroluciferin as a luciferase substrate. Reporter assays using 5'-fluoroluciferin have greater stability and increased tolerance to media components.

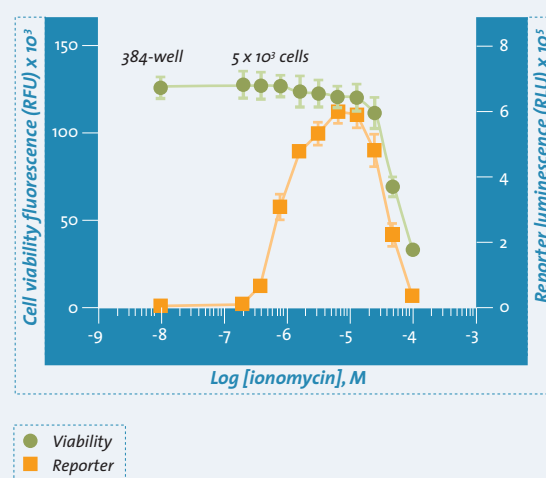
Assay features

Assay type	Fluorescent (AFC 400 _{Ex} /505 _{Em}); luminescent (glow-type)
Markers	Live-cell protease; luciferase reporter gene
Applications	Luciferase reporter gene activity + cell viability
Cell type	Cell lines
Implementation	Homogeneous, two-step assay
Time required	0.6–3 hours
Robustness	96- to 384-well plates



5'-fluoroluciferin as a substrate for the firefly luciferase reaction

Multiplexing for improved interpretation of reporter gene data



GloResponse™ NFAT-RE-luc2P HEK293 reporter gene cells (384-well plate) were treated in the presence of PMA with increasing concentrations of ionomycin. Up to a specific concentration of ionomycin, the two substances cause stimulation of NF-AT-dependent luciferase gene expression. Higher concentrations of ionomycin have a cytotoxic effect on the cells and result in diminished reporter gene expression.

Mitochondrial ToxGlo™ Assay

Cell-based

Applications

Predicting mitochondrial toxicity; drug screening; suitable for HTS.

Assay description

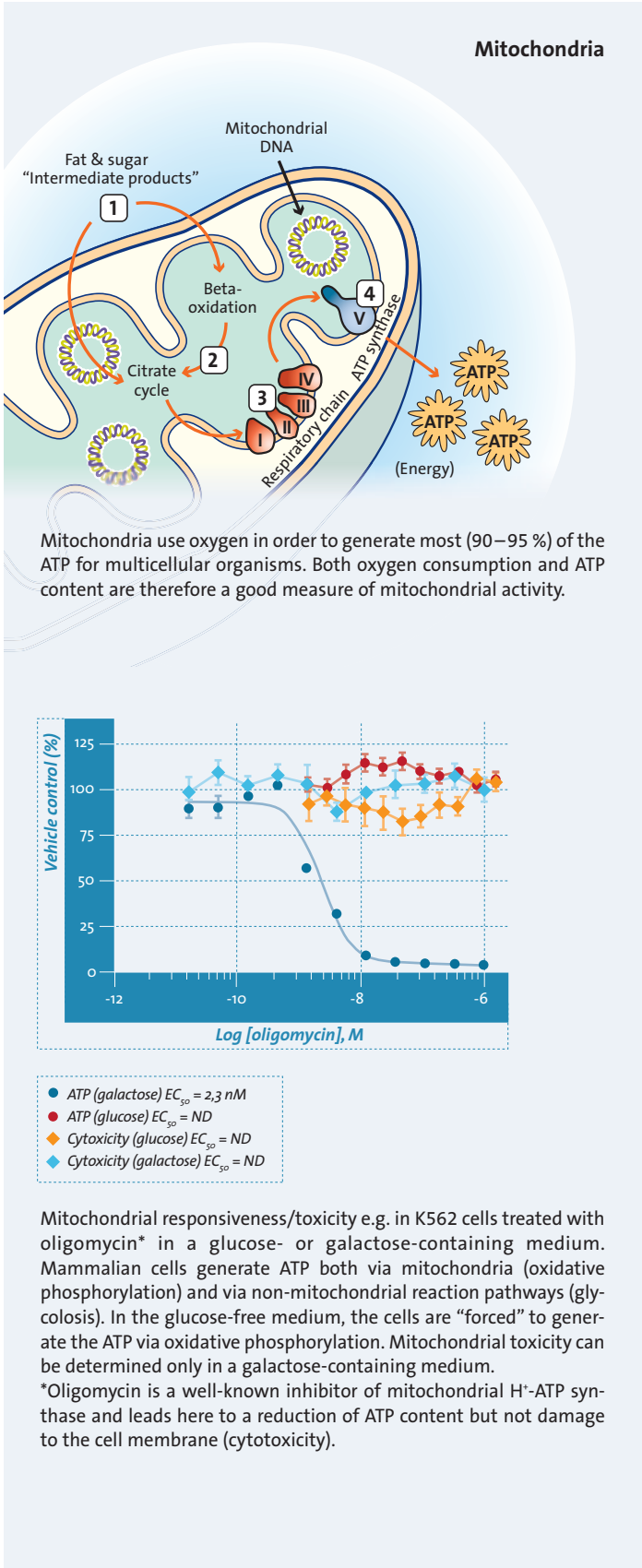
Mitochondrial ToxGlo™ Assay is a cell-based assay for predicting mitochondrial toxicity which may arise e.g. as a result of treatment with xenobiotics. The assay is performed directly in the cell culture plate. ATP content and membrane integrity are measured. Combining the two data sets makes it possible to differentiate between mitochondrial dysfunction and non-mitochondrial cytotoxic mechanisms.

Assay principle

The assay is based on the sequential measurement of two biomarkers. In the first step, cell membrane integrity is determined by measuring dead-cell protease activity in the medium. For this purpose, a fluorogenic peptide substrate (bis-AFF-R110) is used which cannot permeate the cell membrane, and which therefore does not generate a signal in living cells (cf. CytoTox-Fluor™ Cytotoxicity Assay). In the second step, the ATP content of the cell is determined. The ATP detection reagent leads to lysis of the cells, and the ATP content is measured in a luciferase reaction (cf. CellTiter-Glo® Assay). In order to increase the mitochondrial response, the cells should be cultured in a galactose-containing medium with no glucose supplement.

Assay features

Assay type	Fluorescent (R110 485 _{Ex} /520 _{Em}); Luminescent (glow-type)
Markers	Dead-cell protease, ATP
Applications	Mitochondrial toxicity
Cell type	Cell lines in galactose medium, primary cells
Implementation	Homogeneous, two-step assay
Time required	0.6–3 hours
Robustness	High Z' factor, 96- to 384-well formats



III Cell Metabolism

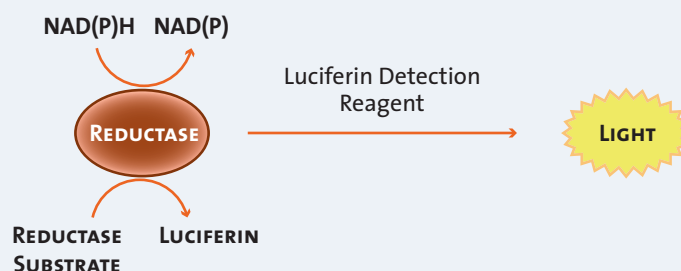
Request
Cellular Metabolism Brochure
for more assays

Cancer is a disease of uncontrolled cell growth that requires cancer cells to alter metabolic pathways to survive and proliferate. The principal mechanisms underlying this metabolic reprogramming by oncogenes and tumor suppressor genes is still poorly understood. Nicotinamide adenine dinucleotides (NAD^+ , NADH , NADP^+ and NADPH) are fundamental co-factors of cellular energy metabolism. These dinucleotides are essential for macromolecule biosynthesis and the maintenance of the cellular redox potential. In addition NAD-dependent signaling pathways (e.g., mono- and poly- ADP ribosylation, protein deacetylation) are involved in regulating other processes linked to cancer development, including epigenetic regulation, cell cycle pro-

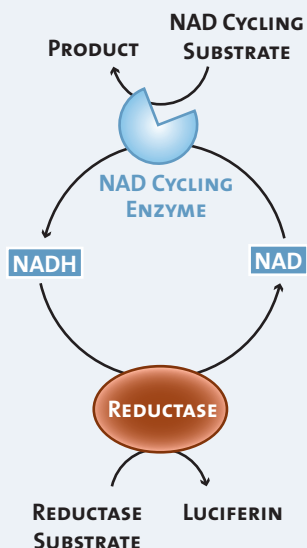
gression, DNA repair, and circadian rhythm. The central role of NAD^+ , NADH , NADP^+ and NADPH in cellular energy metabolism and signaling makes them important target-independent nodes that link the metabolic state of cells with energy homeostasis and gene regulation. Rapid, easy-to-use assays for measuring these dinucleotides would provide a convenient tool for investigating their role in these processes.

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

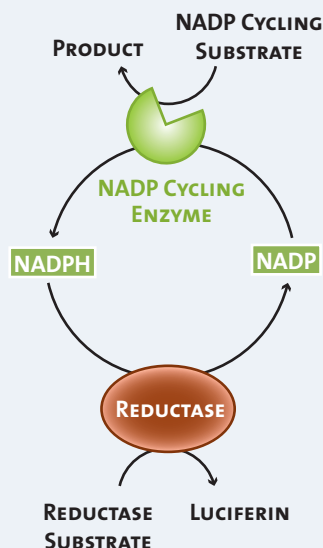
NAD(P)H-Glo™ Detection System – detects NADH and NADPH (Biochemical assay)



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 0.1 μM to 25 μM NAD(P)H. Reactions are scalable and can be performed at low volumes in 96-, 384- and 1536-well plates.

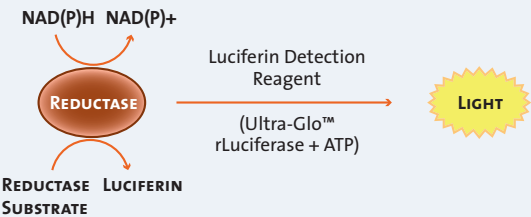
Assay principle

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.

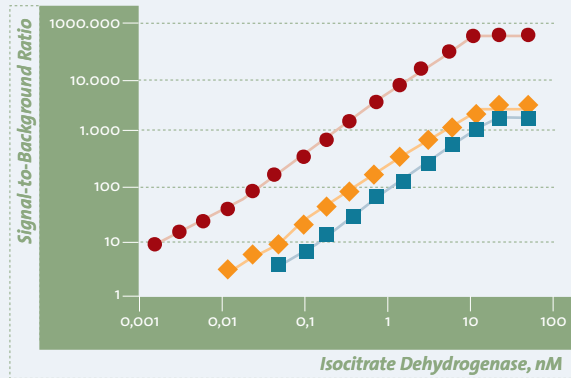
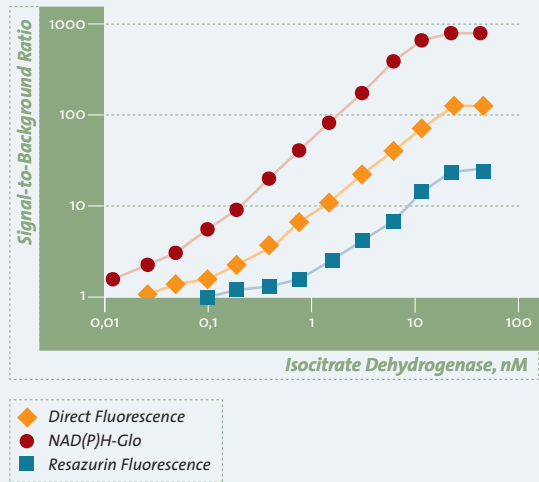
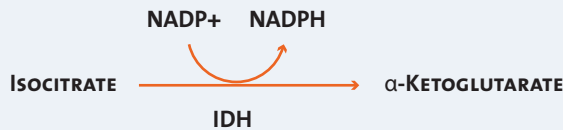
Assay features

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
Linearity	Detects 0.1 μM to 25 μM NAD(P)H
Sensitivity	The limit of detection is ≤ 0.1 μM NADH, with a maximum assay window (i.e., signal-to-background ratio) of 250. The system detects 1 μM with a signal higher than five fold over background.
Robustness	Z' factor > 0.7, 96- to 1536-well formats

NAD(P)H™ Detection System Assay-Principle



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



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

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

Cell-based/Biochemical

Both assays use the same technology (see Figure):

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

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

Applications

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

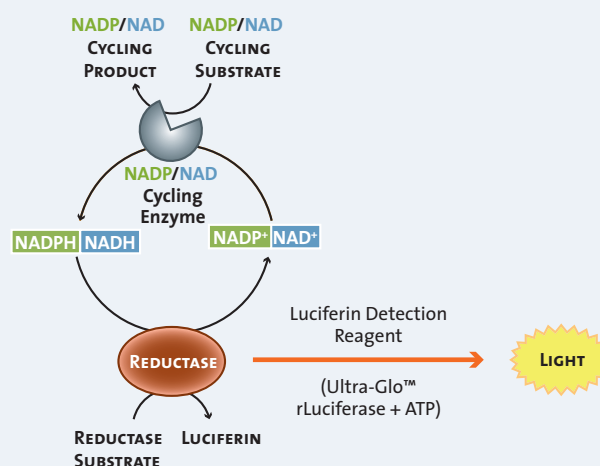
Assay description

The NAD⁺/NADH-Glo™ Assay is a bioluminescent, homogeneous single-reagent-addition assay for detecting total oxidized and reduced nicotinamide adenine dinucleotides (NAD⁺ and NADH, respectively) and determining their ratio in biological samples or in defined enzyme reactions. Cycling between NAD⁺ and NADH by the NAD Cycling Enzyme and Reductase increases assay sensitivity and provides selectivity for the nonphosphorylated NAD⁺ and NADH compared to the phosphorylated forms NADP⁺ and NADPH. The NAD/NADH-Glo™ Assay detects 10nM to 400nM 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.

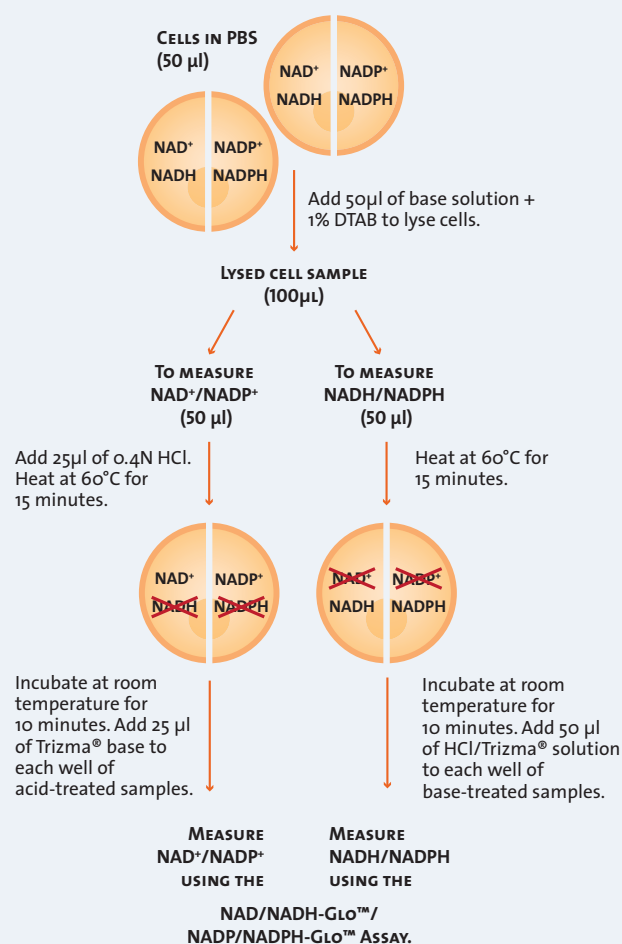
Assay principle

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.

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



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

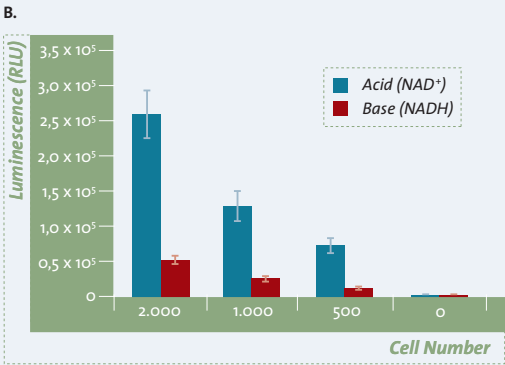
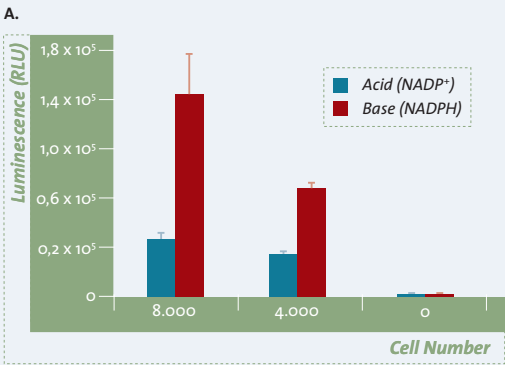


Luciferin then is quantified using Ultra-Glo™ Recombinant Luciferase, and the light signal produced after an incubation of 30–60 minutes is proportional to the amount of NAD⁺ and NADH in the sample. Detergent present in the reagent lyses cells, allowing detection of total cellular NAD⁺ and NADH in a multiwell format with addition of a single reagent. An accessory protocol is provided to allow separate measurements of NAD⁺ and NADH, and calculation of the NAD⁺ to NADH ratio.

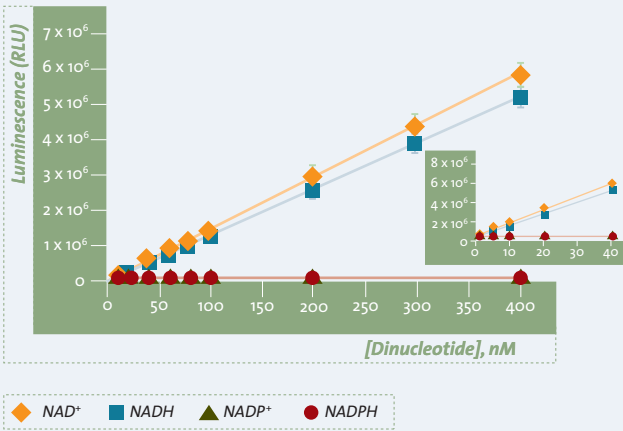
Assay features

Assay type	Luminescent (glow-type)
Markers	NAD ⁺ and NADH
Applications	Monitoring changes in cellular levels of total NAD ⁺ and NADH; determining NAD ⁺ /NADH ratios; monitoring the effects of small molecule compounds on NAD ⁺ and NADH levels in enzymatic reactions or directly in cells in high-throughput formats.
Cell type/Sample	Cells and Enzyme preparations, tissues, plasma
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.
Linearity and Sensitivity	Detects 10nM to 400 nM NAD ⁺ or NADH. The assay detects 100nM with a signal higher than fivefold over background and an assay window (maximum signal-to-background ratio) of ≥ 100.
Robustness	Z' factor > 0.7, 96- to 384-well formats

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



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



Individual purified nicotinamide adenine dinucleotides were assayed following the protocol described in Section 3.C. NADH, NADPH, NAD⁺ and NADP⁺ stocks were prepared freshly from powder (Sigma Cat.# N6660, N9910, N8285 and N8035, respectively) and diluted to the indicated concentrations in phosphate-buffered saline (PBS). Fifty microliter samples at each dinucleotide concentration were incubated with 50 μl of NAD/NADH-Glo™ Detection Reagent in white 96-well luminometer plates.

IV Oxidative stress

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

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

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

cess. 98% of glutathione in the body occurs in the reduced form GSH.

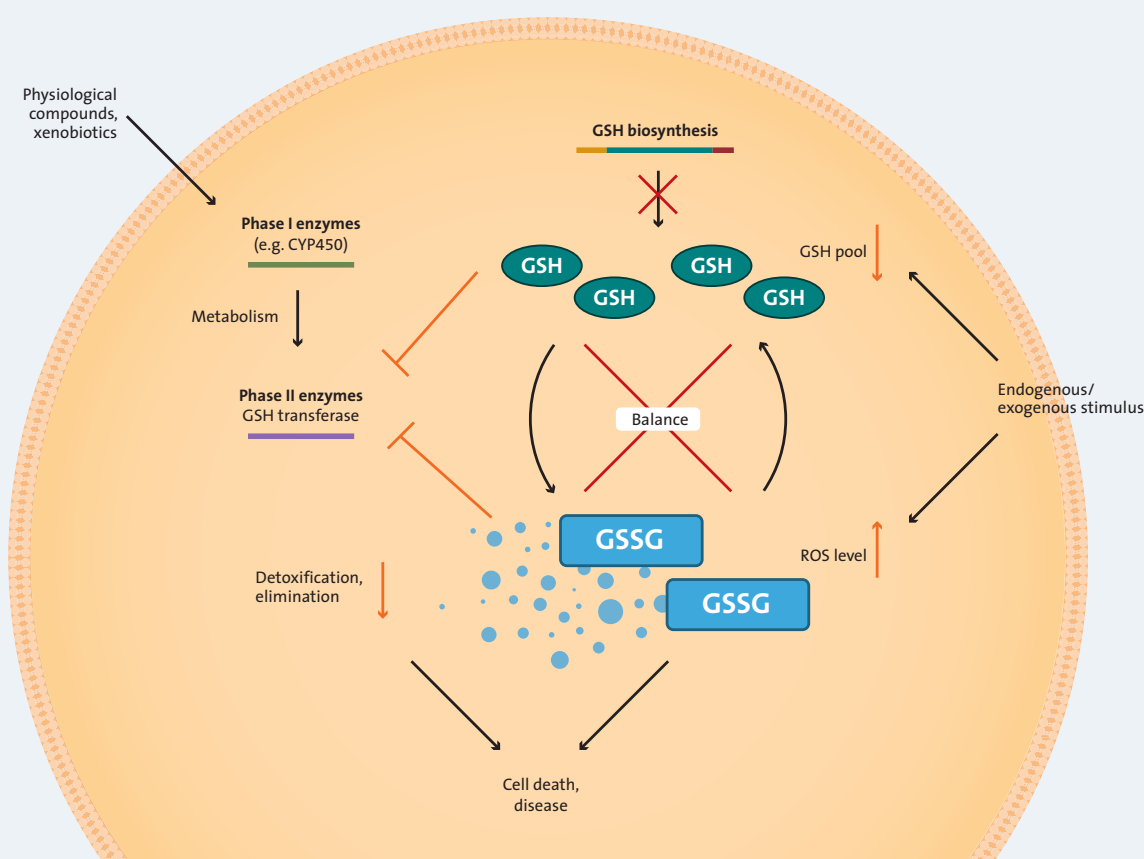
Promega offers two assays for analyzing GSH and one assay for the detection of H_2O_2 -levels:

1. **GSH-Glo™ Glutathione Assay** for quantitatively determining reduced GSH.
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. **ROS-Glo™ H_2O_2 Assay** for the detection of reactive oxygen species (H_2O_2) in cells.

GSH-Glo™ Glutathione Assay

GSH/GSSG-Glo™ Assay

ROS-Glo™ H_2O_2 Assay



ROS-Glo™ H₂O₂ Assay

Cell-based/Biochemical

Applications

Measure changes in hydrogen peroxide (H₂O₂) levels directly in cell culture samples; measure the activity of enzymes that generate or eliminate H₂O₂; 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™ H₂O₂ Assay** is a homogeneous, fast and sensitive bioluminescent assay that measures the level of H₂O₂, a reactive oxygen species (ROS), directly in cell culture or in defined enzyme reactions. The ROS-Glo™ H₂O₂ Substrate reacts directly with H₂O₂, 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.

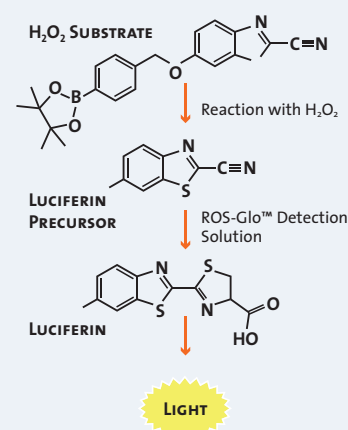
Assay principle

The homogeneous assay is performed following a simple two-reagent-addition protocol that does not require sample manipulation. A H₂O₂ substrate is incubated with sample and reacts directly with H₂O₂ 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 H₂O₂ present in the sample. The assay can be completed in less than 2 hours after reagent addition.

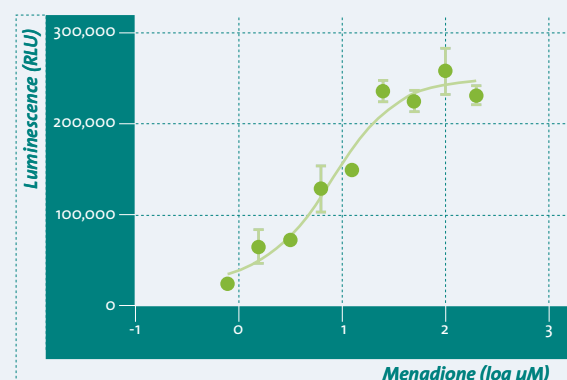
Assay features

Assay type	Luminescent (glow-type), two-step assay, obviating the need for HRP
Markers	H ₂ O ₂
Applications	Measure changes in H ₂ O 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 1536-well plates

ROS-Glo™ H₂O₂ Assay-Principle

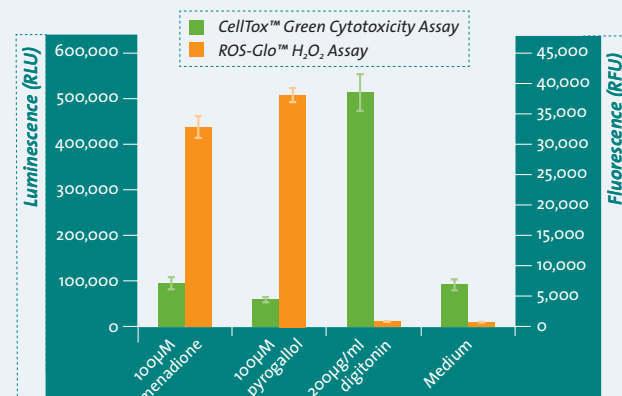


ROS induction in cultured cells



K562 cells were treated with menadione and the ROS-Glo™ H₂O₂ Assay was used to determine ROS production. Menadione resulted in a concentration-dependent ROS increase.

Multiplex with real-time CellTox™ Green Cytotoxicity Assay



HepG2 cells were plated at 2,000 cells/well in a 384-well plate and incubated overnight. The cells were then treated with either 100 μM menadione, 100 μM pyrogallol or 200 μg/ml digitonin and incubated at 37°C in 5% CO₂ for 2 hours. 1X CellTox™ Green Dye and 25 μM H₂O₂ Substrate were added to the cell culture at the time of dosing. After incubation the CellTox™ Green fluorescence signal was first measured and afterwards H₂O₂ levels using ROS-Glo™.

GSH-Glo™ Glutathione Assay

Cell-based

Applications

Quantification of reduced glutathione (GSH) in cells or tissue extracts as an indicator of cell viability; screening of drugs for regulating GSH levels in cells, tissues or blood samples.

Assay description

The **GSH-Glo™ Assay** is a sensitive, luminescent two-step assay, which is suitable for HTS applications and provides a simple and fast alternative to conventional colorimetric and fluorescence-based methods. Since the cells are not transferred, the loss of glutathione is minimal compared with that in conventional assays. Interference by oxidized glutathione (GSSG) or reducing agents is eliminated.

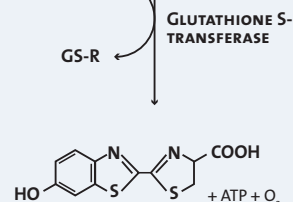
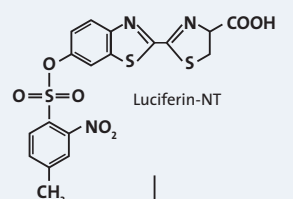
Assay principle

The assay couples the glutathione S-transferase (GST) reaction to the luciferin reaction. In the process, GST detaches a non-functional group from a luciferin derivative and couples this to the reduced glutathione. The freed luciferin enters the luciferase reaction. The light signal generated in this reaction is directly proportional to the level of GSH in the cell. The sample medium has to be removed, but there is no need for the laborious task of removing proteins from the lysate. A stable luminescent signal can be detected in less than 60 minutes with an excellent signal-to-noise ratio.

Assay features

Assay type	Luminescent (glow-type)
Markers	GSH
Applications	Quantification of reduced glutathione (GSH)
Sample material	Cells, tissue extracts or blood samples
Implementation	Homogeneous, two-step assay
Time required	45 minutes

Schematic representation of the GSH-Glo™ Glutathione Assay

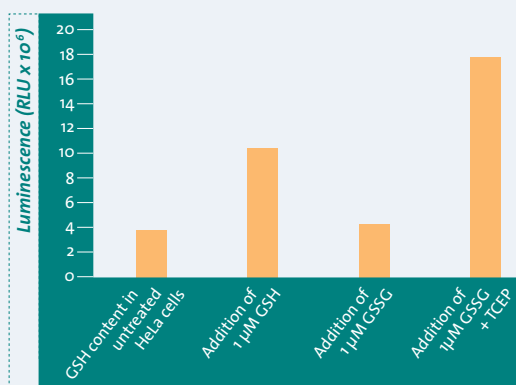


LIGHT

- Step 1:
- Lysis of mammalian cells in the presence of luciferin-NT and glutathione S-transferase
 - Generation of luciferin as a function of the GSH concentration

- Step 2:
- Detection of luciferin via the luciferase reaction
 - Light signal is proportional to the concentration of GSH in the cell

Measurement of reduced glutathione in HeLa cells



Using the GSH-Glo™ Assay, GSH levels were determined in untreated HeLa cells and after the addition of GSH, GSSG and GSSG treated with the reducing agent TCEP.

The GSH-Glo™ Assay measures only reduced glutathione (GSH) and is not affected by the presence of oxidized glutathione (GSSG). The addition of a reducing agent (e.g. TCEP) enables measurement of total glutathione content due to the conversion of GSSG to GSH.

Suitable for
3D-microtissues

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

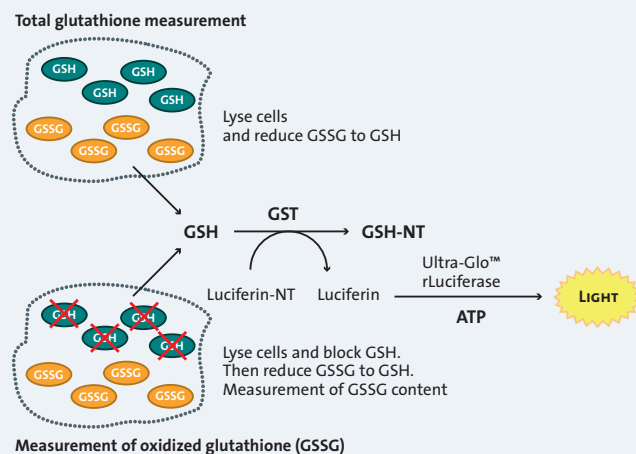
Since the cells are not transferred, the loss of GSH and GSSG is minimal compared with that in conventional assays. The GSH/GSSG-Glo™ assay can readily be adapted for 96-well and 384-well formats.

Assay principle

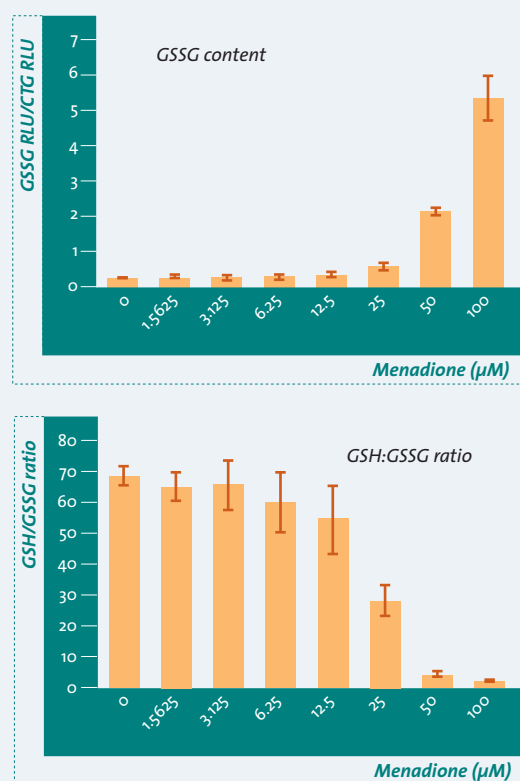
Total glutathione and GSSG are determined in two parallel reactions. The GSH level can be calculated by subtracting the GSSG level from the total glutathione level.

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 freed 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.



Measurement of oxidative stress in A549 lung carcinoma cells



Top: 5,000 cells/well were treated with a series of dilutions of menadione (60 minutes, 37°C). After measurement of GSSG, the data was normalized against a viability measurement using CellTiter-Glo™ (CTG) in order to compensate for the influence of toxic effects due to menadione and of experimental fluctuations. Menadione has a toxic effect in concentrations > 49 μM where incubation times are longer. Below: Determination of the GSH/GSSG ratio by measuring GSSG and total glutathione in A549 cells. It can be seen that, at higher concentrations, the menadione has a significant effect on the redox status of the cells.

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

V Epigenetic assays

“Epigenetics” is the term used to refer to changes in gene expression caused by mechanisms which are not determined by the DNA sequence. These regulatory mechanisms can switch individual genes and/or gene segments on and off without any change in the DNA sequence. Higher-level expression patterns emerge, which can be passed onto daughter cells. Some of the most important epigenetic regulatory mechanisms are DNA methylation, RNA interference and the modification of histones.

One of the goals of current research is to gain an understanding of the epigenetic regulatory processes during cellular differentiation. This knowledge will form the basis for new therapeutic concepts (“epigenetic therapy”) for the treatment of cancer, e.g. myelodysplastic syndrome, as well as for the diagnosis of cancer and hereditary diseases.

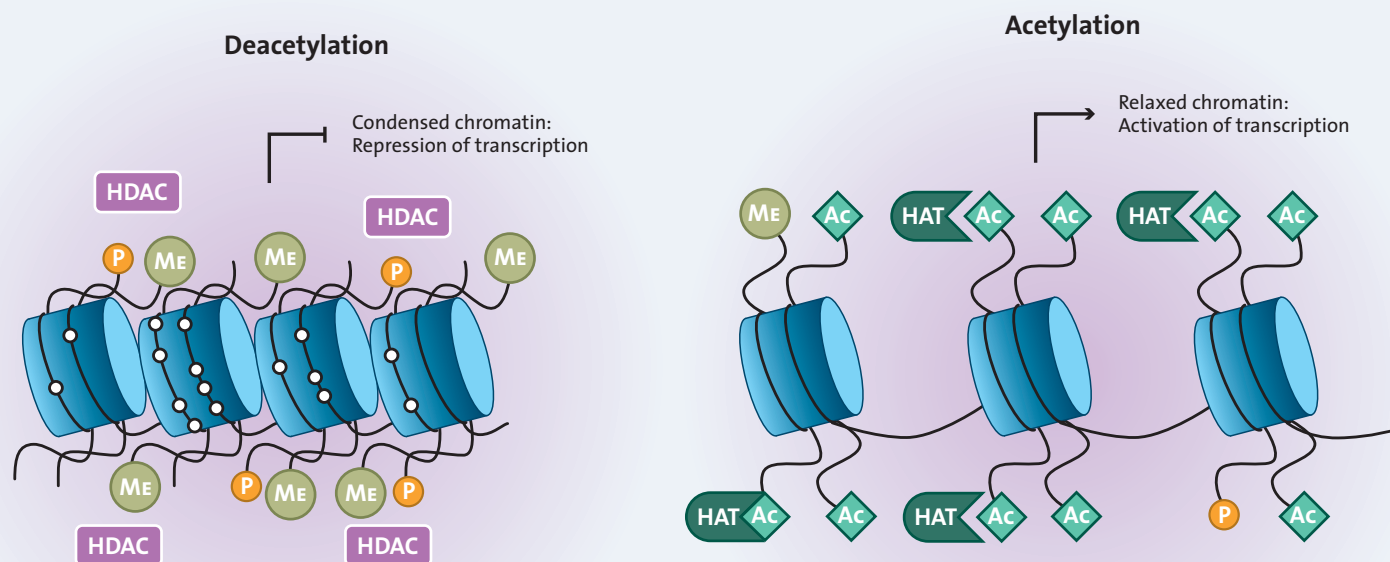
The histone-deacetylases (HDACs) are a promising therapeutic target in the treatment of tumors.

HDACs and the NAD⁺-dependent sirtuins (class III histone-deacetylases) are histone-modifying enzymes which catalyze the deacetylation of lysine residues in histones and thereby influence their activity. They play an important role in numerous biological processes. As anti-cancer agents, HDAC inhibitors arrest the cell cycle in tumour cells, contribute toward tumour differentiation and induce apoptosis. The development of new HDAC-selective inhibitors is a key research topic in both pure and applied research.

HDAC-Glo™ 2 Assay

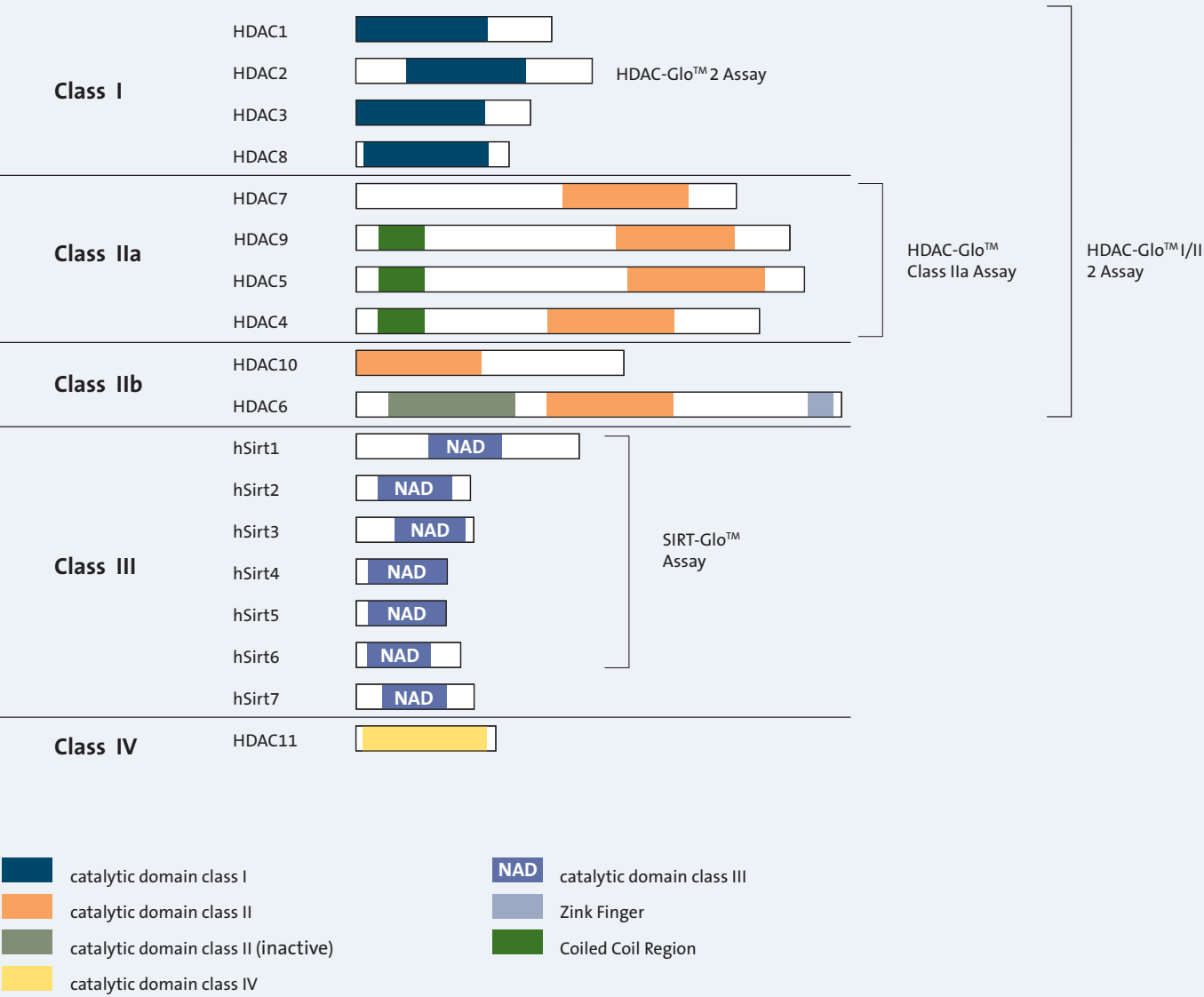
HDAC-Glo™ I/II Assay & HDAC-Glo™ I/II Screening Systems

SIRT-Glo™ Assay



Activation of histone-deacetylases (HDACs) causes lysine residues to be deacetylated at the N-terminal end of the histones. The associated change in the charge of the lysine residue means that the DNA is no longer accessible for transcription and the chromatin is condensed. The enzyme histone acetyltransferase (HAT) reverses this process, neutralizing the lysine residues through acetylation and rendering the DNA accessible for transcription.

Histone deacetylases (HDACs) classes from higher eukaryotes



Members of HDAC families. (Left) Schematic representation of domain structure of HDAC enzymes. (Right) Corresponding cell-based/biochemical assays for the determination of HDAC activity.

(Modified from: Hess-Stumpp, H. et al. (2007) MS-275, a potent orally available inhibitor of histone deacetylases--the development of an anticancer agent. *Int J Biochem Cell Biol.* 2007;39 (7-8):1388-1405.)

HDAC-Glo™ 2 Assay

Cell-based/Biochemical

Applications

Determination of HDAC inhibitor potency using purified enzymes, extracts or cells directly in culture plates; selectively profile HDAC inhibitors with purified enzymes; correlation of HDAC inhibitor potency with cellular fate in same-well multiplexed viability assays; determination of off-target HDAC effects of compounds.

Assay description

The **HDAC-Glo™ 2 Assay** is a single-reagent addition, homogeneous, luminescence assay that selectively measures the relative activity of HDAC2 enzyme from cells, extracts or recombinant sources. The assay uses an isoenzyme-selective, acetylated, live-cell-permeant, luminogenic peptide substrate that can be deacetylated by HDAC2 activity. A maximum signal is generated in as little as 20 minutes with persistent, “glow-type” steady state signal half-life. The assay provides 100-fold or better sensitivity than comparable fluorescence methods

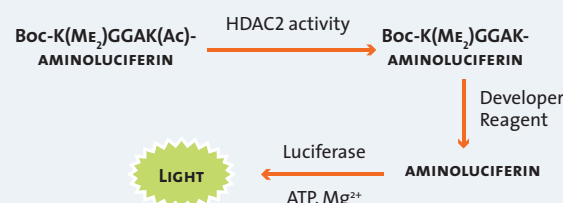
Assay principle

HDAC2 enzyme deacetylates the luminogenic substrate, Boc-K(Me₂)GGAK(AC)-aminoluciferin, making the peptide sensitive to a specific proteolytic cleavage event. Deacetylation of the peptide substrate is measured using a coupled enzymatic system in which the protease in the Developer Reagent cleaves the peptide from aminoluciferin, which is quantified in a reaction using Ultra-Glo™ Recombinant Luciferase. The three enzymatic events occur in a coupled, nearly simultaneous reaction that is proportional to deacetylase activity.

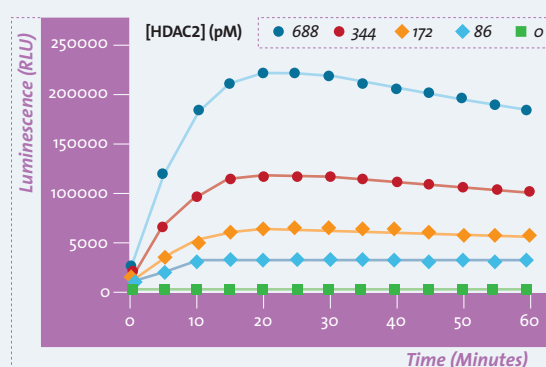
Assay features

Assay type	Luminescent (glow-type)
Markers	HDAC2
Applications	Determination of HDAC inhibitor potency using purified enzymes, extracts or cells directly in culture plates
Cell type/Sample	Cells or extracts, enzyme preparations
Implementation	Homogeneous, one-step assay
Time required	20–30 minutes
Robustness	Easily scalable from 96- to 384-well plate formats

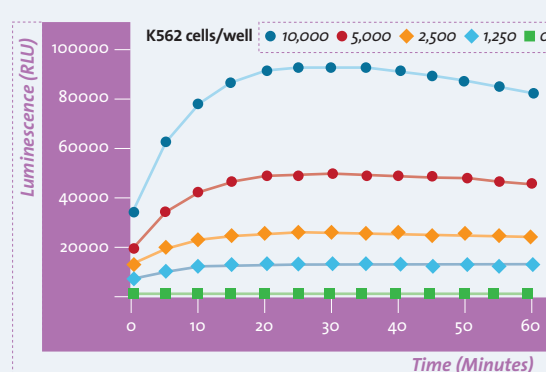
HDAC-Glo™ 2 Assay Principle



A. Activity Measurement of Purified HDAC2 Enzyme



B. Measurement of HDAC2 Enzyme Activity in Cells



HDAC2-mediated luminescent signal is proportional to deacetylase activity and is persistent (A) in a biochemical assay and (B) in a cell-based lytic assay. Enzymatic steady state (between deacetylase, developer enzyme and luciferase) is typically achieved within 20 minutes and has a half-life of ~ 60–90 minutes.

SIRT-Glo™ Assay

Biochemical

Applications

Determination of sirtuin enzyme activity; screening of SIRT inhibitors.

Assay description

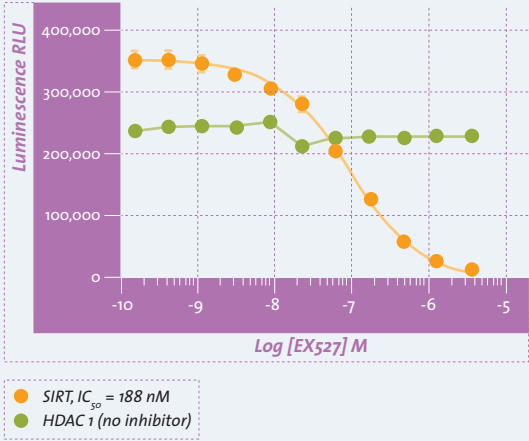
Determination of the enzyme activity of NAD⁺-dependent (class III histone deacetylase) sirtuins (SIRT 1-6). This assay is available as a biochemical assay.

Assay principle

The assay is based – like the HDAC assay – on enzymatic coupling of SIRT activity to the luciferase reaction. The reagents contain a cell-permeable luminogenic peptide substrate which contains the lysine peptide sequence derived from p53 and which is coupled to aminoluciferin.

Assay features

Assay type	Luminescent (glow-type)
Applications	Determination of SIRT 1–6 enzyme activities
Starting material	Purified enzymes
Implementation	Homogeneous, one-step assay, automatable
Time	15–45 minutes
Robustness	96- to 1536-well formats



EX-527 – a potent and very specific SIRT inhibitor – was serially diluted in SIRT-Glo buffer or HDAC-Glo buffer. Recombinant SIRT1 (Enzo) or HDAC1 (Enzo) enzyme was diluted and added to the EX-527 dilution series. The mixtures were incubated for 30 minutes at room temperature. SIRT-Glo™ Assay reagent or HDAC-Glo™ I/II Assay reagent was added and after 30 minutes measured in the luminometer.

This data shows that sirtuin inhibitors do not also inhibit HDAC I/II enzymes – even through there are exceptions. HDAC I/II enzymes are typically inhibited by compounds which act as chelating agents for zinc from the active centre of the enzyme. These compounds in turn do not inhibit sirtuins, as the latter use a mechanism for deacetylation that requires NAD⁺ as a cofactor.

VI Cell signaling pathways

The study of cell signaling pathways is of particular importance to the understanding of cellular processes. These involve numerous proteins and secondary messengers which translate extracellular information via signaling cascades and relay it into the cell interior. Cellular processes such as proliferation, differentiation and apoptosis are controlled in this way. Signaling cascades are generally induced through the binding of extracellular ligands, such as growth factors, cytokines, neurotransmitters or hormones, to the relevant cell receptors. G-protein-coupled receptors (GPCRs) constitute one of the largest families of receptors within the human genome. More than half of all the active substances that have been launched on the market are targeted at these. The main class of GPCRs influences cellular levels of cAMP, a secondary messenger which influences numerous effector systems and, *inter alia*, gene transcription. Following treatment of cells with GPCR modulators, cAMP levels in cells can be determined using the cAMP-Glo™ Assay. The secondary messenger cAMP activates protein kinase A (PKA), which in turn via protein phosphorylation assists signal transduction. Protein phosphorylation is the most common post-translational modification leading to the transduction and amplification of primary signals. Both kinases and phosphatases therefore play a key role in many sig-

naling pathways. The activities of purified kinases and ATPases can be determined using the ADP-Glo™ or Kinase-Glo® Assays. The phosphodiesterases (PDEs) are a further important group involved in signal transduction. They regulate signal transduction through degradation of the secondary messengers cAMP and cGMP. The activity of purified PDEs can be determined using the PDE-Glo™ Phosphodiesterase Assay. GTPases play a major role in various cellular functions such as cell signaling, cell proliferation, cell differentiation, cytoskeleton modulation, and cell motility. GTPases are an important part of cell signaling since they are acting as molecular switches that cycle between an activated GTP-bound state and an inactive GDP-bound state. Deregulation or mutation of these proteins has considerable consequences resulting in multiple pathological conditions.

cAMP-Glo™ Max Assay

ADP-Glo™ Kinase Assay

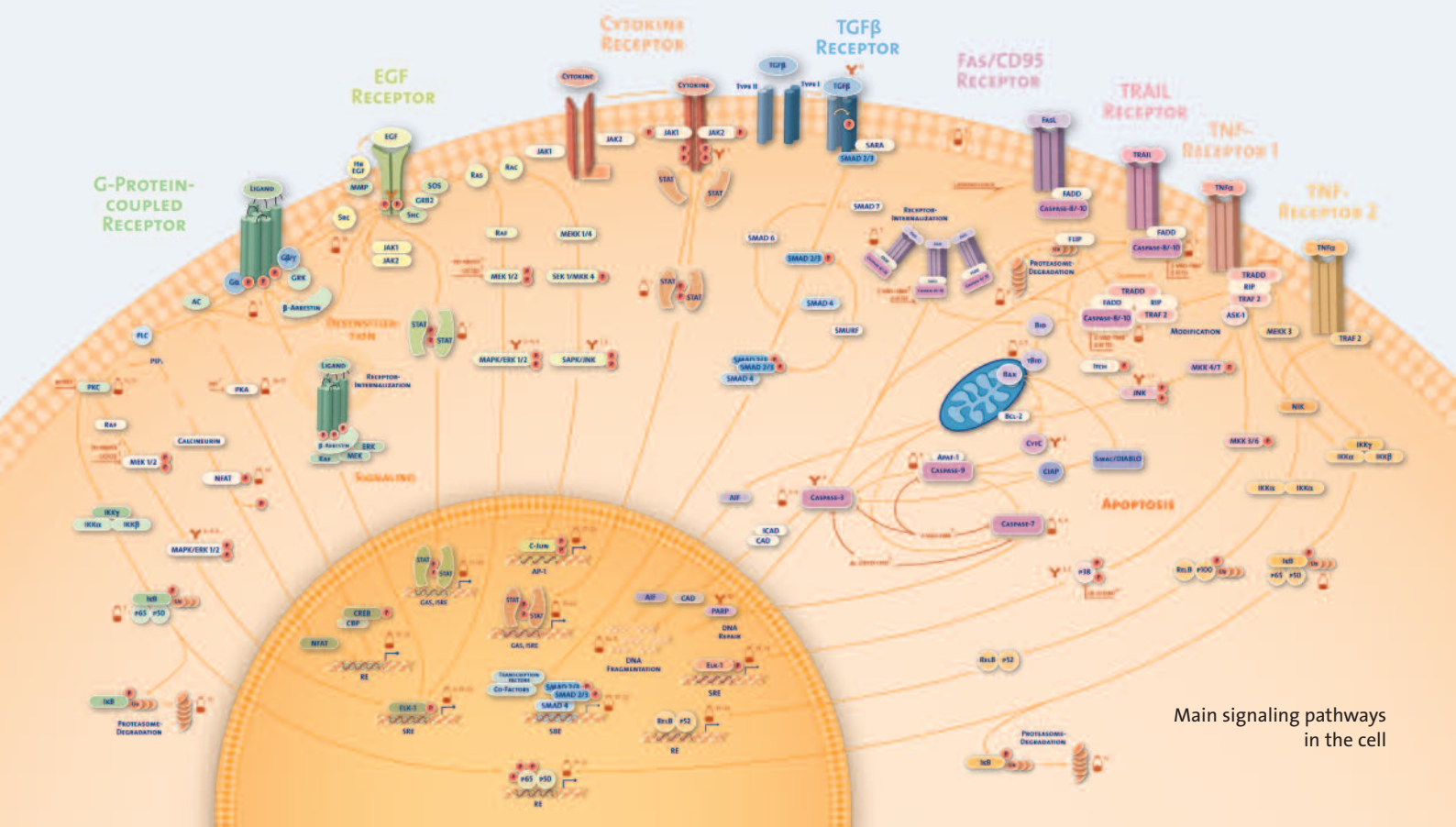
ADP-Glo™ Max Assay

Kinase-Glo® Luminescent Kinase Assay

AMP-Glo™ Assay

PDE-Glo™ Phosphodiesterase Assay

GTPase-Glo™ Assay



Main signaling pathways in the cell

cAMP-Glo™ Max Assay (GPCR signaling pathway)

Cell-based/Biochemical

Applications

High-throughput analysis of library compounds to identify modulators of GPCR activity; quantitation of cAMP levels in broad range of cell types.

Assay description

The **cAMP-Glo™ Max Assay** is a homogeneous, bioluminescent and high-throughput assay to measure cyclic AMP (cAMP) levels in cells. Compounds that modulate GPCRs coupled with adenylate cyclase typically alter intracellular cAMP levels. The cAMP-Glo™ Max Assay monitors cAMP levels in cells in response to the effect of agonists, antagonists or test compounds on G protein-coupled receptors (GPCRs). The assay is based on the principle that cyclic AMP (cAMP) stimulates protein kinase A (PKA) holoenzyme activity, decreasing available ATP and leading to decreased light production in a coupled luciferase reaction. This improved version combines the lysis and cAMP reaction buffers into the cAMP-Glo™ ONE Buffer.

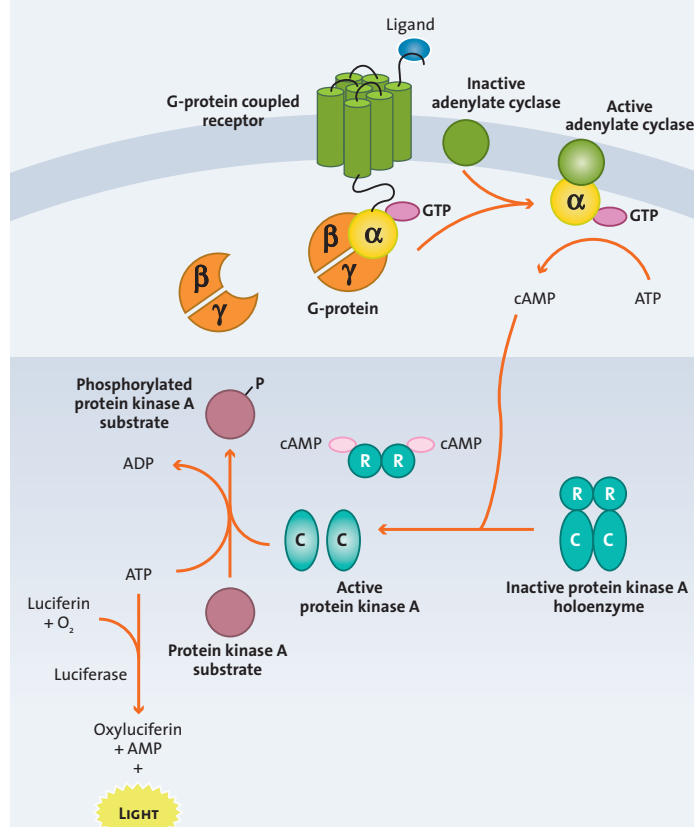
Assay principle

Cells are induced with a test compound for an appropriate period of time to modulate cAMP levels. After induction, cells are lysed, and the cAMP released stimulates protein kinase A in the reagent. The Kinase-Glo® Reagent is then added to terminate the PKA reaction and detect the remaining ATP via a luciferase reaction. The half-life for the luminescent signal is greater than 4 hours providing ample time to read the plates and eliminating the need for luminometers with reagent injectors.

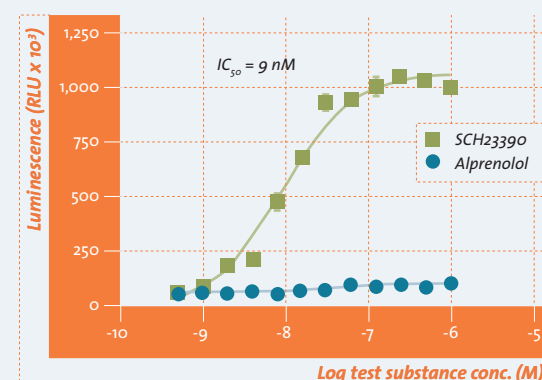
Assay features

Assay type	Luminescent (glo-type; T1/2 > 4 h)
Applications	Quantitative determination of cAMP content
Cell type	Cell lines
Implementation	Homogeneous, two-step assay, improved protocol: lysis and cAMP detection steps combined (cAMP-Glo™ ONE Buffer).
Time required	30 minutes
Signal-to-background	> 200 (with cAMP), >15 (on cells)
Robustness	Reactions are scalable in 96-, 384-, 1536-well plates

Representation of cAMP formation in the cell and the assay principle of the cAMP-Glo™-Assay



Determination of cAMP content after treatment of cells with GPCR modulators



Antagonist-dependent dose-effect curve based on the example of the dopamine D1 receptor. D293 cells stably expressing the dopamine D1 receptor (5,000 cells/well) were treated in the presence of 100 nM dopamine (agonist) with various concentrations of the test substance SCH23390 (antagonist). In the control mixture, the substance alprenolol was used. IC₅₀ values obtained correlate with competitive radioactive binding assays.

ADP-Glo™ Kinase Assay

Biochemical

Applications

Determination of the effects of kinase activators or inhibitors; identification of selective active substances against the target kinases; determination of the activity of immunoprecipitated kinases.

Assay description

The universal **ADP-Glo™ Kinase Assay** family is used for detecting kinase activities and ATPases and has proven particularly successful with difficult kinases such as receptor tyrosine kinases. The **ADP-Glo™ Assay** is a simple, fast and highly sensitive *in vitro* method for which no radioactivity is required and which can be carried out with any kinase substrate (lipid, peptide, protein or sugar). In this assay, the ATP can be added to the kinase reaction over a very broad linear range of concentrations (micromolar to millimolar). This makes it possible to distinguish between competitive and noncompetitive inhibitors. High signal-to-noise ratios can thus be achieved even at low ATP-to-ADP conversion (0.2 pmol ADP) and enable automation and miniaturization of the assay at optimal Z' values > 0.7.

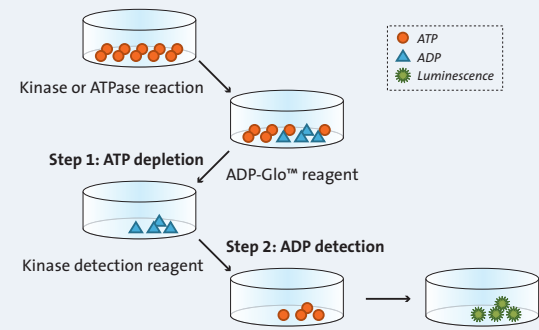
Assay principle

The **ADP-Glo™ Assay** is performed in two steps. In the first step, addition of the ADP-Glo™ assay reagent terminates the kinase reaction and depletes the ATP remaining in the reaction mixture. In the second step, the ADP which has been produced by the kinase activity is converted into ATP. This newly synthesized ATP provides a limiting factor for the subsequent luciferase reaction. The stable light signal is directly proportional to the kinase activity.

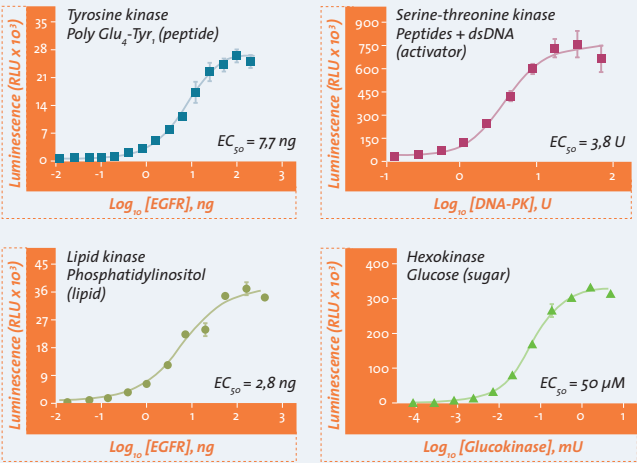
Assay features

Assay type	Luminescent (glow-type)
Applications	Quantitative determination of kinase activity
Sample material	Purified kinases
Implementation	Homogeneous, two-step assay
Time required	60–90 minutes
Sensitivity	Detection even at very low enzyme concentrations: 0.2 pmol ADP
Robustness	Reactions are scalable in 96-, 384-, 1536-well plates

Easy to perform:



The ADP-Glo™ Kinase Assay is suitable for detecting a wide variety of kinases, irrespective of the substrate class



Determination of various kinase activities using the ADP-Glo™ Kinase Assay

ADP-Glo™ Max Assay

Biochemical

Applications

Determination of ATP-depleting enzymes with high K_m values for ATP.

Assay description

ADP-Glo™ Max Assay is a further development of the ADP-Glo™ Kinase Assay. Whereas in the ADP-Glo™ Kinase Assay up to 1 mM ATP can be used, the **ADP-Glo™ Max** tolerates concentrations up to 5 mM ATP and is therefore particularly suitable for enzymes with high K_m values for ATP, such as ATPases.

Assay principle

The assay principle and method of implementation of the **ADP-Glo™ Max Assay** are identical to those of the ADP-Glo™ Kinase Assay.

Assay features

Assay type	Luminescent (glow-type)
Applications	Quantitative determination of ATP-depleting enzymes with a high K_m value for ATP
Sample material	Purified kinases, ATPases
Implementation	Homogeneous, two-step assay
Time required	60–90 minutes
Sensitivity	Detection even at very low enzyme concentrations
Robustness	Reactions are scalable in 96-, 384-, 1536-well plates

Kinase-Glo® Luminescent Kinase Assay

Biochemical

Applications

Screening and identification of kinase inhibitors; differentiation of ATP-competitive and non-competitive inhibitors.

Assay description

The **Kinase-Glo® Luminescent Kinase Assay** family is used for determining the activity of purified kinases by quantifying the levels of ATP remaining in the reaction mixture. The assays in this family are homogeneous, easy to perform and non-radioactive. Furthermore, any kinase substrate (peptide, protein, lipid or sugar) can be used in this universal assay format. **Kinase-Glo® Assays** are miniaturizable and suitable for high-throughput methods, having long signal stability ($T_{1/2} > 5$ h) and excellent Z' values. The Kinase-Glo® platform consists of three assay formats, which can be used to determine the kinase activities within defined ATP concentration ranges:

Kinase-Glo® Assay: 10 μ M ATP

Kinase-Glo® Plus Assay: 100 μ M ATP

Kinase-Glo® Max Assay: 500 μ M ATP

Assay principle

The addition of the Kinase-Glo® reagent terminates the kinase reaction. ATP which has not been consumed by the kinase is converted by Ultra-Glo™ Luciferase into a stable light signal. This signal is inversely proportional to the kinase activity.

Assay features

Assay type	Luminescent (glow-type)
Applications	Quantitative determination of kinase activities on the basis of residual ATP content
Sample material	Purified kinases
Implementation	Homogeneous, one-step assay
Linearity	Linear up to an ATP concentration of 500 μ M ATP
Time required	10–15 minutes
Robustness	Reactions are scalable in 96-, 384-, 1536-well plates

AMP-Glo™ Assay

Biochemical

Applications

Quantitatively monitor the concentration of AMP in a biochemical reaction; screen library compounds for effects on target enzymes in high-throughput formats

Assay description

The **AMP-Glo™ Assay** is a homogeneous assay that generates a luminescent signal from any biochemical reaction that produces AMP as a reaction product. This versatile system can measure the activity of a broad range of enzymes, such as cyclic AMP-specific phosphodiesterases, aminoacyl-tRNA synthetases, DNA ligases and ubiquitin ligases or enzymes modulated by AMP. The stable luminescent signal allows batch-mode processing of multiple plates. The assay can be used to determine the AMP produced either in the presence or absence of ATP as a substrate.

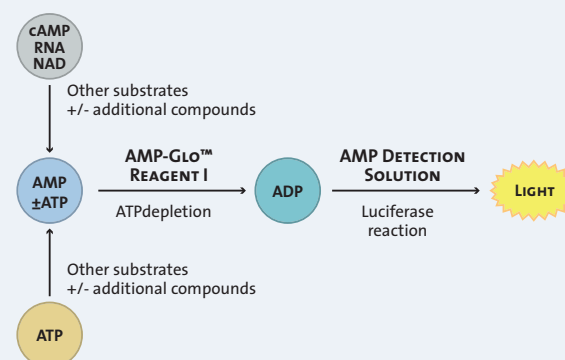
Assay principle

The assay contains two reagents: one to terminate the AMP-generating enzymatic reaction and simultaneously remove ATP and convert AMP produced into ADP, and a second reagent that converts the ADP to ATP followed by conversion of the ATP into a luminescent signal using the luciferin/luciferase reaction. The assay also is well suited for monitoring AMP produced in biochemical reactions catalyzed by enzymes that do not use ATP as a substrate, such as cAMP-dependent phosphodiesterases (PDE) and bacterial DNA ligases.

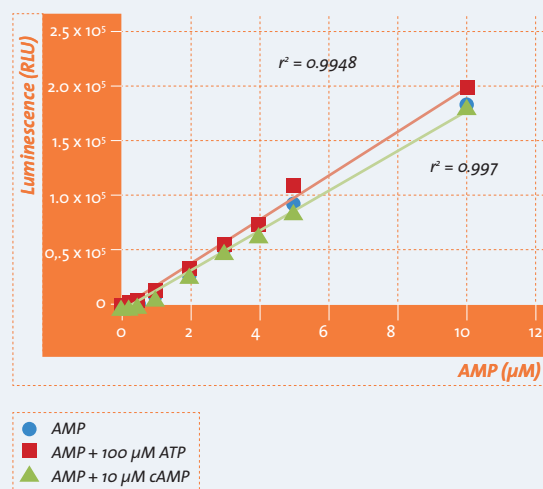
Assay features

Assay type	Luminescent (glo-type)
Applications	Quantitative determination of AMP
Sample type	Ubiquitin ligases, cyclic AMP-specific phosphodiesterases, aminoacyl-tRNA synthetases, DNA ligases, poly(A) deadenylases, demethylases (indirect method)
Implementation	Homogeneous, two-step assay
Time required	2 hours
Signal Strength at Low Substrate	Determination of enzyme activity that more closely mimics physiological conditions—suited for low-activity enzymes
Conversion	
Robustness	Minimal false hits and Z' values greater than 0.7, reactions are scalable in 96-, 384-, 1536-well plates

Substrates for enzymes that do not use ATP.



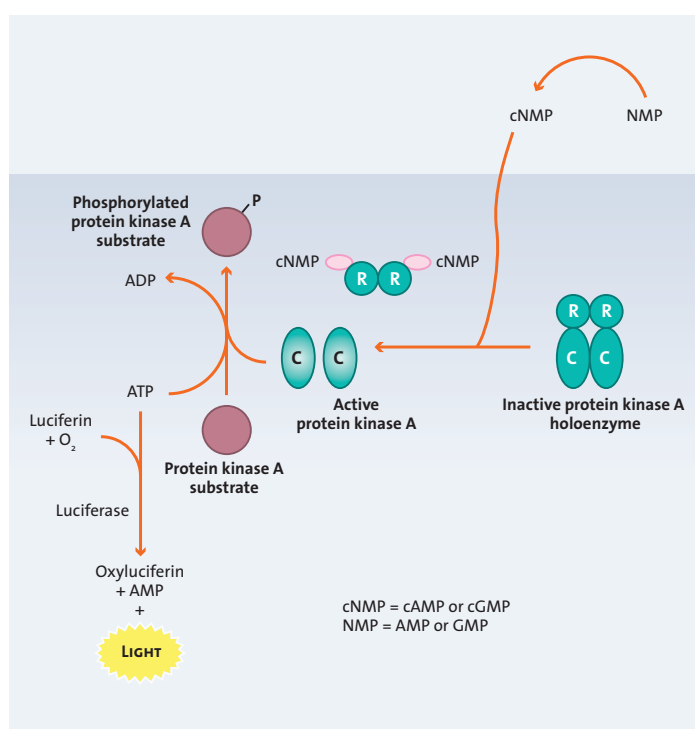
No Interference by ATP or cAMP



Titration of purified AMP. Reactions were assembled with the indicated concentrations of pure AMP in a low-volume, 384-well plate. AMP was titrated alone, with 100 μM ATP or with 10 μM cAMP. Data were collected using a plate-reading luminometer. Each point represents the average of four separate reactions.

Phosphodiesterases

Cyclic nucleotide phosphodiesterases (PDEs) occur in different tissues and organs and hydrolyze the second-messenger signaling molecules cAMP and cGMP. Due to this ability, PDEs are involved in innumerable cellular processes and have been linked to various disease patterns such as asthma and autoimmune disorders. The availability of selective PDE inhibitors has facilitated the study of the effects of cyclic nucleotide signaling. This has made it possible to investigate the role of PDEs in cellular or tissue changes.



Assay features

Assay type	Luminescent (glow-type)
Applications	Quantitative determination of phosphodiesterase activities
Sample material	Purified phosphodiesterases
Implementation	Homogeneous, three-step assay
Time required	1 hour
Robustness	Suitable for HTS; excellent signal-to-background ratio

PDE-Glo™ Phosphodiesterase Assay

Biochemical

Applications

Determination of cyclic nucleotide phosphodiesterase activity.

Assay description

PDE-Glo™ Phosphodiesterase Assay is a robust and reliable assay for determining the activity of purified PDEs. The assay is optimized for the use of both cAMP-specific and cGMP-specific phosphodiesterases. This simple and sensitive assay can be performed in less than 60 minutes. In addition, its miniaturizability, long signal stability and excellent signal-to-noise ratio make it ideal for use in automated high-throughput measurements.

Assay principle

The **PDE-Glo™ Phosphodiesterase Assay** comprises the addition of three different reagents to the reaction mixture. In the first step of the assay, the phosphodiesterase reaction is terminated by the addition of the PDE-Glo™ termination buffer. The PDE detection solution contains inactive protein kinase A (PKA) holoenzyme, a PKA substrate and ATP. The binding of cyclic nucleotide monophosphates (cNMPs) to the inactive PKA holoenzyme causes a conformational change in which the holoenzyme releases its catalytic sub-units, which then catalyze the transfer of the terminal phosphate of ATP to a PKA substrate, consuming the ATP in the process. The residual quantity of ATP can now be determined using the luciferase-based Kinase-Glo® reagent. Since the phosphodiesterases can hydrolyze cAMP and cGMP to AMP and GMP respectively, the quantity of cyclic nucleotide monophosphate decreases. The less cyclic nucleotide monophosphate is present in the reaction mixture, the less PKA can be activated and the less ATP consumed, so the latter is now available for the Ultra-Glo™ Luciferase reaction. The result is increased bioluminescence. This luminescence is thus directly proportional to the residual quantities of ATP, which in turn are inversely proportional to PDE activity.

GTPase-Glo™ Assay

Biochemical

Applications

Measure the effects of protein modulators, such as GAP and GEF proteins, on GTPase activity; can be used for high-throughput applications.

Assay description

GTPase-Glo™ Assay measures intrinsic GTPase activity, GAP-stimulated GTPase activity, GAP activity and GEF activity, which are components of GTPase cycle. GTPase, GAP and GEF activity is inversely correlated to the amount of light produced. A highly active GTPase hydrolyzes more GTP, reducing the amount of ATP produced from GTP and reducing light output. A less active GTPase hydrolyzes less GTP, leaving a larger amount of ATP to be converted to ATP and producing more light.

Assay principle

The **GTPase-Glo™ Assay** is a simple "add and read" method for measuring GTPase activity by detecting the amount of GTP remaining after GTP hydrolysis in a GTPase reaction. The remaining GTP is converted to ATP using the GTPase-Glo™ Reagent, followed by ATP detection using a proprietary thermostable luciferase (Ultra-Glo™ Recombinant Luciferase) and luciferin substrate to produce bioluminescence. The kit contains optimized reaction buffers, GTPase/GAP Buffer and GEF buffer, for performing GTPase/GAP reactions or GEF reactions, respectively.

Assay features

Assay type	Luminescent (glo-type)
Markers	GTPase activity, GAP-stimulated GTPase activity, GAP activity and GEF activity
Applications	Measure the effects of protein modulators, such as GAP and GEF proteins, on GTPase activity; can be used for high-throughput applications.
Implementation	Homogeneous, two-step assay
Time required	GTPase reaction: 60–120 minutes; GTPase detection: 35–40 minutes (after adding the reagent)
Sensitivity	Excellent signal-to-noise ratios at low enzyme concentrations
Robustness	Reactions are scalable in 96-, 384- and 1536-well plates

The GTPase cycle and schematic of the GTPase-Glo™ Assay

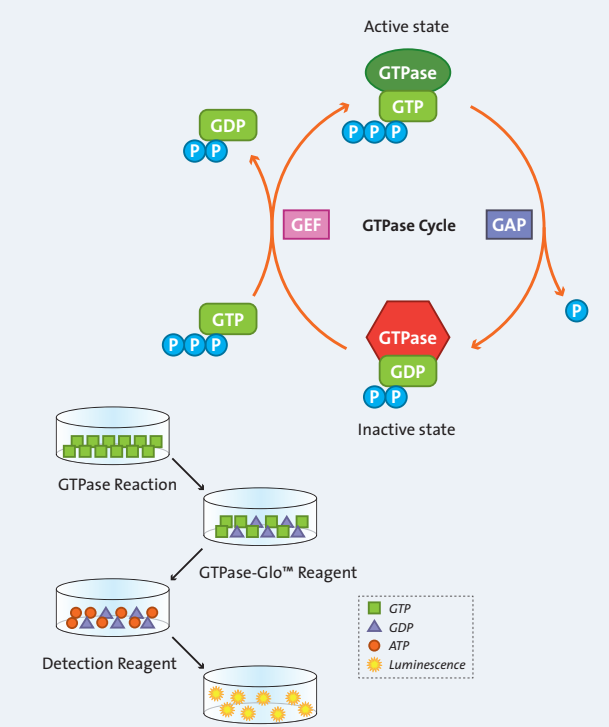
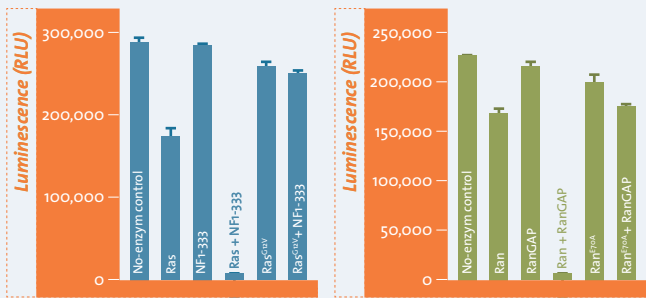


Diagram showing GTPase in active and inactive phases in relation to GDP, GTP. B. A diagram of the GTPase-Glo™ Assay. Following the GTP producing reaction, GTPase-Glo™ Reagent converts the remaining GTP to ATP. Then Detection Reagent is added, converting the ATP to a luminescent signal.

Stimulation of GTPase activity by GAP proteins



A. Reactions contain 2 μM of wildtype or mutant Ras (RasG12V) plus 1 μM of NF1-333 (GAP protein) in GTPase/GAP Buffer. B. Reactions contain 2 μM of wildtype or mutant Ran (RanE70A) plus 1 μM of RanGAP (GAP protein) in GTPase/GAP Buffer. Lower luminescence signals indicate higher GTPase activities in the reaction.

VII Glycobiology

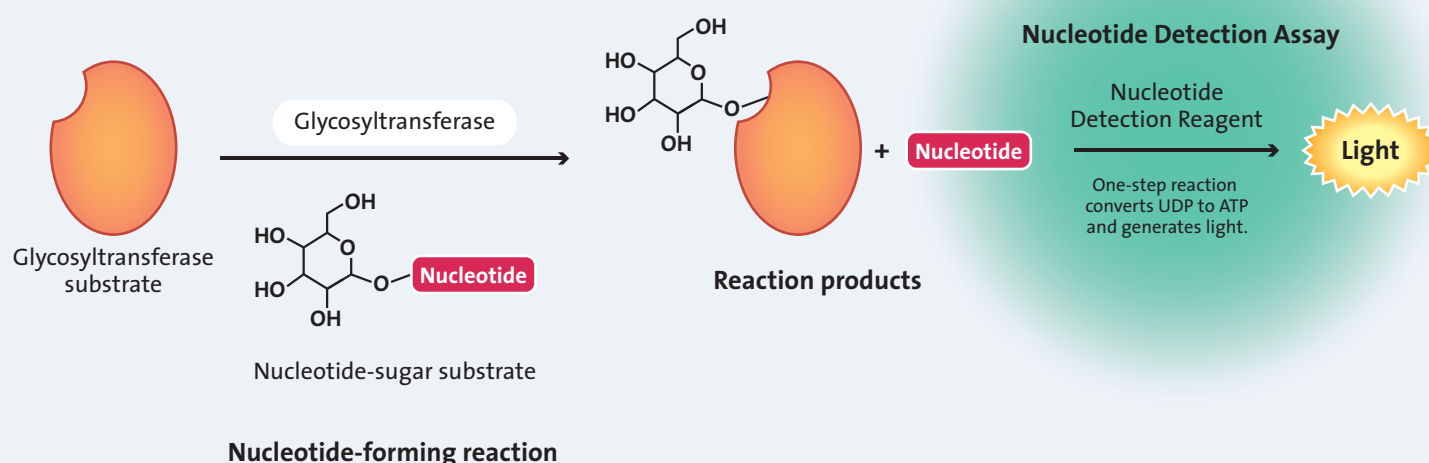
Glycosylation reactions catalyzed by glycosyltransferases are central to many biological processes, including cell:cell interactions, cell signaling and bacterial cell wall biosynthesis. Glycosyltransferases transfer sugar from a nucleotide-glycosyl donor (e.g. UDP-Galactose, UDP-Glucose, UDP-GlcNAc, UDP-GalNAc and UDP-Glucuronic acid) to an acceptor molecule. Other sugars are typical glycosyltransferase acceptor substrates but can also be lipids, proteins, nucleic acids, xenobiotics or other small molecules. Traditional assays for Glycosyltransferase (GT) activity are not easily configured for rapid GT activity detection nor for high throughput screening because they rely on detection of radiolabeled substrate which requires product isolation, the use of non-homogenous antibody-based

assays or mass spectrometry. In a glycosyltransferase reaction, the nucleotide moiety is released as a product; therefore, an assay that detects nucleotides as the universal product of these reactions would be suitable for monitoring the activity of the majority of glycosyltransferases. Promega has developed homogenous, bioluminescent detection assays for measuring glycosyltransferase activities based on UDP, GDP, and CMP quantification.

UDP-Glo™ Glycosyltransferase Assay

GDP-Glo™ Glycosyltransferase Assay

UMP/CMP-Glo™ Glycosyltransferase Assay



UDP-Glo™ Glycosyltransferase Assay

Biochemical

Applications

Profiling glycosyltransferase (GT) specificity for different UDP-sugars; screening library compounds for effects on GT enzyme activity; detection of chemical compound glucuranidation during drug discovery.

Assay description

The UDP-Glo™ Glycosyltransferase Assay is a bioluminescent assay for detecting the activity of GTs that use UDP-sugars as donor substrates and release UDP as a product. Glycosylation reactions catalyzed by GTs are central to many biological processes, including cell:cell interactions, cell signaling and bacterial cell wall biosynthesis. The assay is highly sensitive and robust which is essential for measuring the activity of different UDP-sugar utilizing GTs covering the majority of GT classes. Therefore, the UDP detection assay allows significant savings of enzyme usage in GT reactions.

Assay principle

The UDP-Glo™ Glycosyltransferase Assay is a homogeneous, single-reagent-addition method to rapidly detect UDP formation in GT reactions. GTs transfer sugar from a UDP-glycosyl donor to an acceptor molecule like peptide, protein and lipids. In a GT reaction, the UDP moiety is released as a product, after which an equal volume of UDP Detection Reagent is added to simultaneously convert the UDP to ATP and generate light in a luciferase reaction. The light output is proportional to the concentration of UDP from low nM to 25 μM UDP. The assay is intended for use with purified or immunoprecipitated GTs.

Assay features

Assay type	Luminescent, (glow-type, $T_{1/2} > 3$ h), homogeneous one-step assay
Markers	UDP
Applications	Profiling GT specificity for different sugars
Sample	Native or purified (tagged or affinity bound to beads) GTs
Time required	60 minutes
Linearity	From nM up to 25 μM
Sensitivity	0.1–0.5 pmol of UDP with a more than two fold difference over background
Robustness	Reactions are scalable in 96-, 384- and 1536-well plates

UDP-Glo™ Glycosyltransferase Assay principle

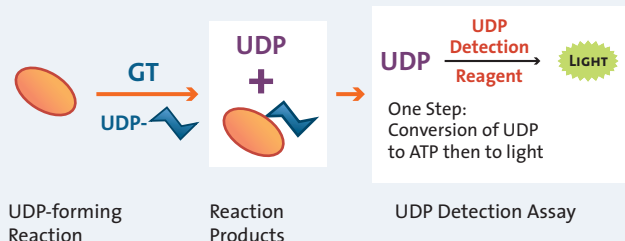
Simple “Add and Read”:
No radioisotopes.
No product separation.
No HPLC

96-well plate

25 μl GT reaction
(UDP-sugar donor + Acceptor substrate + GT)
30–60 min incubation

25 μl UDP Detection Reagent
60 min incubation

Record Luminescence



UDP-Glo™ Glycosyltransferase Assay is especially suitable for screening of selective and potential glycosyltransferase inhibitors.

O-GlcNAc Transferase reaction (25 μl)

50 μM OGT peptide substrate
100 μM UDP-N-Acetylglucosamine
2.5 ng OGT
0–500 μM inhibitors
60 min incubation

25 μl UDP Detection Reagent
60 min incubation

Record Luminescence

OGT-Inhibitors dose response



■ S TO78925 $IC_{50} = 55.5 \mu M$
● S TO45849 $IC_{50} = 57.7 \mu M$

OGT: O-GlcNAc transferase

GDP-Glo™ Glycosyltransferase Assay

Biochemical

Applications

Profiling glycosyltransferase (GT) specificity for any GDP sugars; Screening for specific glycosyltransferase inhibitors and the study of their mode of action; Ideal for low-activity glycosyltransferases

Assay description

The **GDP-Glo™ Glycosyltransferase Assay** is a homogeneous, single-reagent-addition method to rapidly detect GDP formation in glycosyltransferase reactions. The GDP-Glo™ Assay generates a stable luminescent using the thermostable Ultra-Glo™ Recombinant Luciferase, making it convenient to batch-process multiple assay plates across a wide range of assay conditions. The Assay is highly sensitive and can detect low GDP concentrations with high dynamic range and signal-to-background ratio for assaying low-activity glycosyltransferase. The GDP-Glo™ Assay can be used in screening for specific glycosyltransferase inhibitors and the study of their mode of action.

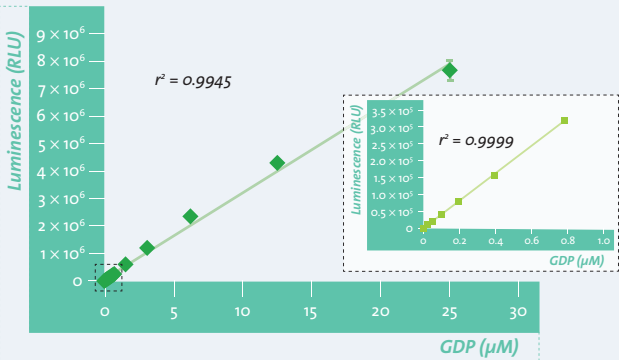
Assay principle

After the glycosyltransferase reaction, an equal volume of GDP Detection Reagent is added to simultaneously convert the GDP product to ATP and generate light in a luciferase reaction. The light generated is detected using a luminometer, and the light output is proportional to the concentration of GDP from low nM to 25µM. Luminescence can be correlated to GDP concentration by using a GDP standard curve.

Assay features

Assay type	Luminescent (glow-type, T _{1/2} > 3 h), one-step assay
Markers	GDP
Applications	Detects any glycosyltransferase that uses GDP-sugar as a substrate
Sample	Native or purified (tagged or affinity bound to beads) GTs
Time required	60 minutes
Linearity	Linear response from low nM to 25 µM
Sensitivity	Detect 0.05–0.1 pmol GDP with a more than twofold difference over background
Robustness	Z' factor values > 0.7, Reactions are scalable in 96-, 384- and 1536-well plates

Linearity and sensitivity of the GDP-Glo™ Glycosyltransferase Assay

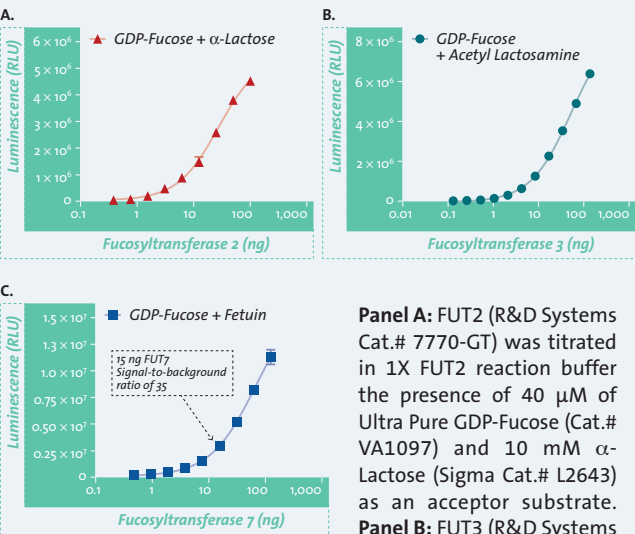


GDP (µM)	25	12.5	6.25	3.13	1.56	0.78	0.39	0.20	0.10	0.05	0.02	0
	35,124	19,758	10,898	5,682	2,965	1,457	715	369	183	96	46	1

Signal-to-Background Ratio at 60 minutes

GDP standard curve was prepared over the indicated range of GDP concentrations in 25 µl of 1X glycosyltransferase reaction buffer in a solid white 96-well plate. Values represent the mean of four replicates. Signal-to-background ratios were calculated for each concentration of the GDP standard curve.

Detection of the activity of various GDP-sugar-utilizing enzymes



Panel A: FUT2 (R&D Systems Cat.# 7770-GT) was titrated in 1X FUT2 reaction buffer the presence of 40 µM of Ultra Pure GDP-Fucose (Cat.# VA1097) and 10 mM α-Lactose (Sigma Cat.# L2643) as an acceptor substrate. **Panel B:** FUT3 (R&D Systems Cat. #4950-GT) was titrated

in 1X FUT3 reaction buffer in the presence of 40 µM of Ultra Pure GDP-Fucose and 100 mM Acetyl Lactosamine (Carbosynth Cat.# OA08244). **Panel C:** FUT7 (R&D Systems Cat.# 6409-GT) was titrated in 1X FUT7 reaction buffer the presence of 40 µM of Ultra Pure GDP-Fucose and 20 µM Fetuin (Sigma Cat# F2379). Each point is an average of two experiments, and the error bars represent the standard deviations. The inserts highlight FUT activity at nanogram amounts with a high signal-to-background ratio.

UMP/CMP-Glo™ Glycosyltransferase Assay

Biochemical

Applications

Detection of sialyltransferases and phosphoglycosyltransferases that use CMP-, CDP- or UDP-sugars as donor substrates; Ideal for low-activity glycosyltransferases

Assay description

The **UMP/CMP-Glo™ Glycosyltransferase Assay** is a homogeneous, single-reagent-addition method to rapidly detect UMP or CMP formation in glycosyltransferase reactions. The UMP/CMP-Glo™ Assay generates a stable luminescent signal using the thermostable Ultra-Glo™ Recombinant Luciferase, making it convenient to batch-process multiple assay plates across a wide range of assay conditions. The Assay is highly sensitive and can detect low UMP/CMP concentrations with high dynamic range and signal-to-background ratio for assaying low-activity glycosyltransferase. The UMP/CMP-Glo™ Assay can be used in screening for specific glycosyltransferase inhibitors and the study of their mode of action.

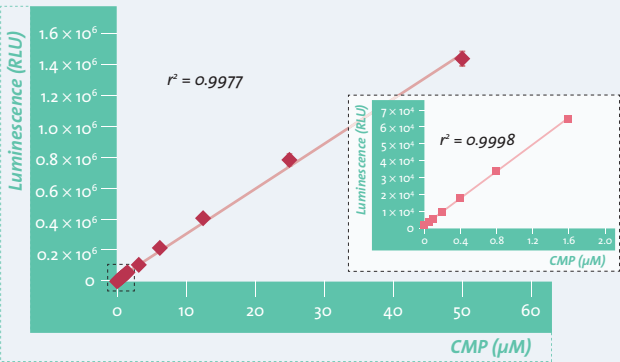
Assay principle

After the glycosyltransferase reaction, an equal volume of UMP/CMP Detection Reagent is added to simultaneously convert the UMP or CMP product to ATP and generate light in a luciferase reaction. The light generated is detected using a luminometer, and the light output is proportional to the concentration of UMP or CMP from low nM to 50μM. Luminescence can be correlated to UMP or CMP concentration by using a UMP or CMP standard curve.

Assay features

Assay type	Luminescent (glow-type, $T_{1/2} > 3$ h), one-step assay
Markers	UMP/CMP
Applications	Detects any glycosyltransferase that uses UMP/CMP-sugar as a substrate
Sample	Native or purified (tagged or affinity bound to beads) GTs
Time required	60 minutes
Linearity	Linear response from low nM to 50 μM
Sensitivity	Detect 1.25–2.5pmol UMP/CMP with a more than twofold difference over background
Robustness	Z' factor values > 0.7; Reactions are scalable in 96-, 384- and 1536-well plates

Linearity and sensitivity for CMP using the UMP/CMP-Glo™ Glycosyltransferase

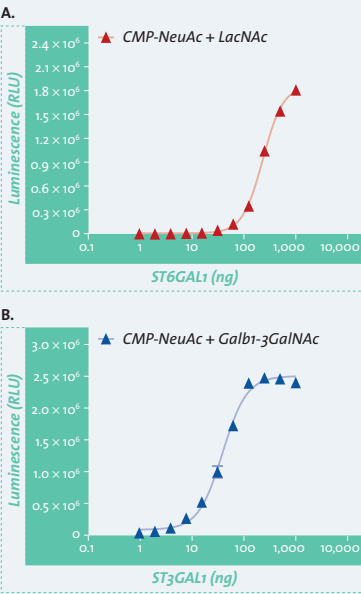


50	25	12.5	6.25	3.13	1.56	0.78	0.39	0.20	0.10	0.05	0
921	503	262	139	70	37	19	10	6	3	2	1

Signal-to-Background Ratio at 60 minutes

CMP standard curve was prepared over the indicated range of CMP concentrations in 25μl of 1X glycosyltransferase reaction buffer in a white 96-well plate. Values represent the mean of two replicates. Signal-to-background ratios were calculated for each concentration of the CMP standard curve.

Detection of the activity of various sialyltransferases



Panel A: ST6GAL1 (R&D Systems Cat.#7620-GT) was titrated in 1X ST6GAL1 reaction buffer in the presence of 100 μM of CMP-NeuAc (Sigma Cat.# C8271) and 1 mM LacNAc (Dextra Cat.# GN204) as an acceptor substrate.

Panel B: ST3GAL1 (R&D Systems Cat.# 6905-GT-020) was titrated in 1X ST3GAL1 reaction buffer in the presence of 200 μM of CMP-NeuAc (Sigma Cat.# C8271) and 0.5 mM β-1,3-galactosyl-N-acetyl galactosamine (Dextra Cat.# GN213). Each point is an average of two experiments, and the error bars represent the standard deviations.

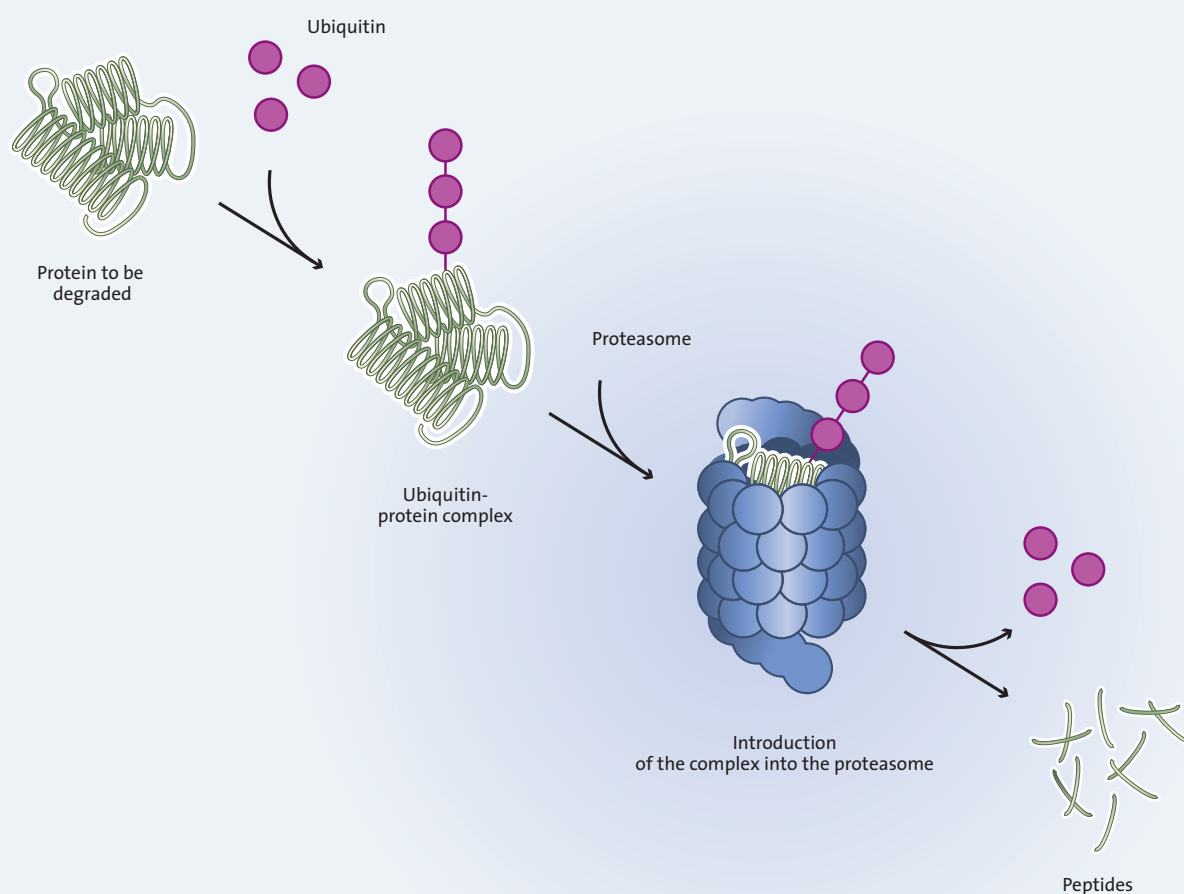
VIII Protease assays

Proteases perform an extremely wide variety of functions in organisms. In the proteasome complex in eukaryotic cells, for example, they are responsible for degrading proteins that are defective or are temporarily no longer needed. The protease calpain is involved in many Ca^{2+} -dependent regulatory processes in a cell. Deubiquitinating proteases play a significant role in protein regulation. Dipeptidyl peptidase (DPPIV) is a serine exopeptidase which performs functions in the immune system, for example, and plays a role in diseases such as cancer and diabetes. DPPIV is already being used as a therapeutic target in the treatment of type II diabetes.

Cell-based Proteasome-Glo™ Assays

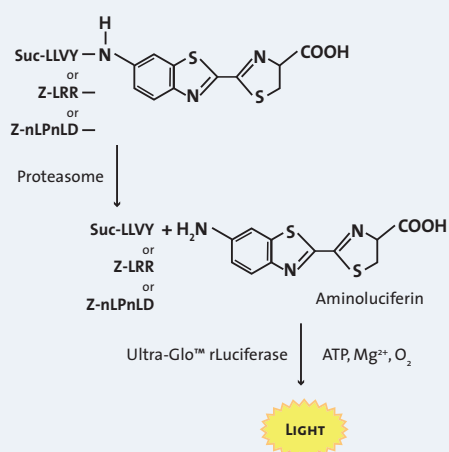
Proteasome-Glo™ Assays

Calpain-Glo™ Protease Assay

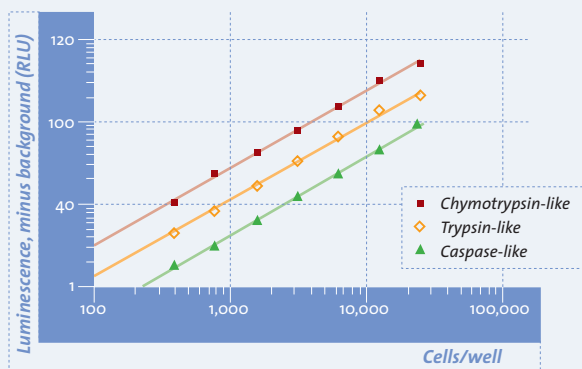


The proteasome

The proteasome is the most important extralysosomal protease of the eukaryotic cell. The 20S subunit contains three proteolytically active centres with chymotrypsin-like, trypsin-like and caspase-like activity. These three activities are of key importance to the homeostasis of the cell and to the maintenance of cellular metabolism (degradation of tumor suppressors, cell cycle, etc.). Proteins which are to be degraded are marked with a polyubiquitin chain, recognized by the proteasome and processed. The use of proteasome inhibitors induces growth inhibition and apoptosis in a range of human tumor cell lines and is consequently of great interest in the development of new cancer therapies.



The luminescence measured is proportional to the cell count



Using the Proteasome-Glo™ Cell-Based Assay, a titration of untreated U266 cells (human myeloma cells from plasma) was performed in a 96-well-plate. To do this, a serial dilution of U266 cells in cell culture medium was created (100 µl/well), the cells incubated for 1.5 hours at 37°C and the various Proteasome-Glo™ Cell-Based Assays performed. 10 minutes after addition of the reagent, the light signal was measured in a luminometer. The light units detected are proportional to the protease activity of the proteasome in the cells.

Cell-Based Proteasome-Glo™ Assay

Cell-based

Applications

Measurement of chymotrypsin-like, trypsin-like and caspase-like activity of the proteasome in cells; screening of substance libraries and measurement of proteasome-regulated protein degradation in cells.

Assay description

The **Proteasome-Glo™ Cell-Based Assay** is a homogeneous assay for independently measuring the individual protease activities associated with the proteasome complex in cultured cells. The assay is based on luminogenic proteasome substrates. The simple “add-mix-measure” format means that the reagent can be added directly to the cells.

The Proteasome-Glo™ Cell-Based 3-Substrate System offers the facility for measuring all three proteasome activities in a single run.

Assay principle

The peptide substrates contained in the **Proteasome-Glo™ Cell-Based Assay reagent** for measuring chymotrypsin-like, trypsin-like and caspase-like activities are Suc-LLVY-aminoluciferin (succinyl-leucine-leucine-valine-tyrosine-aminoluciferin), Z-LRR-aminoluciferin (Z-leucine-arginine-arginine-aminoluciferin) and Z-nLPnLD-aminoluciferin (Z-norleucine-proline-norleucine-aspartate-aminoluciferin). The protease activities can be measured either individually, or in a single run using the Proteasome-Glo™ Cell-Based 3-Substrate System.

The appropriate reagent is added directly to the cells and aminoluciferin is released by the specific proteasome activity. In the subsequent luciferase reaction, a stable luminescent signal that correlates with the enzyme activity is generated after 5 to 10 minutes.

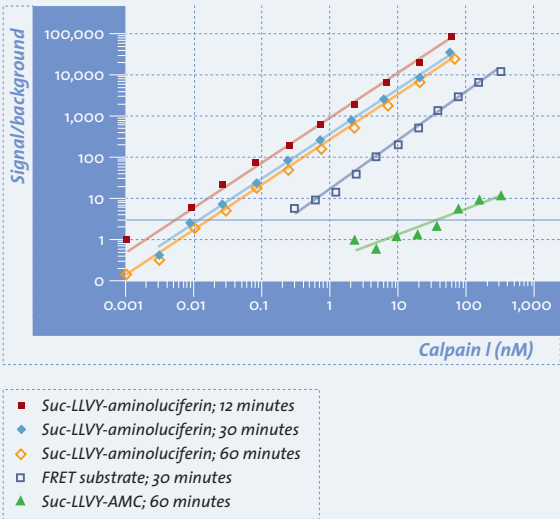
Assay features

Assay type	Luminescent (glow-type)
Applications	Determination of chymotrypsin-like, trypsin-like and caspase-like activity of the proteasome in cells.
Sample material	Cell lines
Implementation	Homogeneous, one-step assay
Time required	30 minutes

Calpain

Calpains belong to the family of Ca²⁺-activated cysteine proteases. They modulate the biological activity of their substrates by targeted proteolysis and are involved in the regulation of numerous Ca²⁺-dependent cellular processes. Their physiological role, however, is not yet fully clear. Calpains play a role in the pathogenesis of various diseases, such as Alzheimer's disease, and in various heart and brain disorders.

Sensitivity of the Calpain-Glo™ Protease Assay compared with fluorescent calpain assays



A calpain I titration was performed in a 96-well plate. Either the Calpain-Glo™ Protease Assay, a Suc-LLVY-AMC fluorescent substrate or the FRET-based substrate H-Lys-(FAM)-EYGMK(Dabcyl)-OH was used. Luminescence or fluorescence was determined at various times after the reagent had been added. The results were shown as a signal-to-noise ratio. The detection limit was defined as a signal-to-noise ratio of >3. The bioluminescent assay had a detection limit of 5 pM within 12 minutes, whereas the FRET-based fluorescent assay reached a detection limit of 200 pM after 30 minutes. The assay with the fluorescent Suc-LLVY-AMC substrate, by comparison, exhibited a limit of 30 nM after 60 minutes. In comparison to fluorescent assays, no accumulation of the calpain-cleaved product is necessary in the Calpain-Glo™ Protease Assay in order to obtain a measurable signal.

Calpain-Glo™ Protease Assay

Biochemical

Applications

Screenings for measuring calpain activities;
identification of calpain inhibitors in a multiwell format.

Assay description

The **Calpain-Glo™ Protease Assay** is a fast and extremely sensitive luminescent assay for measuring the protease activities of calpains I and II. The assay is particularly suitable for rapidly-autolyzing enzymes like calpain. Due to its exceptionally high reaction speed and sensitivity, the test system can be used in high-throughput screening for calpain activities and calpain inhibitors.

Assay principle

The **Calpain-Glo™-Protease Assay** contains a luminogenic succinyl calpain substrate (Suc-LLVY-aminoluciferin) in a buffer system optimized for calpain and luciferase. Proteolysis of the calpain substrate gives rise to aminoluciferin, which is converted by the Ultra-Glo™ Luciferase. The stable light signal associated with the reaction is proportional to the calpain activity. The sensitivity of this assay is unusually high, as it does not depend on accumulation of the calpain-cleaved product. Its immediate conversion by the Ultra-Glo™ Luciferase means that maximum sensitivity is attained just 5 to 10 minutes after incubation with calpain.

Assay features

Assay type	Luminescent (glow-type)
Applications	Quantitative determination of the protease activities of calpain I and II
Sample material	Enzyme preparations
Linearity	Linear over 4 logs of calpain concentration
Sensitivity	1,000 times more sensitive than fluorometric assays (detection of calpain I activity at concentrations < 5 pM)
Implementation	Homogeneous, one-step assay
Time required	10–30 minutes

IX References

Cell viability/proliferation

RealTime-Glo™ Metabolic Cell Viability Assay

Peer-reviewed publications

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CellTiter-Glo® Luminescent Cell Viability Assay

Promega articles

CellTiter-Glo™ Assay: Flexible Luminescent Cell Viability Assay; Randy Hoffman, Natalie Betz and Michael Bjerke; *Promega Notes* **79**, 36–38; **2001**

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In Vitro Toxicology and Cellular Fate Determination Using Promega CellBased Assays; Randy Hoffman; *Promega Notes* **82**, 19–22; **2002**

CellTiter-Glo® Luminescent Cell Viability Assay: Fast Sensitive and Flexible; Terry Riss¹, Rich Moravec¹, Michael Beck¹, Rita Hannah¹, Karen Wilson² and Robert Swanson³; *Promega Notes* **81**, 2–5; **2002**

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Hahn, C.K., Ross, K.N., Warrington, I.M., Mazitschek, R., Kanegai, C.M., Wright, R.D., Kung, A.L., Golub, T.R. and Stegmaier, K.; **Expression-based screening identifies the combination of histone deacetylase inhibitors and retinoids for neuroblastoma differentiation**; *Proc. Natl. Acad. Sci. USA* **105**, 9751–9756; **2008**

Lin, H., Lee, E., Hestir, K., Leo, C., Huang, M., Bosch, E., Halenbeck, R., Wu, G., Zhou, A., Behrens, D., Hollenbogh, D., Linnemann, T., Qin, M., Wong, J., Chu, K., Doberstein, S.K. and Williams, L.T.; **Discovery of a cytokine and its receptor by functional screening of the extracellular proteome**; *Science* **320**, 807–11; **2008**

CellTiter-Glo® 2.0 Cell Viability Assay

Promega articles

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Thorne, N. *et al.* (2010) **Apparent activity in high-throughput screening: Origins of compound-dependent assay interference;** *Curr. Opin. Chem. Biol.* **14**, 315–24

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See also HDAC-Glo™ I/II Assay

SIRT-Glo™ Assays and Screening Systems

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Cell signaling pathways

cAMP-Glo™ Assay

Promega articles

Monitor GPCR Modulation of Cellular cAMP with an HTS Bioluminescence-Based Assay; Said A. Goueli, Kevin Hsaio and Jolanta Vidugiriene; *Promega Notes* **97**, 24–27; **2007**

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ADP-Glo™ Kinase Assay

Promega articles

Screening and Profiling Kinase Inhibitors with a Luminescent ADP Detection Platform; Hicham Zegzouti, Juliano Alves, Tracy Worzella, Gediminas Vidugiris, Gregg Cameron, Jolanta Vidugiriene and Said Goueli; **2011**

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Kinase-Glo® Luminescent Kinase Assay

Promega articles

Introducing the Kinase-Glo® Luminescent Kinase Assay; Richard Somberg, Becky Pferdiert and Kevin Kupcho; *Promega Notes* **83**, 14–17; **2003**

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Baki, A., Bielik, A., Molnár, L., Szendrei, G. and Keserü, G.M. (2007) A high throughput luminescent assay for glycogen synthase kinase-3 β inhibitors; *Assay and Drug Development Technologies* **5**, 75–83

PDE-Glo™ Phosphodiesterase Assay

Peer-reviewed publications

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Lehnart, S.E. *et al.* (2005) Phosphodiesterase 4D deficiency in the ryanodine-receptor complex promotes heart failure and arrhythmias; *Cell* **123**, 25–35

Glycobiology

UDP-Glo™ Glycosyltransferase Assay

Publications

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GDP-Glo™ Glycosyltransferase Assay

Publications

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UMP/CMP-Glo™ Glycosyltransferase Assay

Publications

Walvoort, M. T. C.; Lukose, V.; Imperiali, B., A Modular Approach to Phosphoglycosyltransferase Inhibitors Inspired by Nucleoside Antibiotics. *Chemistry – A European Journal* **2016**, 22 (11), 3856–3864.

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Protease assays

Cell-based Proteasome-Glo™ Assays/Proteasome-Glo™ Assays

Promega articles

New Bioluminescent Cell Based Assays to Measure All Three Proteasome Protease Activities; Rich Moravec, Martha O'Brien, Bill Daily, Mike Scurria, Laurent Bernad, Sandy Hagen, Alyssa TenHarmsel, Neal Cosby and Terry Riss; *Cell Notes* **21**, 10–12; **2008**

Monitoring Proteasome Activity with a Cell-Based Assay Using a Single-Addition Luminescent Method; Rich Moravec¹, Martha O'Brien¹, Bill Daily², Mike Scurria², Laurent Bernad², Brad Larson¹, Tracy Worzella¹, Kay Rashka¹, Jeri Culp¹, Brian McNamara¹ and Terry Riss¹; *Cell Notes* **15**, 4–7; **2006**

Peer-reviewed publications

Groll, M., Schellenberg, B., Bachmann, A.S., Archer, C.R., Huber, R., Powell, T.K., Lindow, S., Kaiser, M. and Duler, R. (2008) **A plant pathogen virulence factor inhibits the eukaryotic proteasome by a novel mechanism;** *Nature* **452**, 755–758

Filimonenko, M., Stuffers, S., Railborg, C., Yamamoto, A., Malerod, L., Fisher, E.M.C., Isaacs, A., Brech, A., Stenmark, H. and Simonsen, A. (2007) **Functional multivesicular bodies are required for autophagic clearance of protein aggregates associated with neurodegenerative disease;** *J. Cell Biol.* **179**, 485–500

Calpain-Glo™ Protease Assay

Promega articles

Screen for Calpain Inhibitors Using a Cell-Based, High-Throughput Assay; Katheleen Seyb^{1,2}, Jake Ni¹, Mickey Huang¹, Eli Schuman¹, Mary L. Michaelis² and Marcie A. Glicksman¹; *Cell Notes* **18**, 6–8; **2007**

A Bioluminescent Assay for Calpain Activity; Martha O'Brien¹, Mike Scurria², Kay Rashka¹, Bill Daily² and Terry Riss¹; *Promega Notes* **91**, 6–9; **2005**

Peer-reviewed publications

Seyb, K.I., Schuman, E.R., Ni, J., Huang, M.M., Michaelis, M.L. and Glicksman, M.A. (2008) **Identification of small molecule inhibitors of α -amyloid cytotoxicity through a cell-based high-throughput screening platform;** *J. Biomol. Screening* **13**, 870–878
Notes: This paper demonstrates use of a calpain assay in a cell-based format. (Calpain-Glo™ Assay).

Boehmerle, W., Zhang, K., Sivula, M., Heidrich, F.M., Lee, Y. Jordt, S-E. and Ehrlich, B.E. (2007) **Chronic exposure to paclitaxel diminishes phosphoinositide signaling by calpain-mediated neuronal calcium sensor-1 degradation;** *Proc. Natl. Acad. Sci. USA* **104**, 11103–11108

X Overview of products

Viability and proliferation

Product	Quantity	Catalog No.
RealTime-Glo™ MT Cell Viability Assay	100 reactions	G9711
	10 x 100 reactions	G9712
	1,000 reactions	G9713
CellTiter-Glo® Luminescent Cell Viability Assay	10 ml	G7570
	10 x 10 ml	G7571
	100 ml	G7572
	10 x 100 ml	G7573
CellTiter-Glo® Cell Viability 2.0 Assay	10 ml	G9241
	100 ml	G9242
	500 ml	G9243
CellTiter-Glo® 3D Viability Assay	10 ml	G9681
	10 x 10 ml	G9682
	100 ml	G9683
CellTiter 96® AQueous One Solution Cell Proliferation Assay	200 assays	G3582
	1,000 assays	G3580
	5,000 assays	G3581
CellTiter-Blue® Cell Viability Assay	20 ml	G8080
	100 ml	G8081
	10 x 100 ml	G8082
CellTiter-Fluor™ Cell Viability Assay	10 ml	G6080
	5 x 10 ml	G6081
	2 x 50 ml	G6082
	10 ml	G8230
BacTiter-Glo™ Microbial Cell Viability Assay	10 x 10 ml	G8231
	100 ml	G8232
	10 x 100 ml	G8233

Cytotoxicity

Product	Quantity	Catalog No.
LDH-Glo™ Cytotoxicity Assay	10 ml	J2380
	50 ml	J2381
CellTox™ Green Cytotoxicity Assay	10 ml	G8741
	100 ml	G8742
	500 ml	G8743
CellTox™ Green Express Cytotoxicity Assay	200 µl	G8731
CytoTox-Fluor™ Cytotoxicity Assay	10 ml	G9260
	5 x 10 ml	G9261
	2 x 50 ml	G9262
CytoTox-Glo™ Cytotoxicity Assay	10 ml	G9290
	5 x 10 ml	G9291
	2 x 50 ml	G9292
CytoTox-ONE™ Homogeneous Membrane Integrity Assay	200–800 assays	G7890
	100–4,000 assays	G7891
CytoTox-ONE™ Homogeneous Membrane Integrity Assay, HTP	1000–4,000 assays	G7892
Viral ToxGlo™ Assay	10 ml	G8941
	10 x 10 ml	G8942
	100 ml	G8943

Apoptosis

Product	Quantity	Catalog No.
RealTime-Glo™ Annexin V Apoptosis and Necrosis Assay	100 assays	JA1011
	1000 assays	JA1012
Caspase-Glo® 3/7 Assay	2.5 ml	G8090
	10 ml	G8091
	10 x 10 ml	G8093
	100 ml	G8092
Caspase-Glo® 3/7 3D Assay	10 ml	G8981
	100 ml	G8982
	10 x 10 ml	G8983
Caspase-Glo® 6 Assay	10 ml	G0970
	50 ml	G0971
Caspase-Glo® 8 Assay	2,5 ml	G8200
	10 ml	G8201
	100 ml	G8202
Caspase-Glo® 9 Assay	2.5 ml	G8210
	10 ml	G8211
	100 ml	G8212
Apo-ONE® Homogeneous Caspase-3/7 Assay	1 ml	G7792
	10 ml	G7790
	100 ml	G7791
Apo-ONE® Homogeneous Caspase-3/7 Buffer	100 ml	G7781
CaspACE™ FITC-VAD-FMK in situ Marker	50 µl	G7461
	125 µl	G7462

Autophagy

Product	Quantity	Catalog No.
HEK293 Autophagy LC3 HiBiT Reporter Cell Line and Detection System	-	GA1040
U2OS Autophagy LC3 HiBiT Reporter Cell Line and Detection System	-	GA1050
Autophagy LC3 HiBiT Reporter Vector and Detection System	-	GA2550

Inflammasom and immunogenic cell death

Product	Quantity	Catalog No.
Caspase-Glo® 1 Inflammasome Assay	10 ml	G9951
	5 x 10 ml	G9952
RealTime-Glo™ Extracellular ATP Assay	200 assays	GA5010
	2,000 assays	GA5011
	10 x 200 assays	GA5012
Lumit™ HMGB1 Human/Mouse Immunoassay	100 assays	W6110
	500 assays	W6112

Multiplexing

Product	Quantity	Catalog No.
Multitox-Fluor Multiplex Cytotoxicity Assay	10 ml	G9200
	5 x 10 ml	G9201
	2 x 50 ml	G9202
Multitox-Glo Multiplex Cytotoxicity Assay	10 ml	G9270
	5 x 10 ml	G9271
	2 x 50 ml	G9272
ApoLive-Glo™ Multiplex Assay	10 ml	G6410
	5 x 10 ml	G6411
Apotox-Glo™ Triplex Assay	10 ml	G6320
	5 x 10 ml	G6321

Multiplexing

Product	Quantity	Catalog No.
ONE-Glo™ + Tox Luciferase Reporter and Cell Viability Assay	1 plate	E7110
	10 plates	E7120
Mitochondrial ToxGlo™ Assay	10 ml	G8000
	100 ml	G8001

Cell Metabolism

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

Oxidative stress

Product	Quantity	Catalog No.
ROS-Glo™ H ₂ O ₂ Assay	10 ml	G8820
	50 ml	G8821
GSH-Glo™ Glutathione Assay	10 ml	V6911
	50 ml	V6912
GSH/GSSG-Glo™ Assay	10 ml	V6611
	50 ml	V6612

Epigenetic assays

Product	Quantity	Catalog No.
HDAC-Glo™ 2 Assay	10 ml	G9590
HDAC-Glo™ I/II Assay	10 ml	G6420
	5 x 10 ml	G6421
	100 ml	G6422
HDAC-Glo™ I/II Screening Systems	10 ml	G6430
	5 x 10 ml	G6431
SIRT-Glo™ Assay	10 ml	G6450

Cell signaling pathways

Product	Quantity	Catalog No.
cAMP-Glo™ Max Assay	2 plates	V1681
	20 plates	V1682
	10 x 20 plates	V1683
ADP-Glo™ Kinase Assay	1,000 assays	V9101
	10,000 assays	V9102
	100,000 assays	V9103
ADP-Glo™ Max Assay	1,000 assays	V7001
	10,000 assays	V7002
Kinase-Glo® Luminescent Kinase Assay	10 ml	V6711
	10 x 10 ml	V6712
	100 ml	V6713
	10 x 100 ml	V6714
Kinase-Glo® Max Luminescent Kinase Assay	10 ml	V6071
	10 x 10 ml	V6072
	100 ml	V6073
	10 x 100 ml	V6074

Cell signaling pathways

Product	Quantity	Catalog No.
Kinase-Glo® Plus Luminescent Kinase Assay	10 ml	V3771
	10 x 10 ml	V3772
	100 ml	V3773
	10 x 100 ml	V3774
AMP-Glo™ Assay	1,000 assays	V5011
	10,000 assays	V5012
PDE-Glo™ Phosphodiesterase Assay	1,000 assays	V1361
	10,000 assays	V1362
GTPase-Glo™ Assay	1,000 assays	V7681
	10,000 assays	V7682

Glycobiology

Product	Quantity	Catalog No.
UDP-Glo™ Glycosyltransferase Assay	200 assays	V6961
	400 assays	V6962
	4,000 assays	V6963
UDP-Glo™ Glycosyltransferase Assay + UDP-GlcNAc	200 assays	V6971
	400 assays	V6972
UDP-Glo™ Glycosyltransferase Assay + UDP-GalNAc	200 assays	V6981
	400 assays	V6982
UDP-Glo™ Glycosyltransferase Assay + UDP-Glucose	200 assays	V6991
	400 assays	V6992
UDP-Glo™ Glycosyltransferase Assay + UDP-Galactose	200 assays	V7051
	400 assays	V7052
GDP-Glo™ Glycosyltransferase Assay	200 assays	VA1090
	400 assays	VA1091
	4,000 assays	VA1092
UMP/CMP-Glo™ Glycosyltransferase Assay	200 assays	VA1130
	400 assays	VA1131
	4,000 assays	VA1132

Protease assays

Product	Quantity	Catalog No.
Proteasome-Glo™ Chymotrypsin-Like Cell-Based Assay	10 ml	G8660
	5 x 10 ml	G8661
	2 x 50 ml	G8662
Proteasome-Glo™ Trypsin-Like Cell-Based Assay	10 ml	G8760
	5 x 10 ml	G8761
Proteasome-Glo™ Caspase-Like Cell-Based Assay	10 ml	G8860
	5 x 10 ml	G8861
Proteasome-Glo™ 3-Substrate Cell-Based Assay System	10 ml	G1180
	50 ml	G1200
Proteasome-Glo™ Chymotrypsin-Like Assay	10 ml	G8621
	50 ml	G8622
Proteasome-Glo™ Trypsin-Like Assay	10 ml	G8631
	50 ml	G8632
Proteasome-Glo™ Caspase-Like Assay	10 ml	G8641
	50 ml	G8642
Proteasome-Glo™ 3-Substrate System	10 ml	G8531
	50 ml	G8532
Calpain-Glo™ Protease Assay	10 ml	G8501
	50 ml	G8502

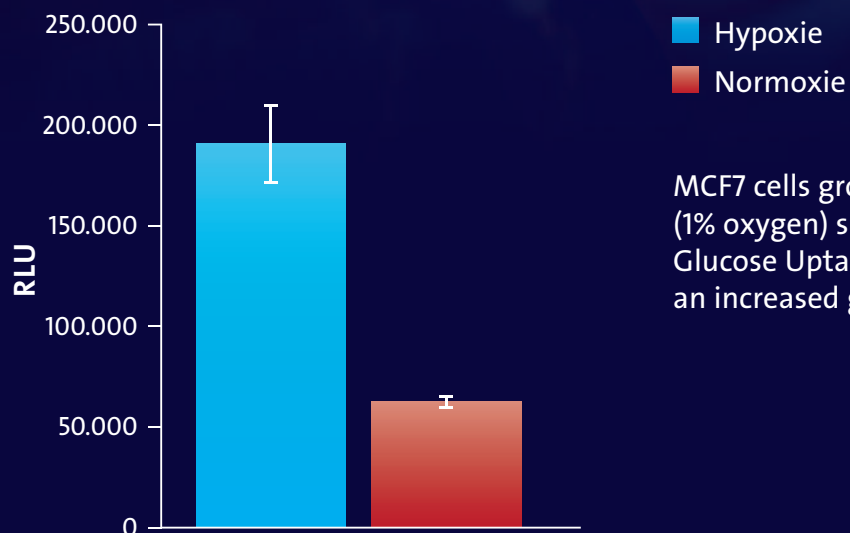
Cellular Metabolism Assays

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- Glutamate
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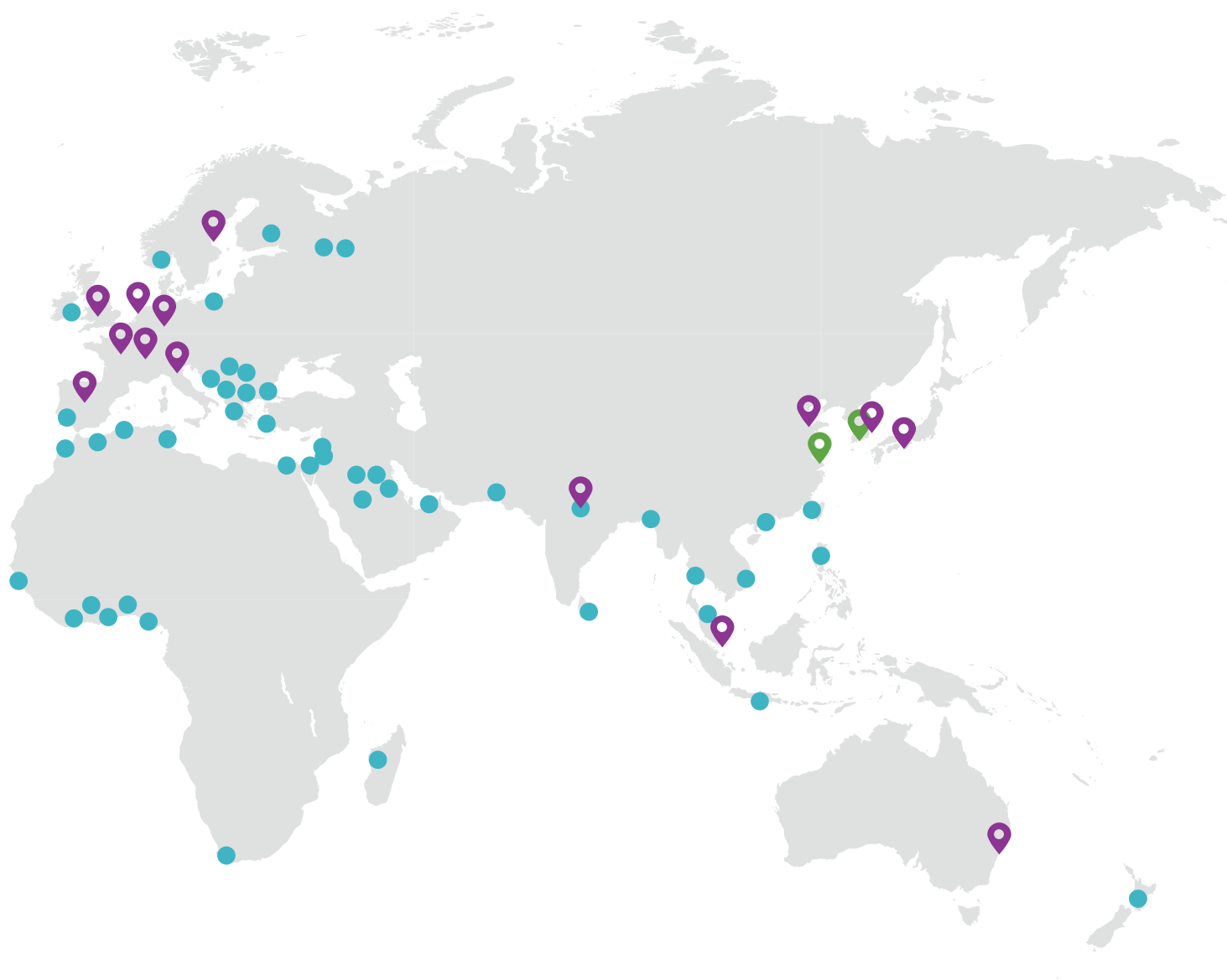
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