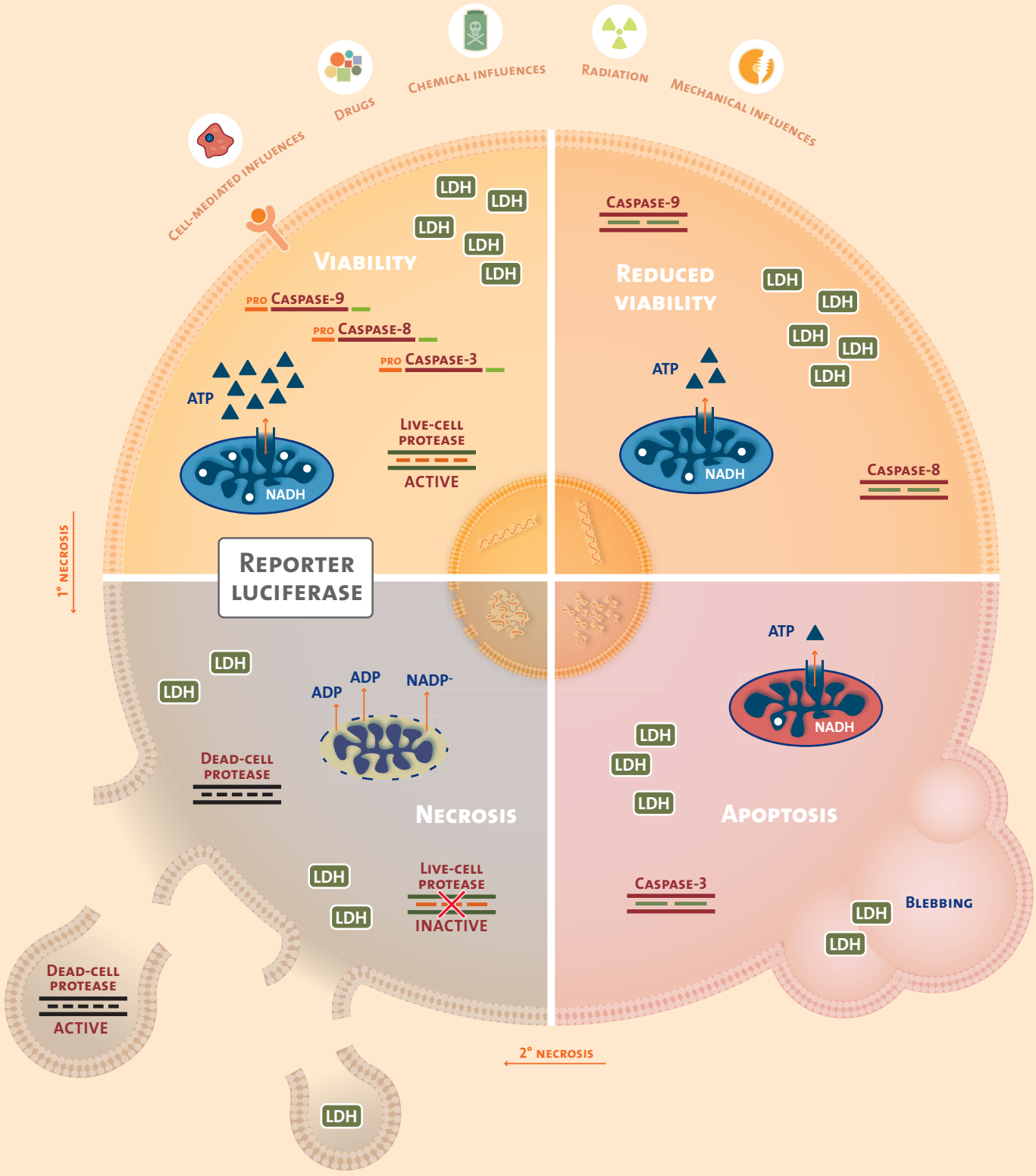
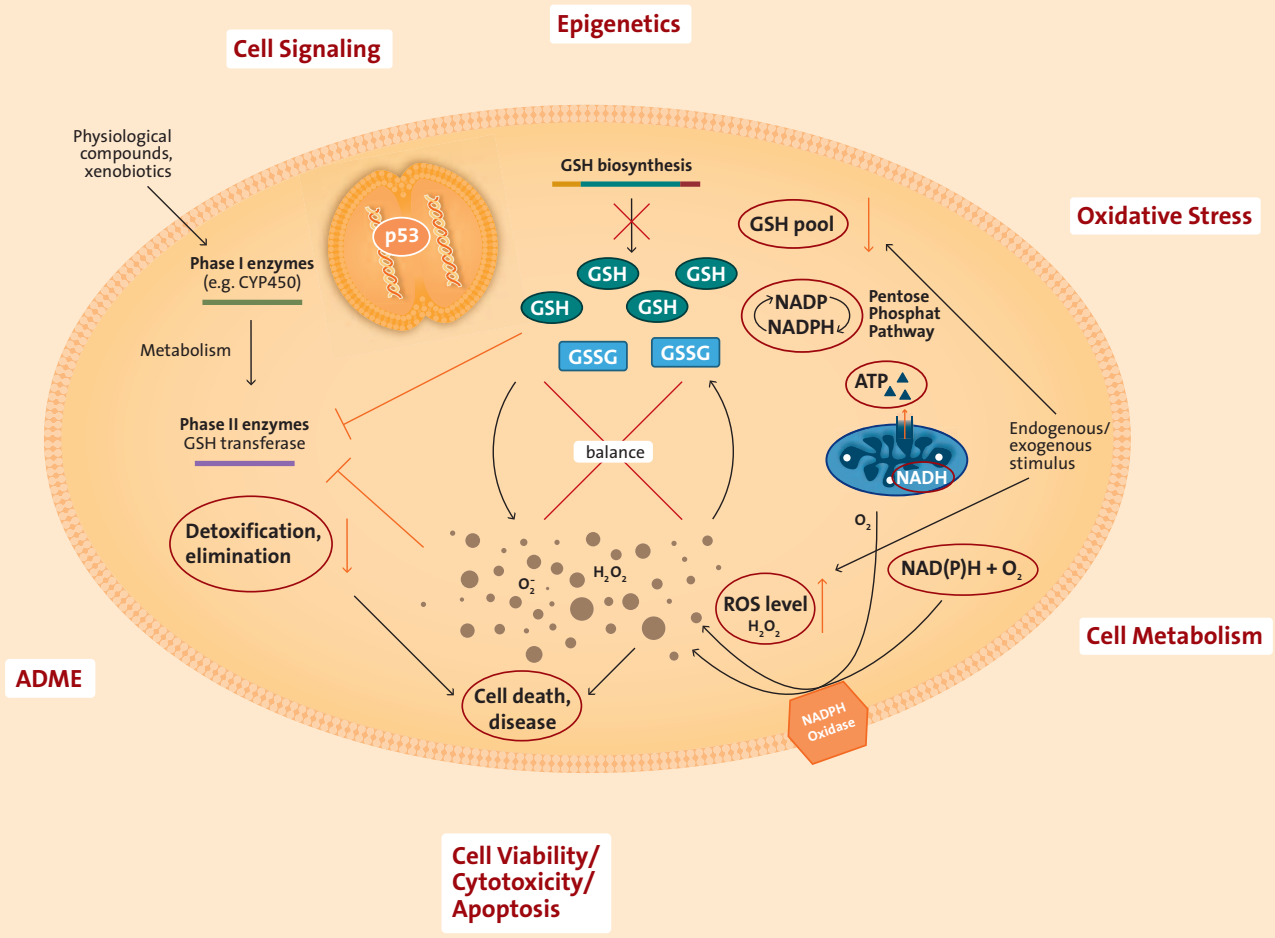
Promega

Cell-Based & Biochemical Assays

Promega



Cellular Parameters for Cell-Based Assays



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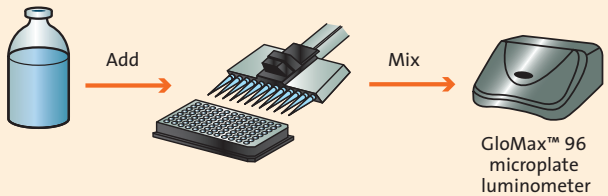
Cell-based and biochemical assay formats

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 G protein coupled receptors signaling pathways and epigenetic changes. 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 indispensible 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 results

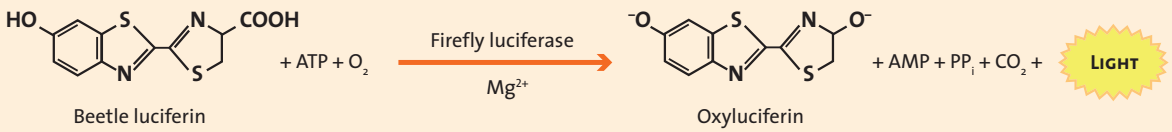
It is often helpful for scientists to be able to obtain multiple data points from a single sample. It is therefore an advantage if an assay is suitable for multiplexing – the analysis of more than one parameter in a single sample. For example, researchers may initially use a fluorescence assay for measuring cytoxicity 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 enzymes relevant. Luminescent assays, on the other hand, are based on the luciferase reaction of the firefly *Photinus pyralis*. 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

Reaction of firefly luciferase



Beetle luciferin

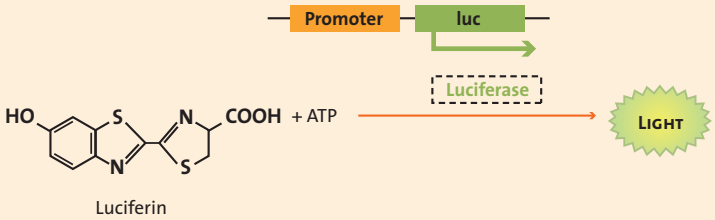
Oxyluciferin

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

Type I:

Measurement of luciferase expression

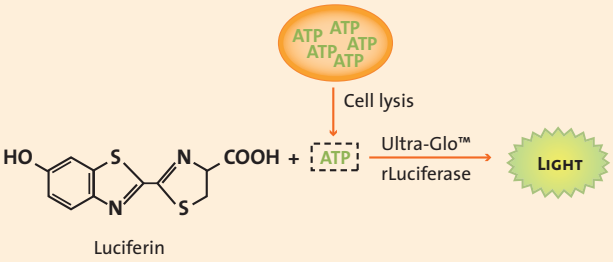
The measurement of luciferase expression is the basis of all reporter gene assays or GPCR assays. The level of luciferin expression is determined by the strength or saturation state of the promoter. The assay reagent combines an excess of luciferin and ATP and therefore the expressed luciferase is the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



Type II:

Measurement of ATP content

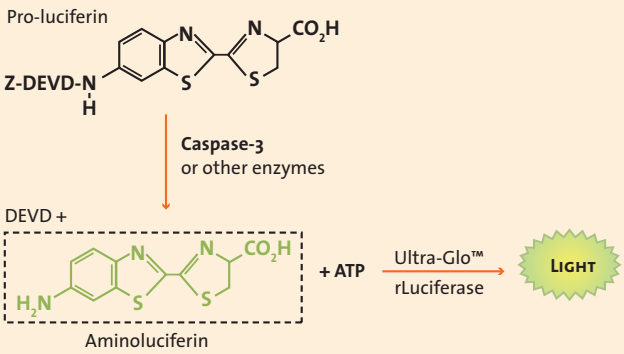
In ATP-dependent assays, the ATP content in a sample is measured. Here, ATP is the limiting factor. It is determined indirectly via the luciferase reaction. This principle is used in the determination of cell viability and in the measurement of kinase activities, for example. The assay reagent contains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



Type III:

Measurement of luciferin release

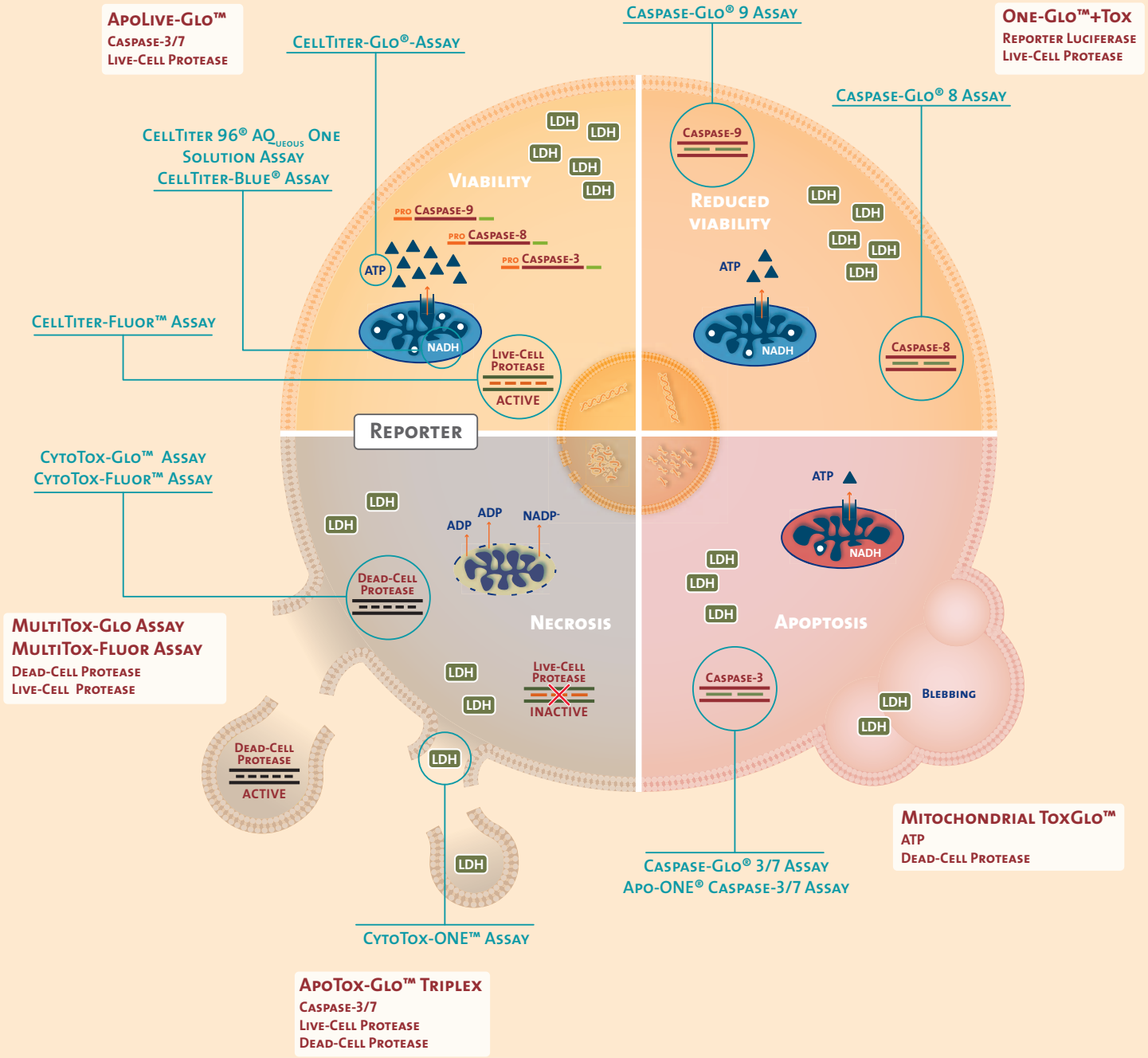
The luciferin-generating assays contain a pro-luciferin with enzyme recognition sequences, such as DEVD tetrapeptide for determining caspase-3/7 activity. Only after the substrate has been released the luminescence signal is generated by the relevant enzyme. The assay reagent contains modified luciferin and an excess of Ultra-Glo™ Luciferase and ATP.



II Cell viability, cytotoxicity, apoptosis and inflammation

In many areas of bioscience, the investigation and determination of cell viability, cytotoxicity, apoptosis 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 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.



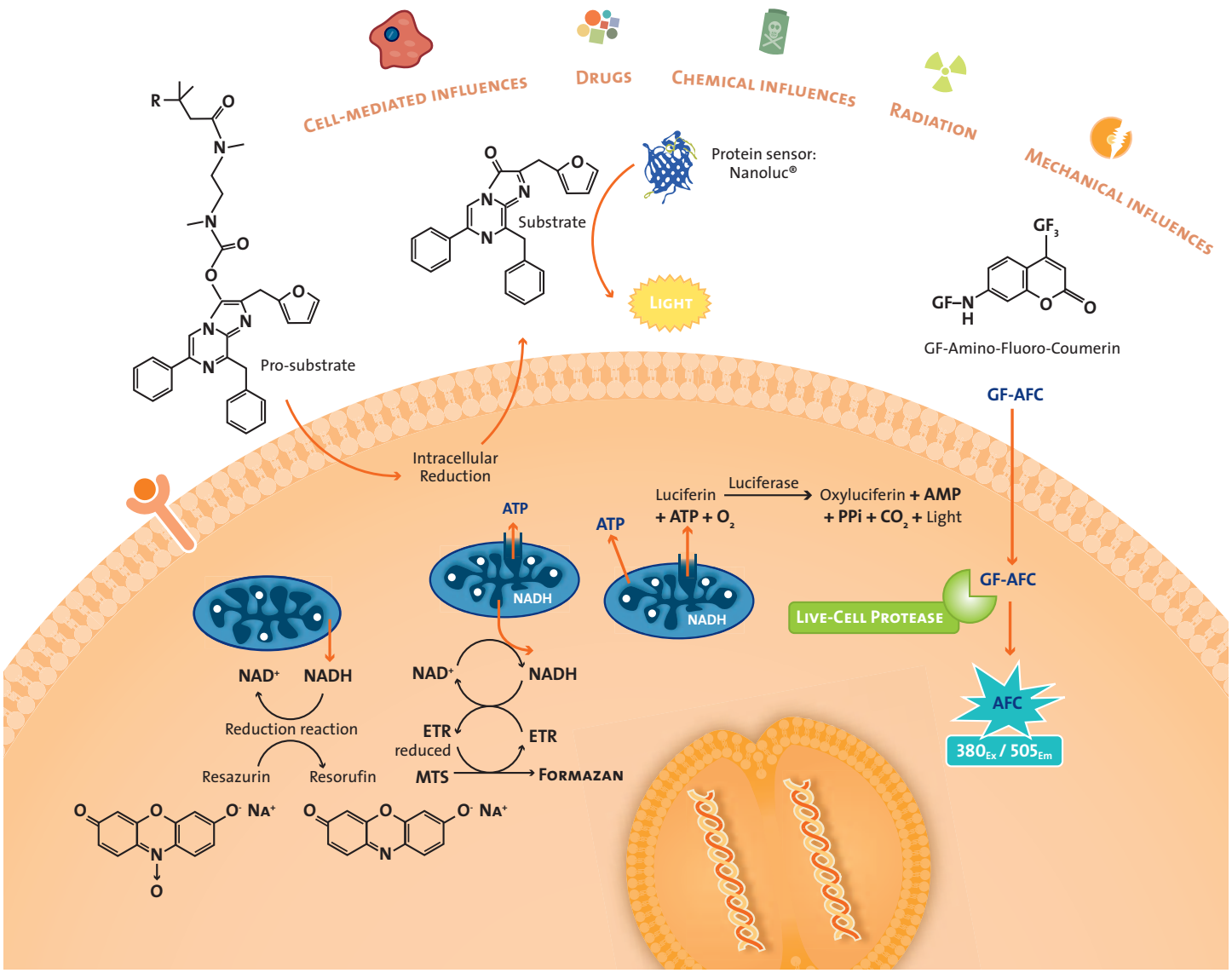
Cell-based assays at a glance for cell viability, cytotoxicity, apoptosis and inflammation

Assay	Parameters/Biomarkers	Time required	96-well sensitivity	Plate format	Instrument
RealTime-Glo™ MT Cell Viability Assay	Reducing capacity of the cells	300 min	< 100 cells/well in 96-well fromat	96/384/1536	Luminometer
CellTiter-Glo® Assay	ATP	10 min	10 living cells	96/384/1536	Luminometer
CellTiter-Glo® 2.0 Assay	ATP	10 min	15 living cells (384-well)	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
CellTox™ Green Assay	DNA	15 min	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
Caspase-Glo® 3/7 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,$499_{Ex}/521_{Em}$
Caspase-Glo® 8 Assay	Caspase-8 activity	0.5 h	~1,000 apoptotic cells	96	Luminometer
Caspase-Glo® 9 Assay	Caspase-9 activity	0.5 h	~1,500 apoptotic cells	96	Luminometer
Caspase-Glo® 2 Assay	Caspase-2 activity	0.5 h	Enzyme preparation	96/384/1536	Luminometer
Caspase-Glo® 6 Assay	Caspase-6 activity	0.5 h	Enzyme preparation	96/384/1536	Luminometer
Caspase-Glo® 1 Assay	Caspase-1 activity	1 h	ND	96/384	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; (Crabtree effect, galactose medium)	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 distinguish whether a reduced cell viability is due to 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® AQ_{ueous} 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

Suitable for 3D-microtissues

Multiplexing with CellTox™ Green

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

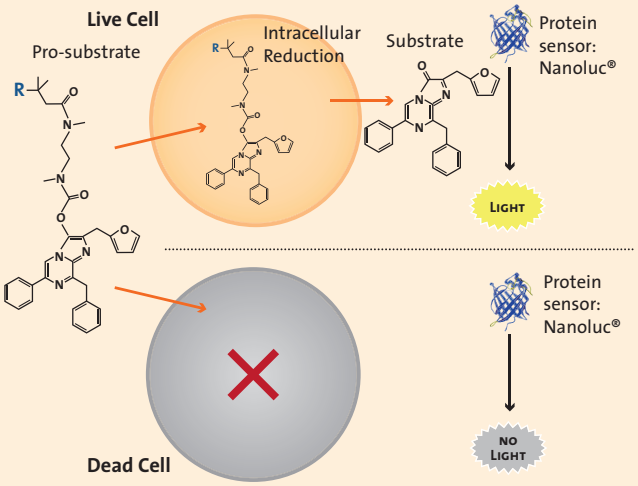
The **RealTime-Glo™ MT Cell Viability Assay** is a non-lytic, homogeneous, bioluminescent method to determine in real time the number of viable cells in culture by measuring the reducing potential of cells and thus metabolism (MT). The reagent is stable and nontoxic to cells for up to 72 hours. No cell 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.

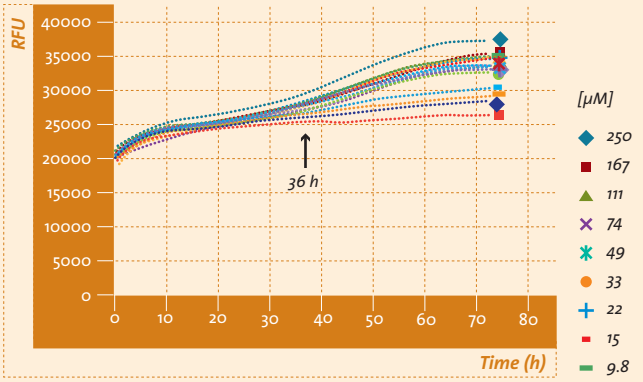
Assay features

Assay type	Luminescent
Markers	Reducing capacity of the cell
Applications	Cell viability in real time, onset of toxicity, analyze potency versus efficacy over time
Cell type	Cell lines and 3D culture (spheroids)
Implementation	Homogeneous, non-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

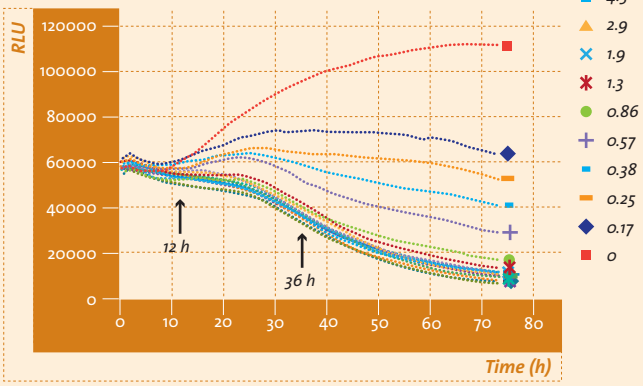


RealTime-Glo™ utilizes NanoLuc® luciferase and a novel pro-furimazine substrate to determine the number of viable cells through measurement of the reducing capacity of the cell.

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 % CO_2) every 1 h for 72 h.

CellTiter-Glo® Luminescent Cell Viability Assay

Cell-based

Applications

Cell viability; proliferation; cytotoxicity.

Assay description

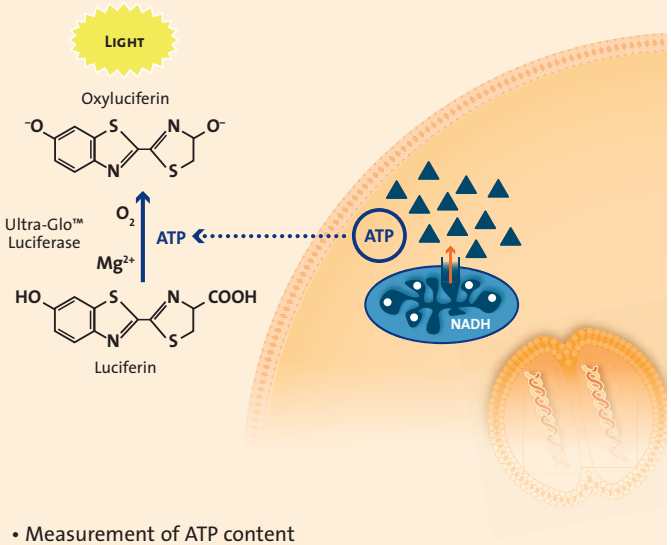
CellTiter-Glo® is the most sensitive cell-based assay for detecting cell viability. It is therefore particularly suitable for use in studies on primary cells. Notable features of the assay include the ease and speed with which it can be performed and the reproducibility of the data (Z' factor > 0.63 in a 1536-well format), as well as the extremely wide linear measurement range of 10–50,000 cells.

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 oxyluferin 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.

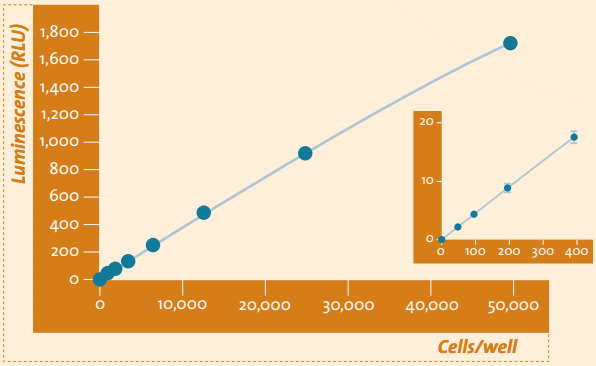
Assay features

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



- Measurement of ATP content
- Based on an ATP-dependent luciferase reaction
- Assay reagent leads to lysis of the cells

Excellent linearity and sensitivity



Dilution series of Jurkat cells in 96-well plates. The number of living cells, from 10 to 50,000 cells per well, is directly proportional to the measured luminescence signal (R = 0.99).

CellTiter-Glo® 2.0 Cell Viability Assay

Cell-based

Stable for 1 week at room temperature!

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.

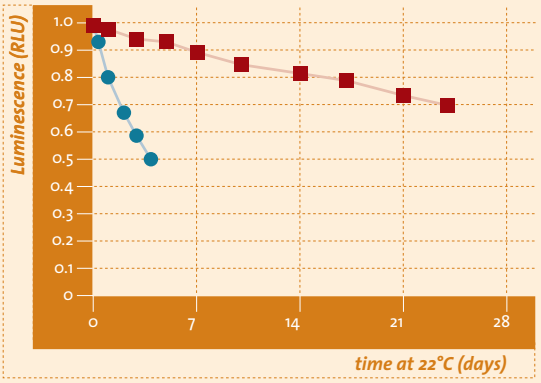
Assay features

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

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 mM ATP in water. Luminescence was recorded after 10 minutes .

Performance with various cells & media

	Cell type	*Luminescence (RLU x 10^6)*		*Signal Half-Life (hours)*	
Medium		**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 in particular 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

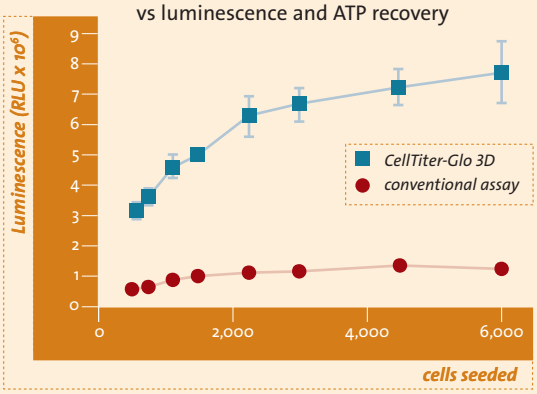
The CellTiter-Glo® 3D Assay is an improved reagent formulation of CellTiter-Glo® for bioluminescent detection of ATP with a more effective lysis. The "add-mix-read" protocol and single-component liquid format make this novel cell viability assay a simple and convenient reagent for assaying the viability 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

CellTiter-Glo® 3D Viability Assays applied on microtissues

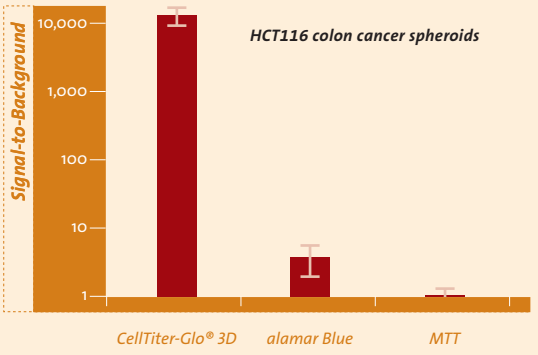
GravityTRAP™ plate, Hanging Drop: cell seeded vs luminescence and ATP recovery

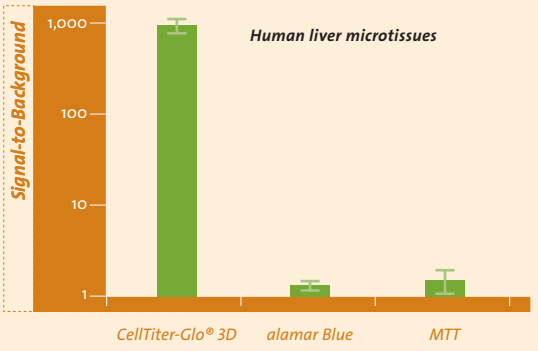


		ATP (nM)	
cells seeded	diameter (µm)	CellTiter-Glo® 3D	conventional assay
6,000	716	1,327	290
2,250	533	1,079	262
1,125	468	799	206
563	355	539	134

HCT116 cells (RPMI +10% FBS) were grown by the hanging drop method for 4 days. The bright field image was taken in a GravityTRAP™ plate (InSphero).

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.

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

Cell-based

Applications

Cell viability; proliferation; cytotoxicity.

Assay description

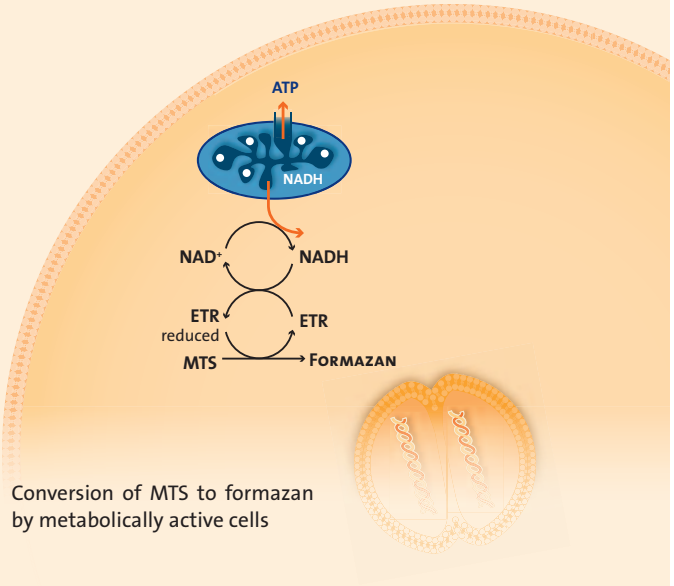
CellTiter 96® AQ_{ueous} One Solution Assay (MTS) is based on a colorimetric method for determining numbers of living cells and is suitable for measuring cell viability, proliferation and indirectly also cytotoxicity. One advantage that this one-step assay has over MTT assays is that the MTS formazan 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 readout is directly proportional to the number of living cells in culture.

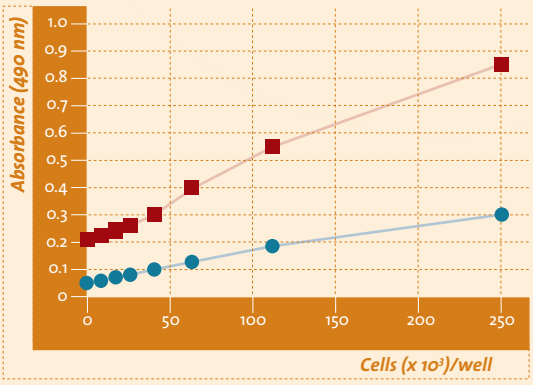
Assay features

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, plants, yeasts, blood lymphocytes
Implementation	Homogeneous, one-step assay
Time required	1–4 hours
Sensitivity	1,000 living cells (96-well format)



Conversion of MTS to formazan by metabolically active cells

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)-2,5-diphenyltetrazolium bromide

CellTiter-Blue® Cell Viability Assay

Cell-based

Applications

Cell viability; proliferation; cytotoxicity; multiplexing.

Assay description

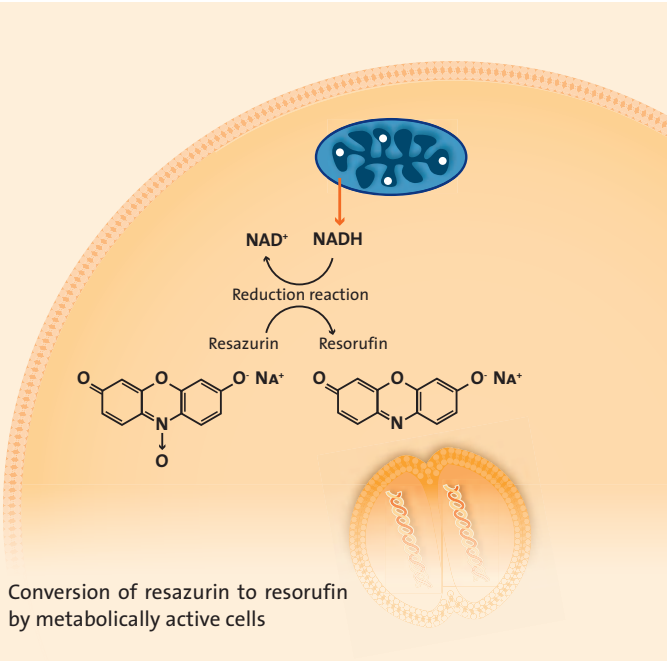
CellTiter-Blue® Assay is a fluorescent, cell-based assay for determining cell viability, proliferation and cytotoxicity. In contrast to comparable assays, the assay reagent resazurin is highly purified and is nontoxic to the cells, so flexible incubation times are possible. The assay can be performed 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).

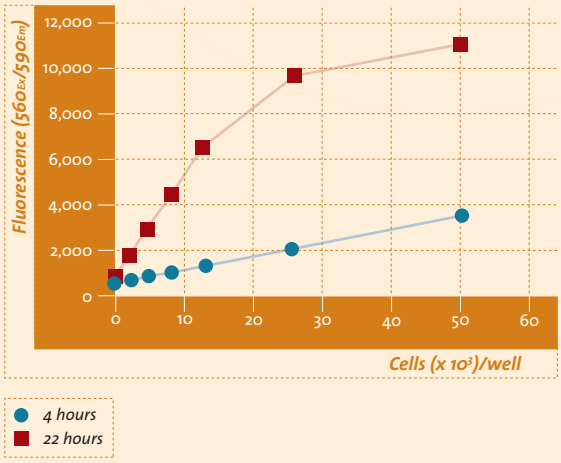
Assay features

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)
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



Conversion of resazurin to resorufin by metabolically active cells

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

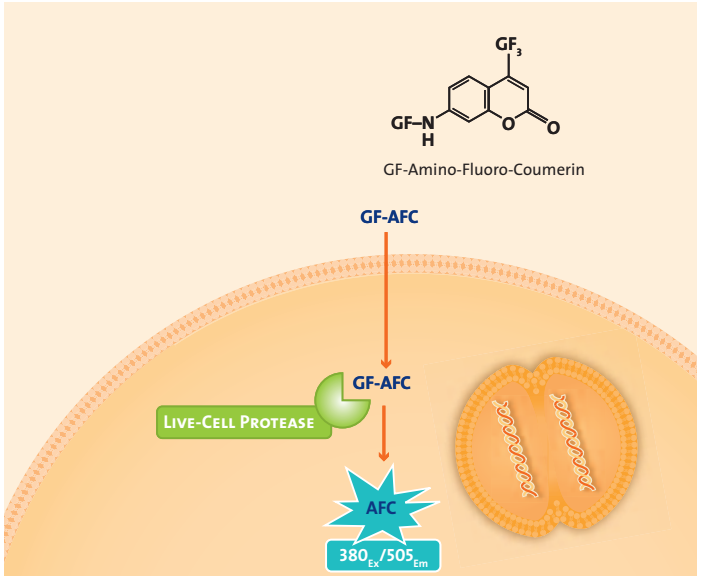
CellTiter-Fluor™ Assay is a fluorescent, cell-based assay for determining cell viability. The assay is particularly suitable for multiplexing, as the cells remain intact. The assay is frequently used for normalizing data, i.e. to compensate for differences between individual wells and plates. The **CellTiter-Fluor™ Assay** is also very well suited to automation.

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 glycylphenylalanyl-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.

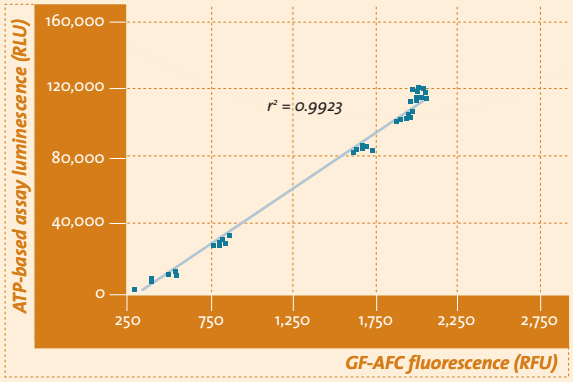
Assay features

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)
Implementation	Homogeneous, one-step assay
Time required	0.5–3 hours
Sensitivity	40 living cells (96-well format)



- Conversion of cell-permeable GF-AFC by the live-cell protease
- The live-cell protease is inactive extracellularly

Excellent correlation between ATP-based and live-cell-protease-based cell viability measurements



CellTiter-Fluor™ Assay exhibits a very good correlation with established methods for detecting cell viability, as can be seen here with the ATP-based CellTiter-Glo® Assay for example.

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.

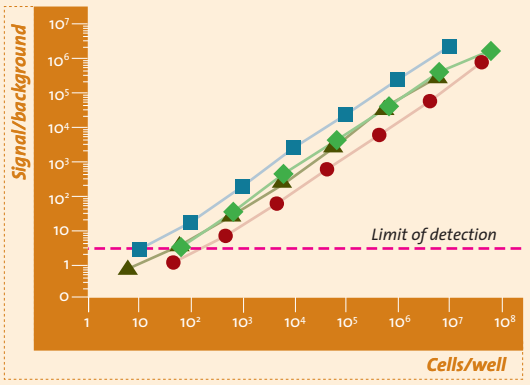
Assay principle

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

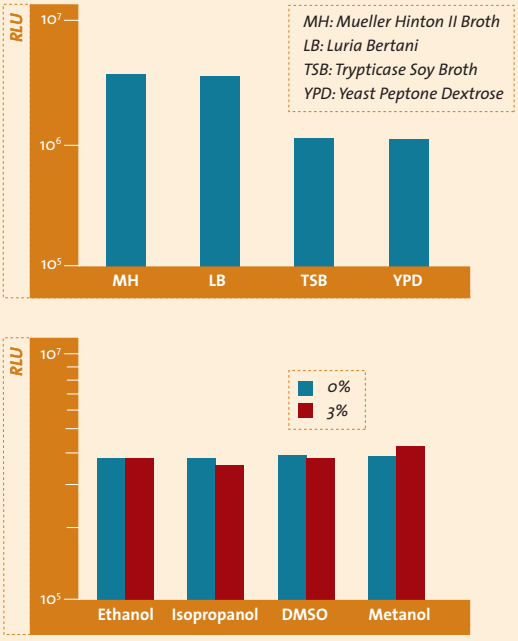
Assay type	Luminescent (glow-type; $T_{1/2}$ > 0.5 h)
Markers	ATP
Applications	Measurement of the viability of bacteria and yeast
Bacteria/Yeasts	Gram-positive bacteria, Gram-negative bacteria, yeast
Implementation	Homogeneous, one-step assay
Time required	5 minutes
Sensitivity	10 bacteria (1,000 times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

Excellent sensitivity and linearity



Correlation between bacterial count and luminescence signal. Depending on the bacterial strain, as few as 10 cells can be detected.

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 ~ 1 × 10^{-12} moles of ATP.

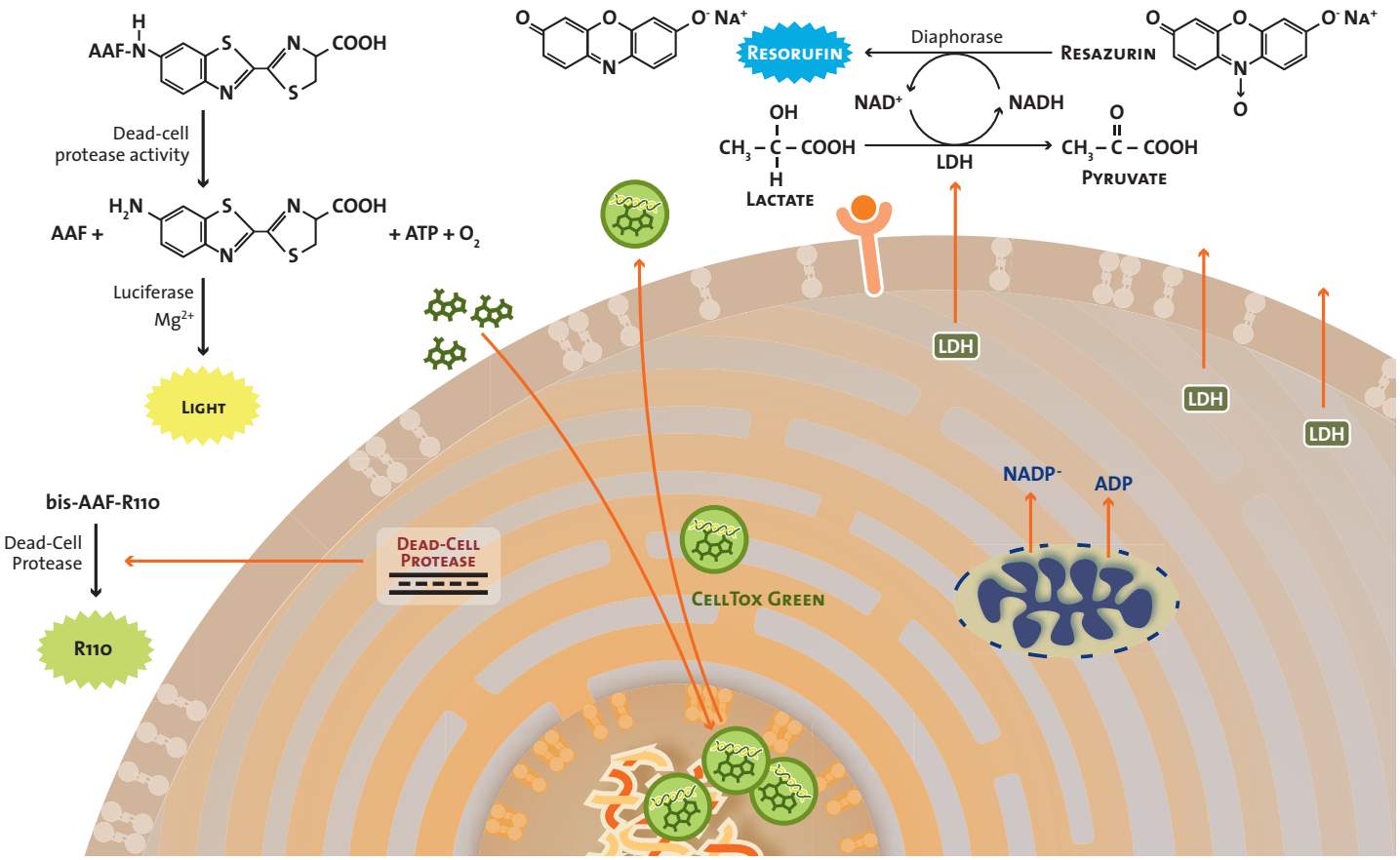
IIb Cytotoxicity

The term “cytoxicity” 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, an image-based method for real-time monitoring of cytoxicity and a ATP-based method.

CellTox™ Green Cytotoxicity Assay
CytoTox-Glo™ Cytotoxicity Assay
CytoTox-Fluor™ Cytotoxicity Assay
CytoTox-ONE™ Homogeneous Membrane Integrity Assay (LDH)
ViralTox-Glo™ Assay



CellTox™ Green Cytotoxicity Assay

Cell-based

Suitable for 3D-microtissues

Multiplexing with CellTiter-Glo® 3D

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 can be multiplexed with other spectrally distinct measures of cell health to provide mechanistic information relating to cytotoxicity.

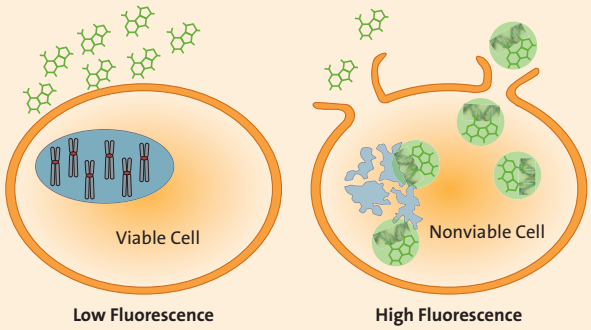
Assay principle

When the dye binds DNA, released from 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.

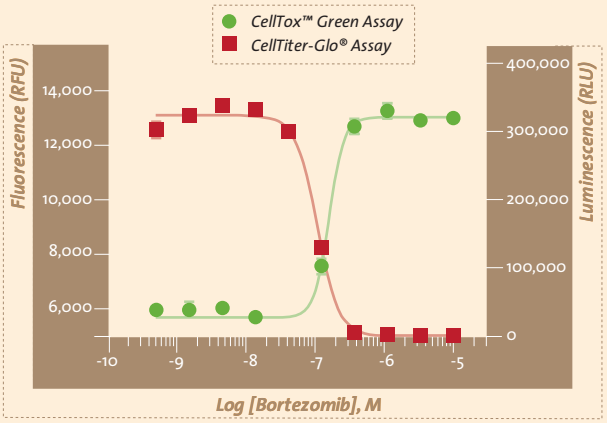
Assay features

Assay type	Stable fluorescence signal over 72 h ($485-500_{Ex}/520-530_{Em}$)
Markers	DNA
Applications	Real-time cytotoxicity measurements; membrane damage; multiplexing with other assays.
Cell type	Cells
Implementation	Add assay reagent directly to cells prior to plating or with dosing media to perform kinetic cytotoxicity measurements, or add diluted dye directly to cell culture wells as an endpoint add-mix-measure assay.
Time required	15 minutes incubation

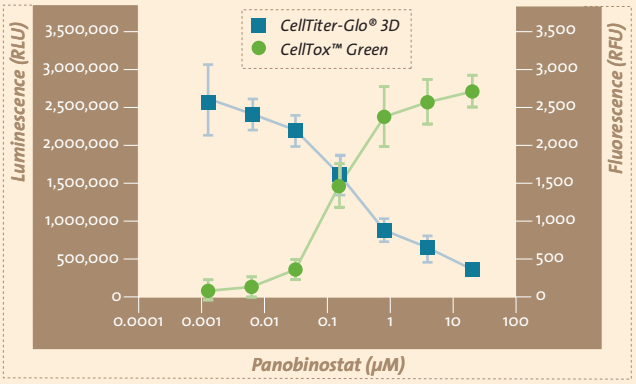
Assay Principle: CellTox™ Green binds DNA of cells with impaired membrane integrity



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

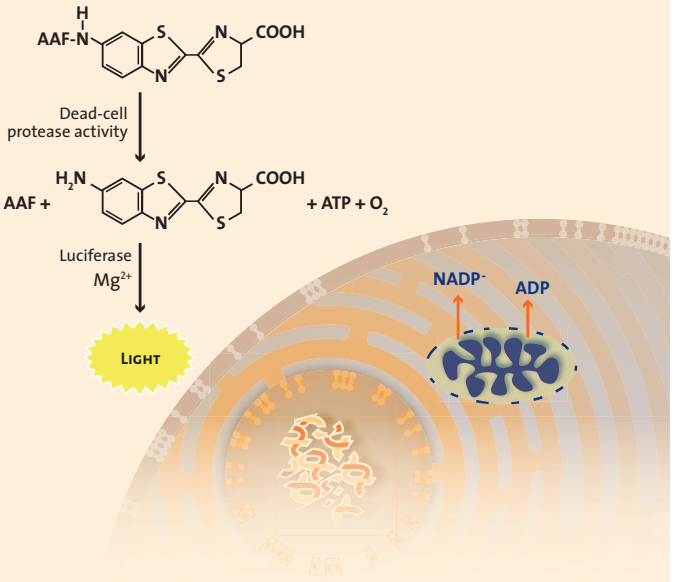
CytoTox-Glo™ Assay is a luminescent cell-based assay for determining cytotoxicity. The assay is particularly suitable for multiplexing, as the cells remain intact. It is frequently used for normalizing data, i.e. to compensate for differences between individual wells and plates. The CytoTox-Glo™ Assay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

Assay principle

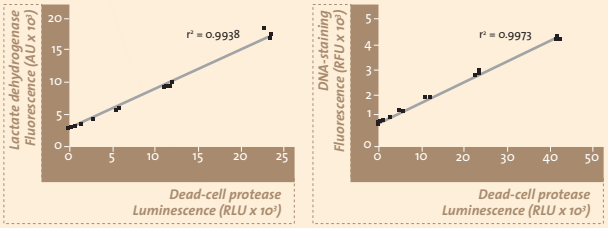
The principle of the **CytoTox-Glo™ Assay** is based on measurement of the activity of dead-cell protease, which is released into the medium following cell membrane damage. Using a luminogenic peptide substrate Ala-Ala-Phe-aminoluciferin (AAF-aminoluciferin), which cannot pass through the cell membrane, 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

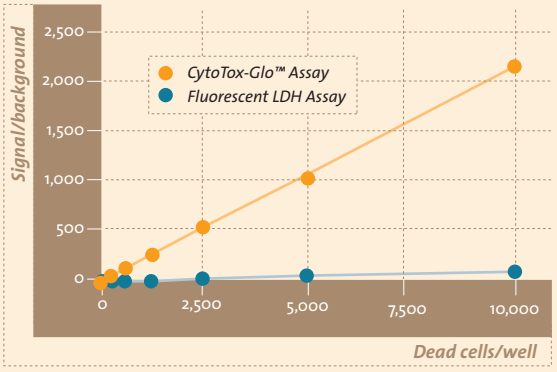
Assay type	Luminescent (glow-type)
Markers	Dead-cell protease
Applications	Cytotoxicity, membrane integrity, multiplexing with other cell-based assays
Cell type	Cell lines
Implementation	Homogeneous, one-step assay
Time required	15 minutes
Sensitivity	10 dead cells (96-well format)



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

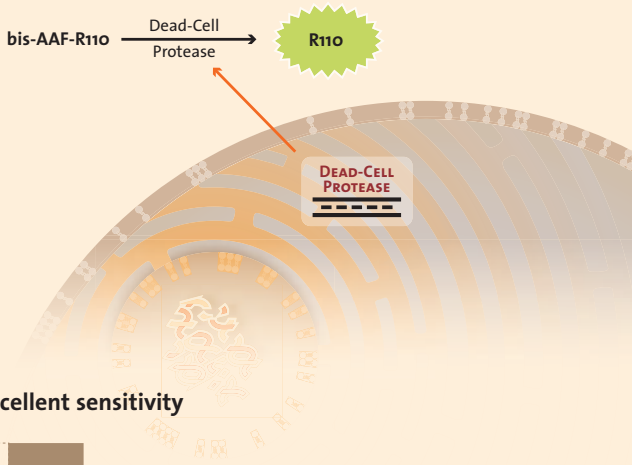
CytoTox-Fluor™ Assay is a fluorescent assay for determining cytotoxicity. It measures the proportion of dead cells in a cell culture sample. This assay is frequently used for multiplexing and is a component of the following multiplexing assays: MultiTox-FluorAssay, ApoTox-Glo™-Triplex Assay, Mitochondrial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

Assay principle

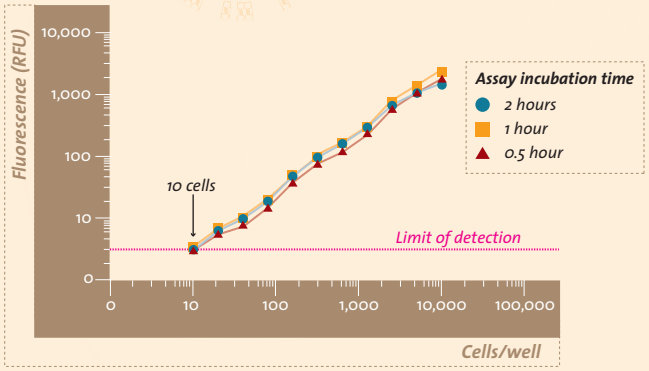
The assay is based on the detection of dead-cell protease, which is released following membrane damage. The fluorogenic cell-impermeable dye bis-Ala-Ala-Phe-rhodamine-110 (bis-AAF-R110) is specifically recognized by the dead-cell protease and converted to fluorescent rhodamine-110. The measured fluorescent 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
Implementation	Homogeneous, one-step assay
Time required	0.5–3 hours
Sensitivity	10 dead cells (96-well format)

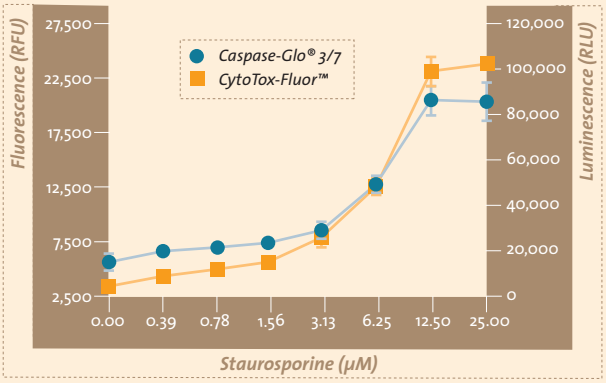


Excellent sensitivity



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

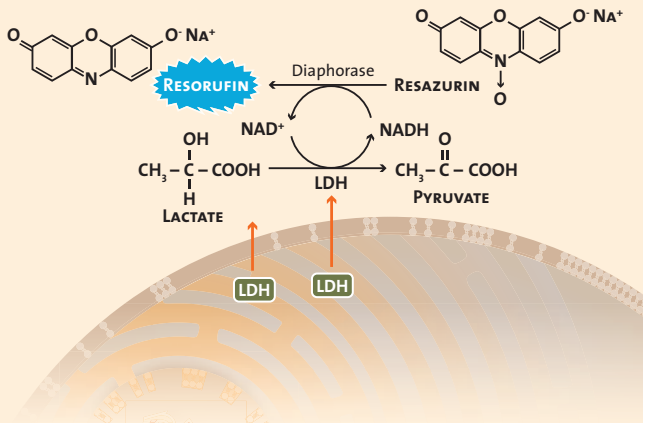
CytoTox-ONE™ assay can be used to measure both the cytotoxic effect of substances on cellular systems and cell-mediated cytotoxicity (e.g. immune response). Where cells are damaged by these processes, the cell membrane loses its integrity and cytosolic proteins, for example, such as lactate dehydrogenase (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

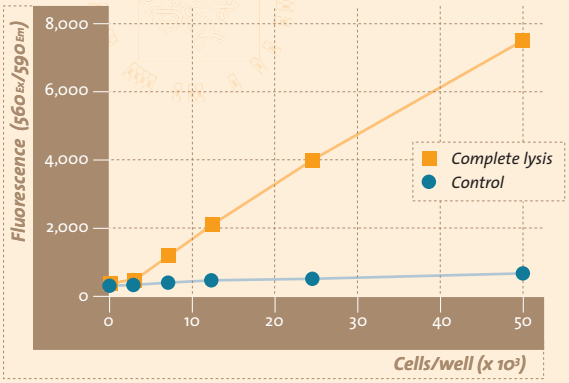
LDH activity in a coupled enzymatic reaction is measured as a gauge of cell damage. The assay reagent contains lactate, NAD^+, resazurin and the enzyme diaphorase. In the first step, lactate is oxidized to pyruvate by the LDH. Here, the reducing equivalents are initially transferred to NAD^+ and then 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 ($560_{Ex}/590_{Em}$)
Markers	LDH
Applications	Cytotoxicity; multiplexing
Cell type	Cell lines
Implementation	Homogeneous, two-step assay
Time required	10 minutes
Sensitivity	200 dead cells (96-well format)

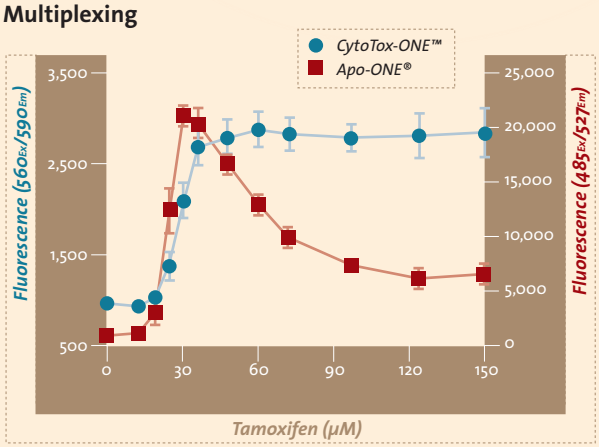


Sensitivity and linearity of the CytoTox-ONE™ Assay



After treatment of L929 cells with Triton X-100 (complete lysis) and with PBS (control), the CytoTox-One™ reagent was added. The reaction was terminated after 10 minutes and the absorbance measured in a fluorometer.

Multiplexing



Determination of membrane integrity and caspase activation in a sample after treatment of HepG2 cells with tamoxifen. Depending on the tamoxifen concentration, apoptosis and necrosis are induced.

Viral ToxGlo™ Assay

Cell-based

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.

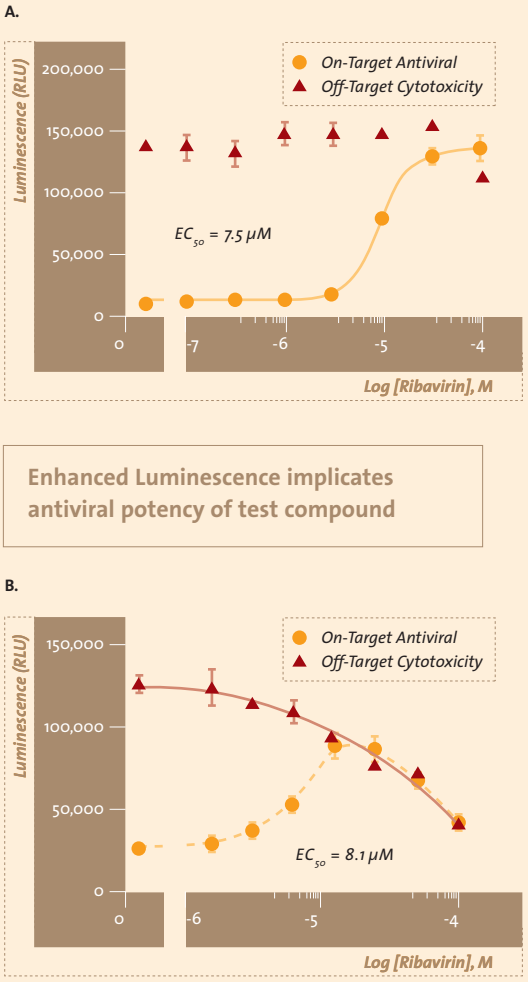
Assay principle

The homogeneous "add-mix-read" assay 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 TCID50; determination of potential antiviral potency or off-target toxicity of test compounds.
Virus	lytic virus, that induce cytophatic 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 plate formats.

Calculation of antiviral potency



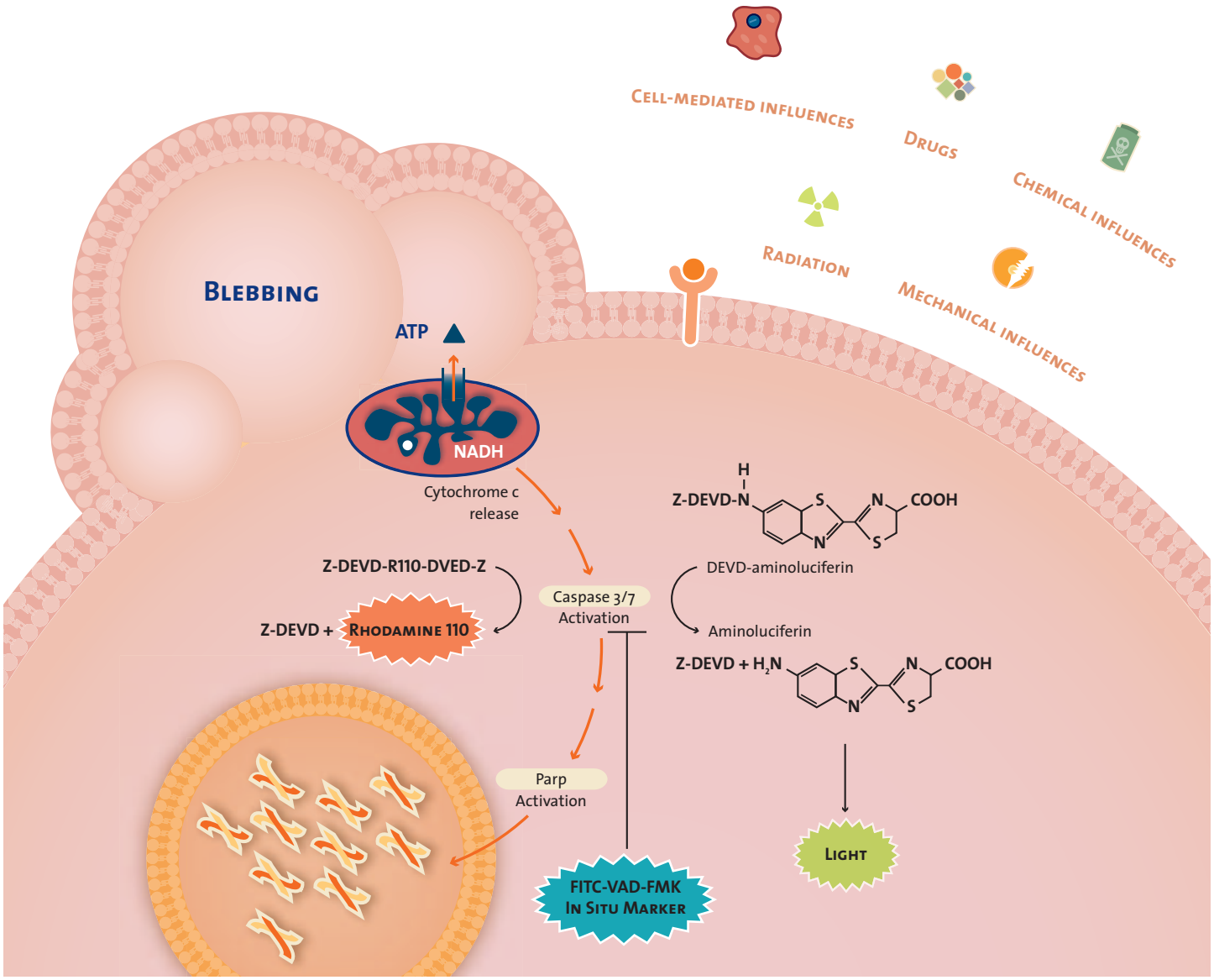
A. A. Half-log (3.16-fold) dilutions of Ribavirin were added to either MDCK cells with 100 TCID50 of H1N1 (on-target) or MDCK cells only (off-target) for 72 hours.
B. Twofold serial dilutions of Ribavirin were added to replicate wells of a 96-well plate containing BHK-21 cell monolayers. Either 100 TCID50 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

Apoptosis, "programmed cell death", is a multi-step process for the removal of the body's own cells. In contrast to cell death through necrosis, apoptosis is a physiological process which is set in motion by external factors (extrinsic) or intracellular influences (intrinsic). Apoptotic cells shrink, with distinguishing features including caspase activation and DNA fragmentation into characteristic lengths. The cell membrane remains intact during these processes. The formation of bulges and blebs in the cytoplasmic membrane (membrane blebbing) leads to the emergence of "apoptotic bodies", which are phagocytosed by macrophages or adjacent cells. Unlike necrosis, apoptosis does not therefore trigger an inflammatory response, and adjacent cells remain intact. Caspases play a key role in apoptosis as "initiator" and "effector" caspases. The detection of these makes it possible for apoptotic processes to be analyzed in detail.

Overview of caspase assays
Caspase-Glo® 3/7 Assay
Apo-One® Homogeneous Caspase-3/7 Assay
CaspACE™ FITC-VAD-FMK *In Situ* Marker



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-2***	Involved in stress-induced and TRAIL-mediated apoptosis.	Caspase-Glo® 2 Assay	Z-VDVAD-aminoluciferin	• Caspase-3/7 inhibitor (Ac-DEVD-CHO) • Proteasome inhibitor (MG-132)
	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	
	Caspase-6*	Plays a role in neurodegenerative diseases, such as Alzheimer's or Huntington's.	Caspase-Glo® 6 Assay	Z-VEID-aminoluciferin	• Caspase-3/7 inhibitor (Ac-DEVD-CHO) • Proteasome inhibitor (MG-132)

Note:
* The Caspase-Glo® 2 and Caspase-Glo® 6 Assays are recommended only for detecting the activity of purified enzyme preparations!
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

Applications

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

Assay description

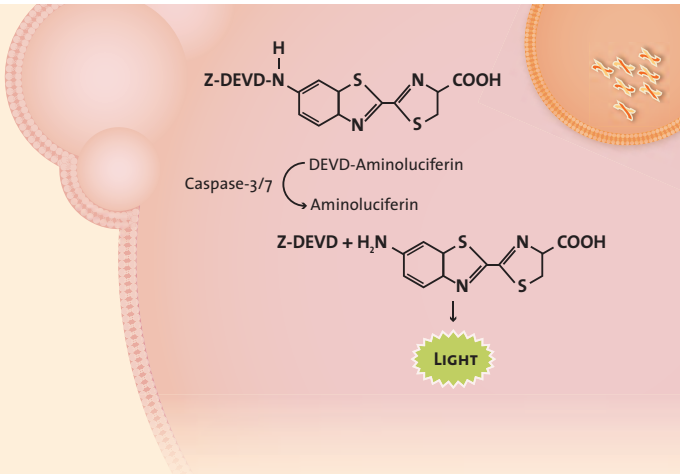
Caspase-Glo® 3/7 Assay is an extremely sensitive test system for determining apoptosis in cell culture or in enzyme preparations. The activity of the effector caspases, caspases -3 and -7, is measured through cleavage of a proluminescent substrate. This contains the tetrapeptide DEVD as a recognition 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

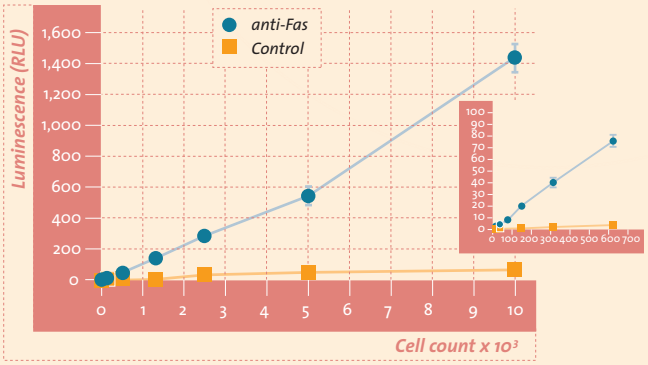
The assay is based on measurement of caspase-3/7 activity via the tetrapeptide DEVD. In this detection process, two enzymatic steps occur in immediate succession: the Caspase-Glo® 3/7 reagent contains a substrate for caspase-3 and -7 (Z-DEVD-aminoluciferin), which is cleaved and releases aminoluciferin. 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, enzyme preparations
Implementation	Homogeneous, one-step assay
Time required	0.5–3 hours
Sensitivity	100 apoptotic cells (96-well format)
Robustness	High Z' factor; excellent signal-to-background ratio

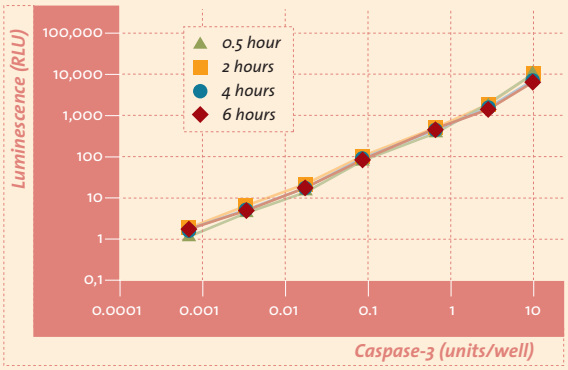


Wide linear measurement range from 20 to 10,000 cells



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

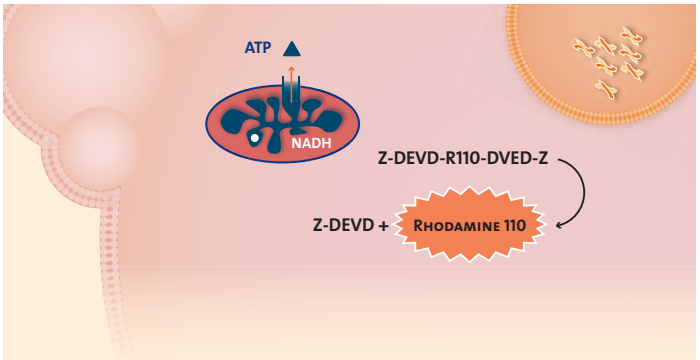
Apo-ONE® Homogenous Caspase-3/7 Assay is a fluorescent cell-based assay for determining caspase-3 and caspase-7 activity. This assay is also suitable for multiplexing.

Assay principle

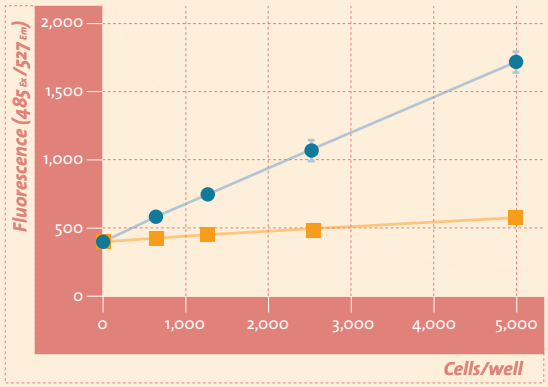
Apo-ONE® Homogeneous Caspase-3/7 Assay uses a fluorescent marked substrate (Z-DEVD-R110-DVED-Z) for detecting active caspase-3 and -7 in a wide variety of starting materials. Coupling rhodamine 110 to the caspase substrate allows a substantially higher degree of sensitivity to be achieved than in comparable fluorescent assays (approx. 600 apoptotic cells in a 96-well format). The fluorescence signal in this case is proportional to the quantity of activated caspases. We recommend using a 96-well or 384-well format.

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)
Robustness	High Z' factor; excellent signal-to-background ratio

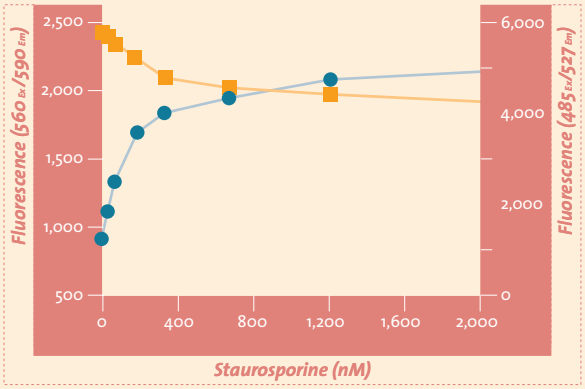


Sensitivity



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



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 *in vivo* detection of caspase activity and can be combined with other markers (e.g. antibodies) for double staining. It is a fluorescent analog of the pan-caspase inhibitor Z-VAD-FMK (carbobenzoxy-valyl-alanyl-aspartyl-[O-methyl]-fluoromethyl-ketone). The carbobenzoxy group is replaced by the fluorescent dye fluorescein isothiocyanate (FITC). This marker is cell-permeable and is delivered direct to the cells.

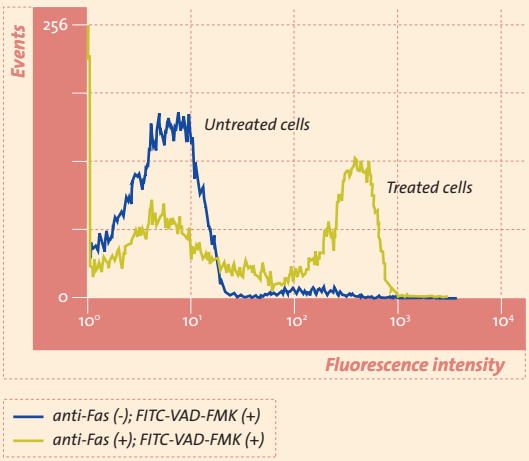
Assay principle

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

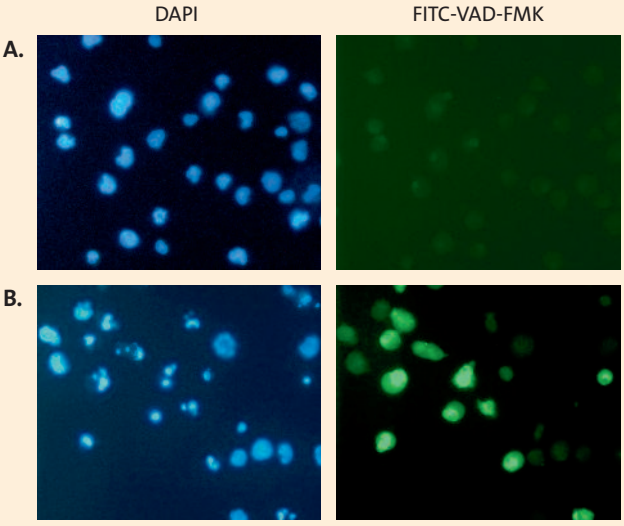
Assay type	Fluorescent
Markers	Pan-caspases
Applications	Apoptosis, can be combined with antibody labeling
Cell type	Cell lines, primary cells
Implementation	Add directly to the medium
Time required	0.5–1 hours
Sensitivity	4,000 apoptotic cells

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

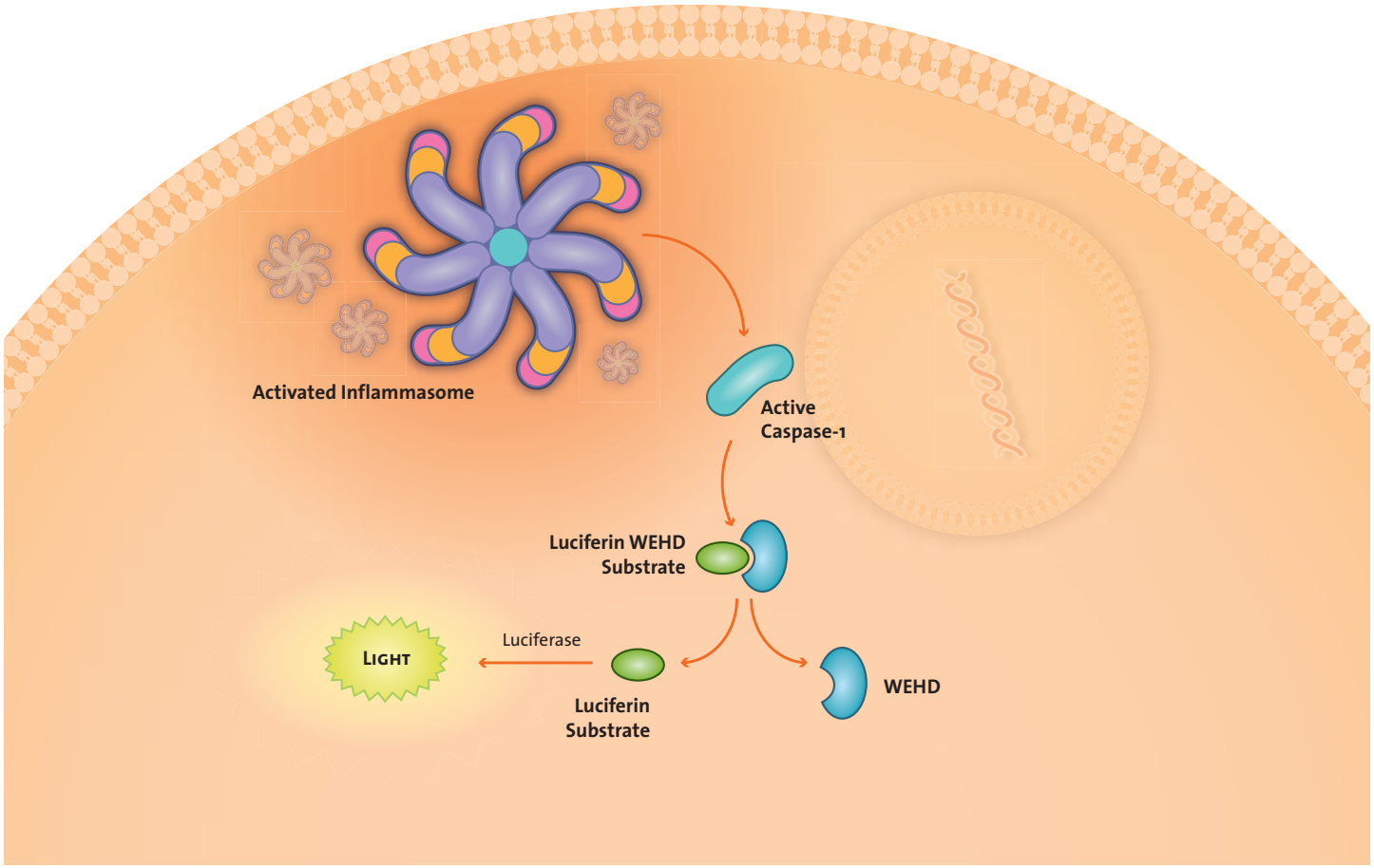


In situ marking with CaspACE™ FITC-VAD-FMK corresponds to the flow cytometric analysis shown above. Jurkat cells were analyzed under a fluorescence microscope. **A:** untreated cells and **B:** cells treated with anti-Fas. Nuclear staining with the DAPI stain confirms activation of apoptosis (condensed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

IId Inflammation

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-1ß and IL-18 and 2) pyroptosis, an immunogenic form of cell death.

Caspase-Glo® 1 Inflammasome Assay



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.

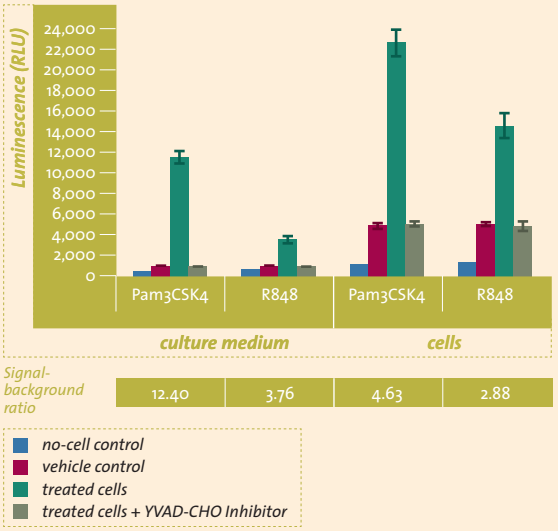
Assay principle

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

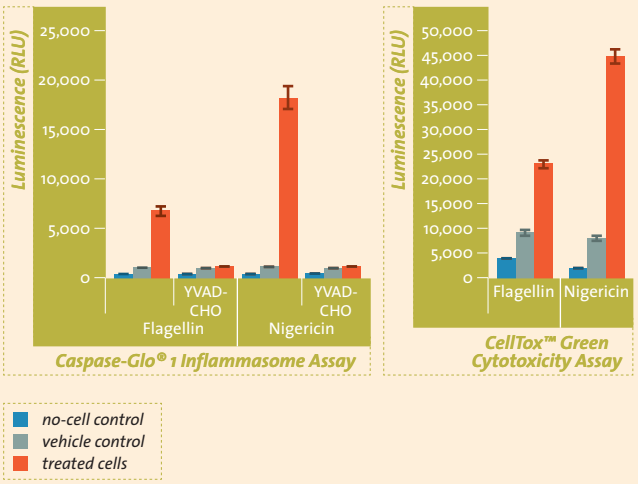
Assay type	Luminescent (glo-type; T1/2 > 3 h)
Markers	Caspase-1 activity
Applications	Determination of inflammasome activity or purified caspase-1 activity.
Cell type	Cells or medium from cultured cells in multiwell plates. No lysate preparation or multiple pipetting steps required.
Implementation	Homogeneous, 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 20 nM 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

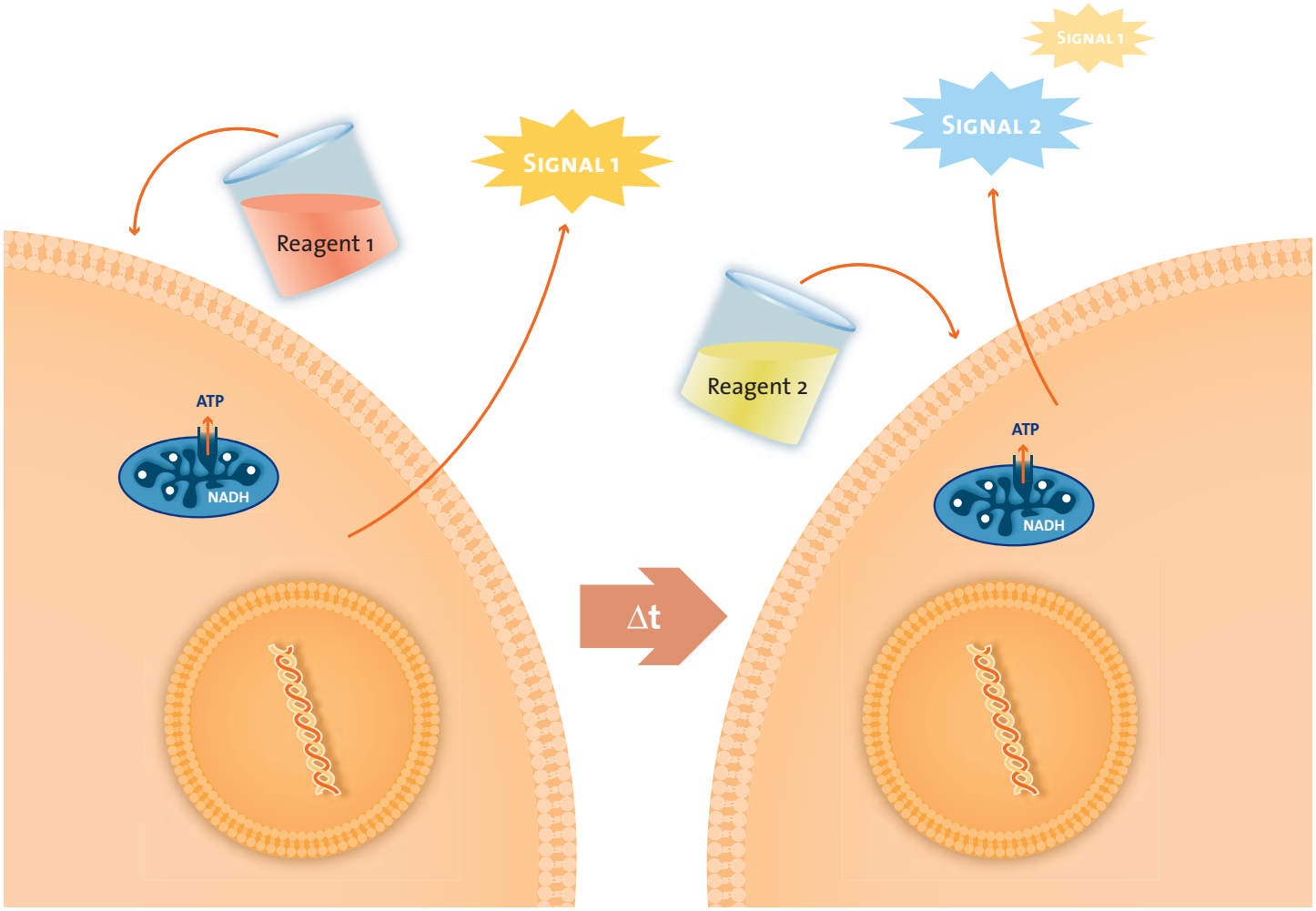


THP-1 cells were added to plates at 5×10^5 cells/ml in 100 µl of medium and differentiated with PMA (20 nM, 3 days) in 96-well plates followed by treatment with flagellin (1 µg/ml, 1 hour) or nigericin (20 µM, 2 hours). Half of the culture was transferred to a separate plate, and Caspase-Glo® 1 Reagent 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.

IIe 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 internal 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	Information obtained by multiplexing	2nd Assay
RealTime-Glo™ MT Cell Viability Assay	**CellTox™ Green Cytoxicity Assay**	**Cell viability and cytotoxicity (membrane integrity)**
	CytoTox-Fluor™ Cytotoxicity Assay	**Cell viability and cytotoxicity (protease release)**
	NAD/NADH-Glo™ Assay	**Cell viability and measurement of NAD^+ and NADH**
	NADP/NADPH-Glo™ Assay	**Cell viability and measurement of $NADP^+$ and NADPH**
	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**
	P450-Glo™ Assay	**Cytochrom P450**
CellTiter-Blue® Assay Fluorescence (Resazurin to Resorufin) $560_{Ex}/590_{Em}$	**CytoTox-ONE™ Assay**	**Cytotoxicity**
	Apo-ONE® Caspase 3/7 Assay	**Apoptosis**

Cytotoxicity Assay

1st Assay	Information obtained by multiplexing	2nd Assay
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	
	Caspase-Glo® 3/7 Assay	**Apoptosis**
	Apo-ONE® Caspase 3/7 Assay	
CytoTox-Fluor™ Assay Fluorescence Dead-Cell Protease-Activity (bis-AAF-R110) $485_{Ex}/520_{Em}$	**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	Information obtained by multiplexing	2nd Assay
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**

Multiplexing Assay

Assay	Information obtained by multiplexing	
MultiTox-Fluor™ Multiplex Assay Fluorescence Cell Viability and Necrosis CellTiter-Fluor™: $400_{Ex}/505_{Em}$ CytoTox-Fluor™: $485_{Ex}/520_{Em}$	**Caspase-Glo® 3/7 Assay** **(available as ApoTox-Glo™ Triplex Assay)**	**Apoptosis**
	GSH-Glo™ Assay	**Oxidative Stress**

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.

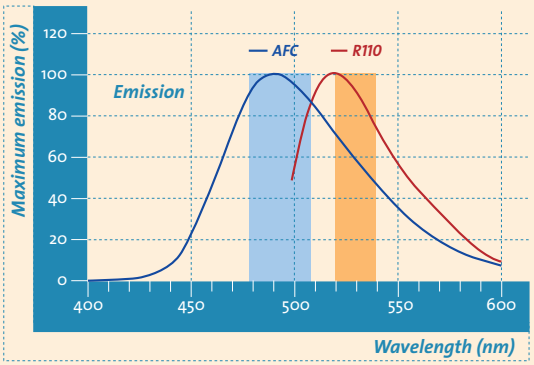
Assay principle

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

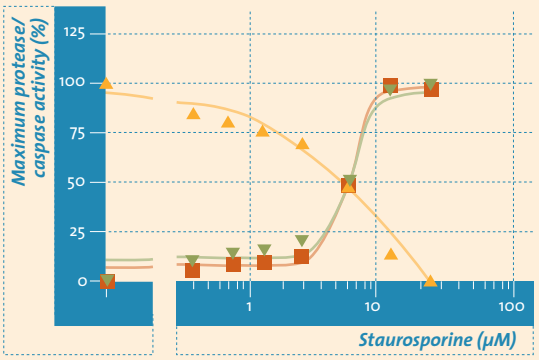
Assay type	Fluorescent (AFC $400_{Ex}/505_{Em}$, R110 $485_{Ex}/520_{Em}$)
Markers	Live-cell protease; dead-cell protease
Applications	Simultaneous measurement of cell viability and cytotoxicity in the same well
Cell type	Human cell lines (adherent or in suspension)
Implementation	Homogeneous, one-step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

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.

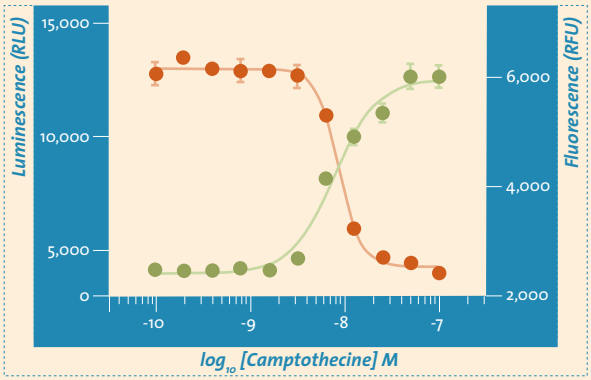
Assay principle

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	Human cell lines (adherent or in suspension)
Implementation	Homogeneous, two-step assay
Time required	0.5 hours
Sensitivity	40 living cells; 10 dead cells (96-well format)

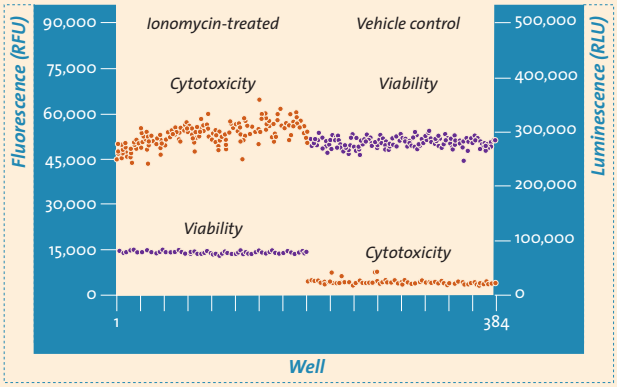
MultiTox-Glo Multiplex Cytotoxicity Assay



- *GF-AFC substrate (viability) EC_{50} = 8.5 nM*
- *AAF-Glo™ substrate (cytotoxicity) EC_{50} = 7.6 nM*

Comparable EC_{50} values through parallel measurement of live-cell and dead-cell protease. Jurkat cells were treated with the alkaloid camptothecine for 24 hours. Cell viability and cytotoxicity were determined using MultiTox-Glo. The EC_{50} values determined are comparable.

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



- *GF-AFC substrate (viability) Z' = 0.85*
- *AAF substrate (cytotoxicity) Z' = 0.75*

Low dispersion of individual measurements using the MultiTox-Glo reagent. In this trial, 5×10^3 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.

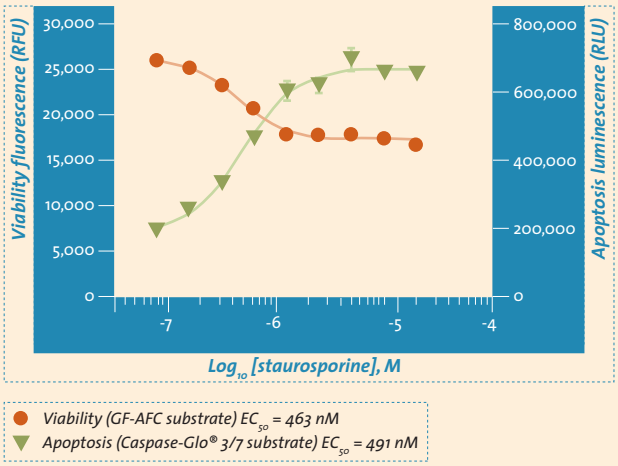
Assay principle

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)
Implementation	Homogeneous, two-step assay
Time required	1–3 hours
Sensitivity	40 living cells; 100 apoptotic cells (96-well format)

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.

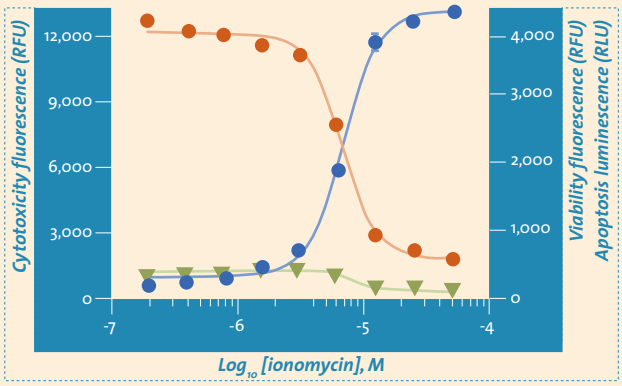
Assay principle

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)
Implementation	Homogeneous, two-step assay
Time required	1–3 hours
Sensitivity	40 living cells; 10 dead cells; 100 apoptotic cells (96-well format)

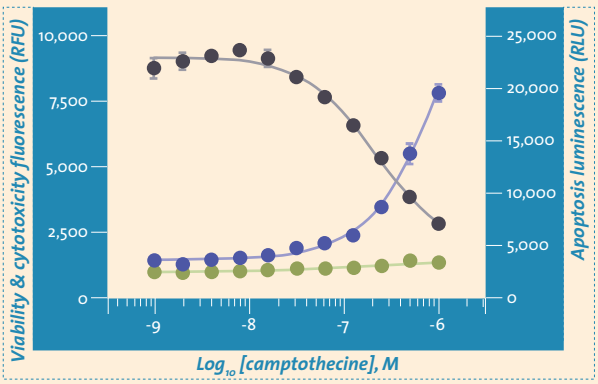
Induction of primary necrosis by ionomycin



Viability (GF-AFC) EC_{50} = 6.89 µM
Apoptosis (caspase-3/7) EC_{50} = N.D.
Cytotoxicity (bis-AAF-R110) EC_{50} = 6.87 µM

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



Viability/cytotoxicity reagent (viability)
Viability/cytotoxicity reagent (cytotoxicity)
Caspase-Glo® 3/7 reagent (apoptosis)

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.

Assay description

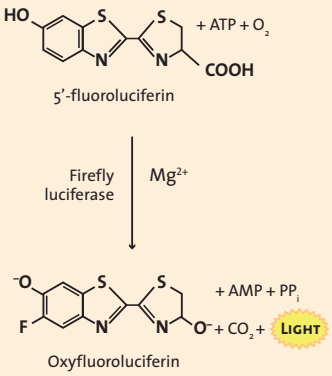
ONE-Glo™ + Tox Luciferase Reporter and Cell Viability Assay is an assay with 2 pipetting steps for simultaneously determining firefly luciferase reporter gene expression and cell viability in the same well. Using this assay significantly simplifies and improves the interpretation of reporter gene expression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

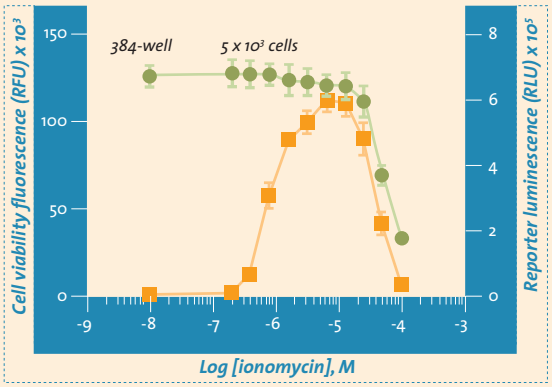
Assay features

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



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 ionoymcin. 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

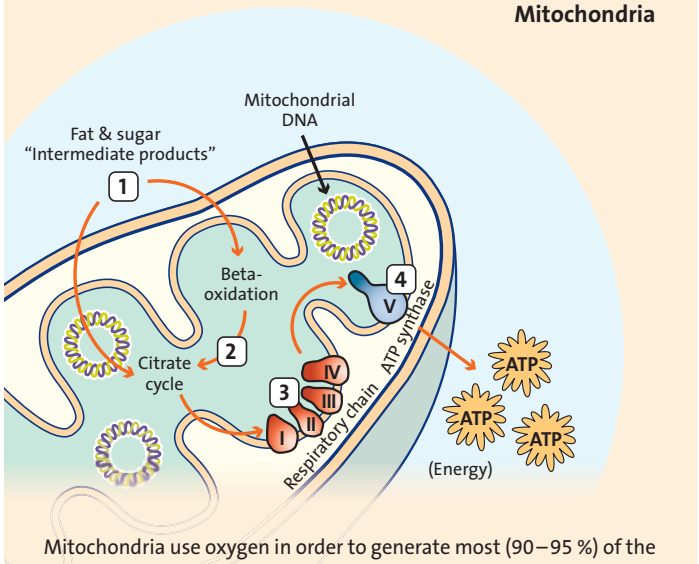
Mitochondrial ToxGlo™ Assay is a cell-based assay for predicting mitochondrial toxicity which may arise e.g. as a result of treatment with xenobiotics. The assay is performed directly in the cell culture plate. ATP content and membrane integrity are measured. Combining the two data sets makes it possible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

Assay principle

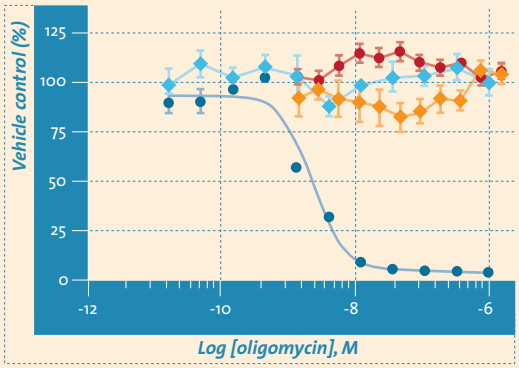
The assay is based on the sequential measurement of two biomarkers. In the first step, cell membrane integrity is determined by measuring dead-cell protease activity in the medium. For this purpose, a fluorogenic peptide substrate (bis-AFF-R110) is used which cannot permeate the cell membrane, and which therefore does not generate a signal in living cells (cf. CytoTox-Fluor™ Cytotoxcicity 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
Implementation	Homogeneous, two-step assay
Time required	0.6–3 hours
Robustness	High Z' factor



Mitochondria use oxygen in order to generate most (90–95 %) of the ATP for multicellular organisms. Both oxygen consumption and ATP content are therefore a good measure of mitochondrial activity.



- *ATP (galactose) EC_{50} = 2,3 nM*
- *ATP (glucose) EC_{50} = ND*
- *Cytoxicity (glucose) EC_{50} = ND*
- *Cytoxicity (galactose) EC_{50} = ND*

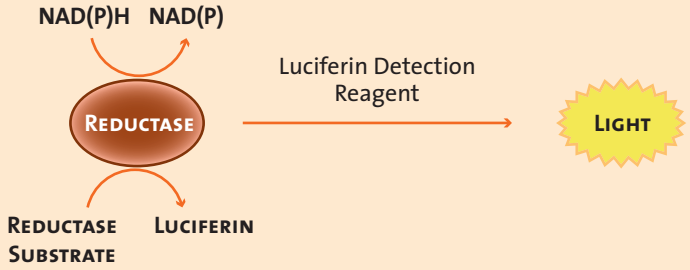
Mitochondrial responsiveness/toxicity e.g. in K562 cells treated with oligomycin* in a glucose- or galactose-containing medium. Mammalian cells generate ATP both via mitochondria (oxidative phosphorylation) and via non-mitochondrial reaction pathways (glycolosis). In the glucose-free medium, the cells are "forced" to generate the ATP via oxidative phosphorylation. Mitochondrial toxicity can be determined only in a galactose-containing medium.
*Oligomycin is a well-known inhibitor of mitochondrial H^+-ATP synthase and leads here to a reduction of ATP content but not damage to the cell membrane (cytotoxicity).

III Cell Metabolism

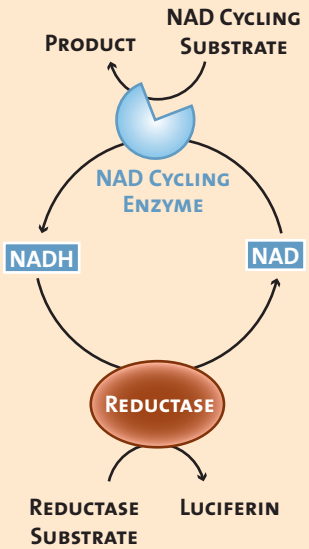
Cancer is a disease of uncontrolled cell growth that requires cancer cells to alter metabolic pathways to survive and proliferate. The principal mechanisms underlying this metabolic reprograming by oncogenes and tumor suppressor genes is still poorly understood. Nicotinamide adenine dinucleotides (NAD^+,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

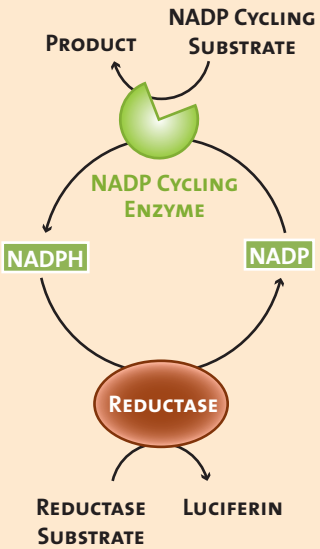
NAD(P)H-Glo™ Detection System – detects NADH and NADPH (Biochemical assay)



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.

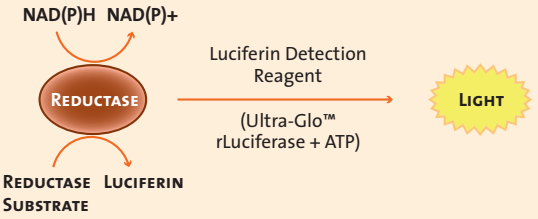
Assay principle

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.

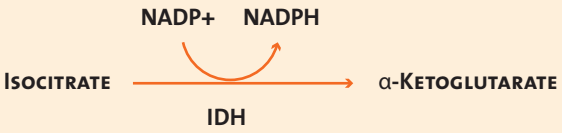
Assay features

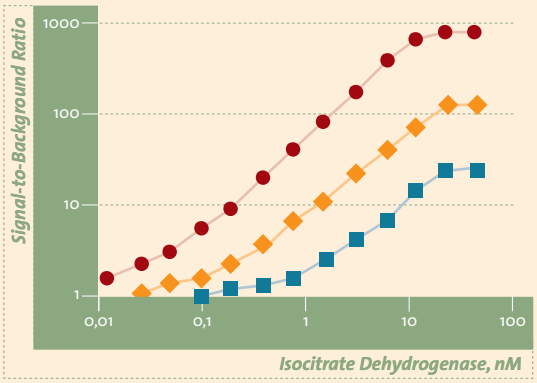
Assay type	Luminescent (glow-type; $T_{1/2}$ > 2 h)
Markers	NADH, NADPH
Applications	Monitoring the activity of enzymes that produce or use NAD(P)H.
Sample	Enzyme preparations
Implementation	Homogeneous, one-step assay with flexible storage capability
Linearity	Detects 0.1 µM to 25 µM NAD(P)H
Sensitivity	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

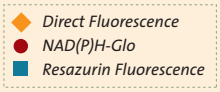
NAD(P)H™ Detection System Assay-Principle

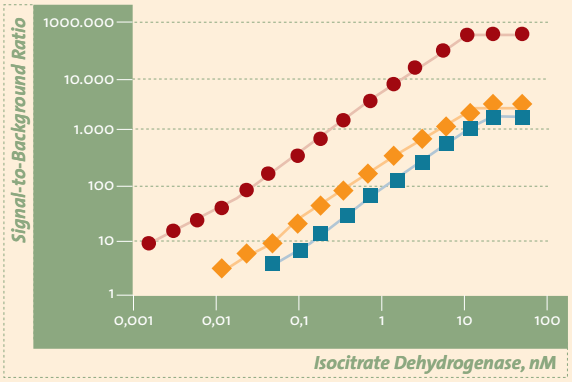


The luminescent NAD(P)H-Glo™ Detection System is more sensitive than alternative fluorescent methods









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

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

Cell-based/Biochemical

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

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

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

Applications

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

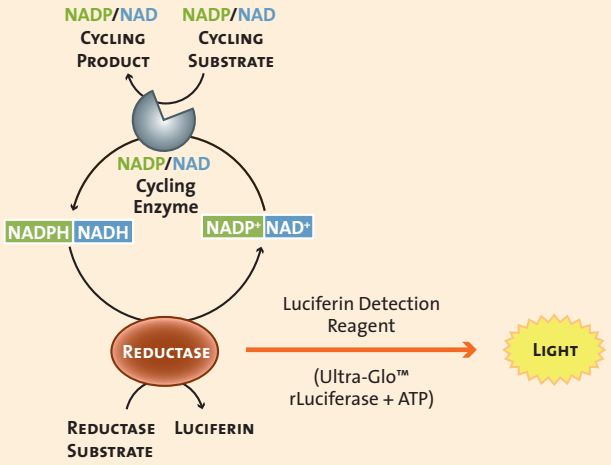
Assay description

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

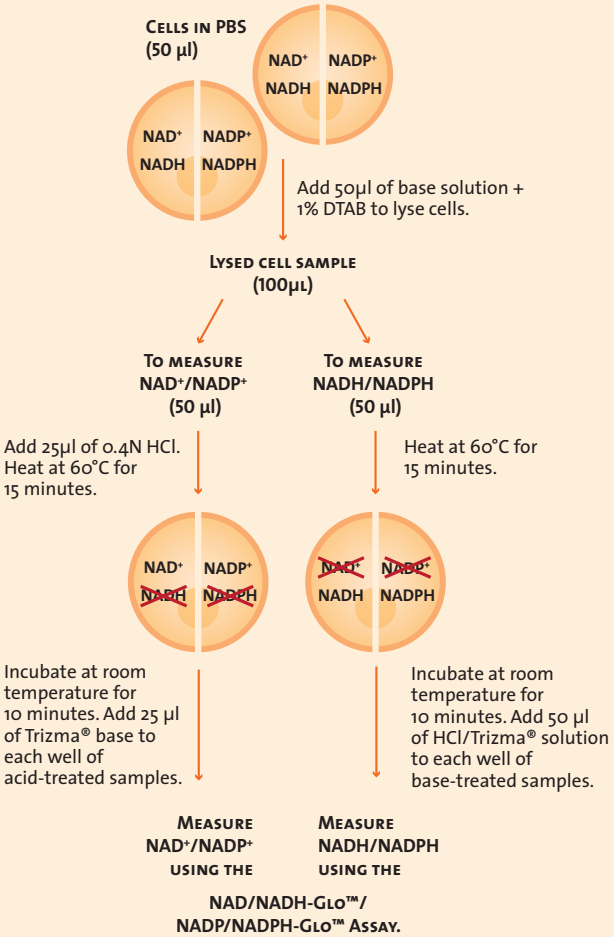
Assay principle

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.

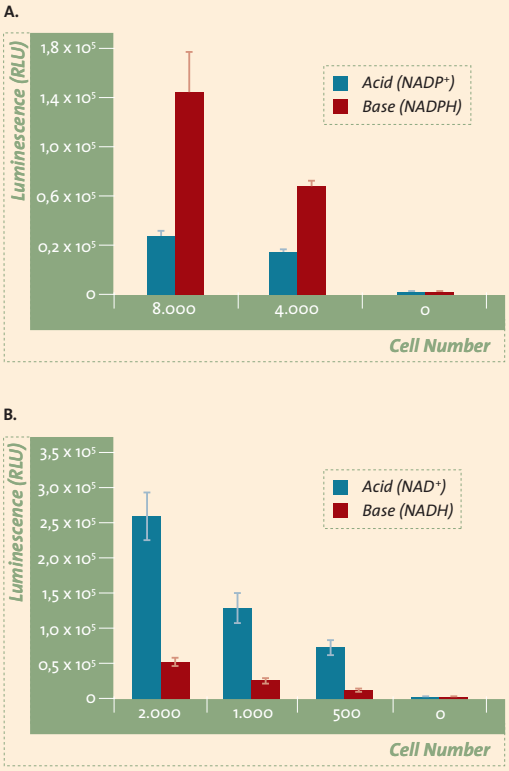


Luciferin then is quantified using Ultra-Glo™ Recombinant Luciferase, and the light signal produced after an incubation of 30–60 minutes is proportional to the amount of NAD^+ and NADH in the sample. Detergent present in the reagent lyses cells, allowing detection of total cellular NAD^+ and NADH in 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.

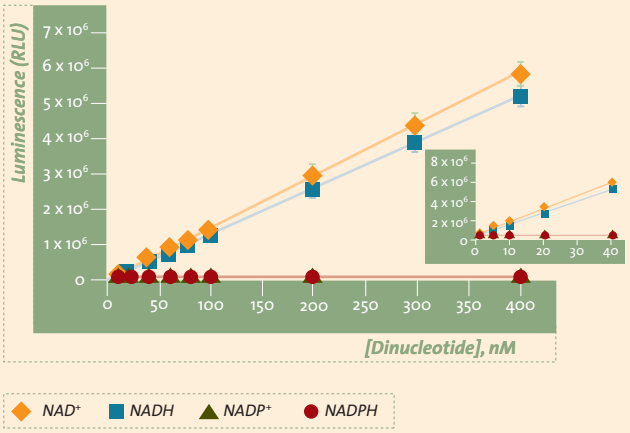
Assay features

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
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

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



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



Individual purified nicotinamide adenine dinucleotides were assayed following the protocol 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). Fiftymicroliter 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.

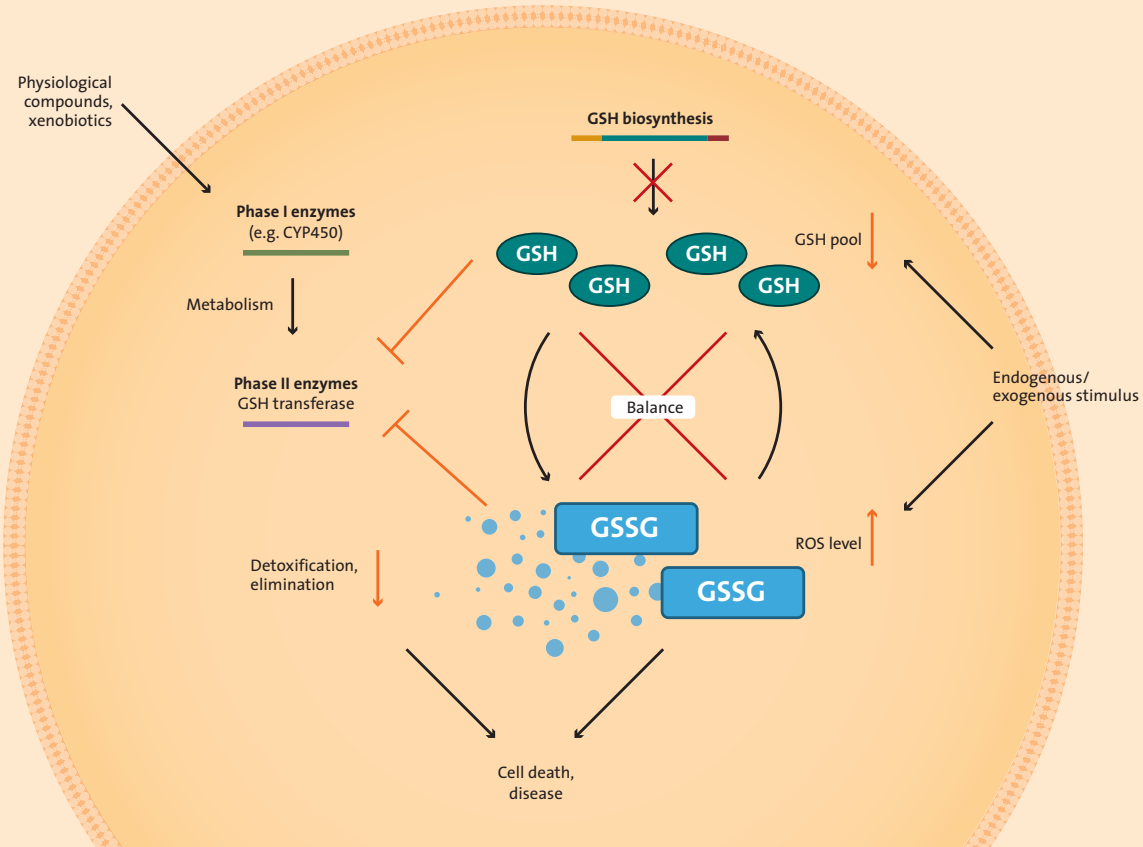
Glutathione consists of the three amino acids glutamic acid, cysteine and glycine. Besides functioning as the main component of the reductive pool, GSH probably constitutes the most important reserve of the amino acid cysteine.

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

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

1. **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™ H_2O_2 Assay

Cell-based/Biochemical

Applications

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

Assay description

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

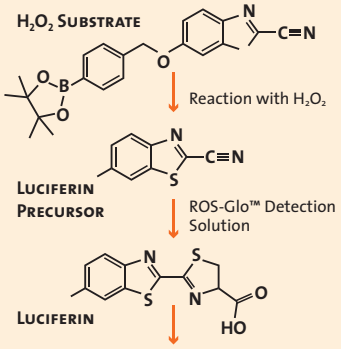
Assay principle

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

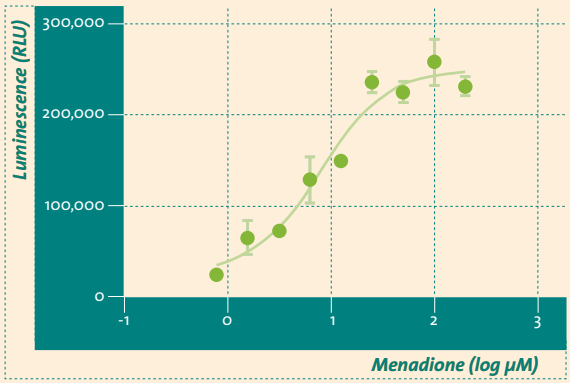
Assay features

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

ROS-Glo™ H_2O_2 Assay-Principle

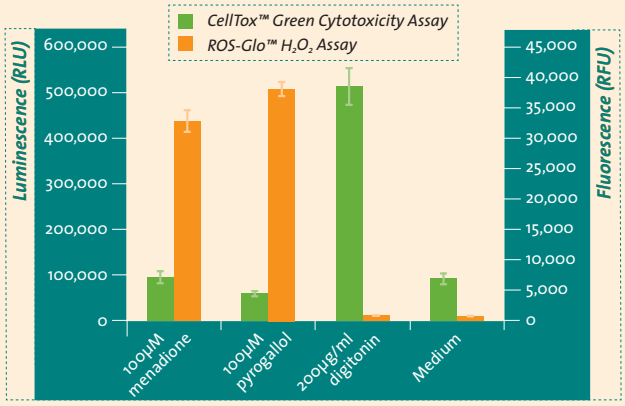


ROS induction in cultured cells



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

Multiplex with real-time CellTox™ Cytotoxicity Assay



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

GSH-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.

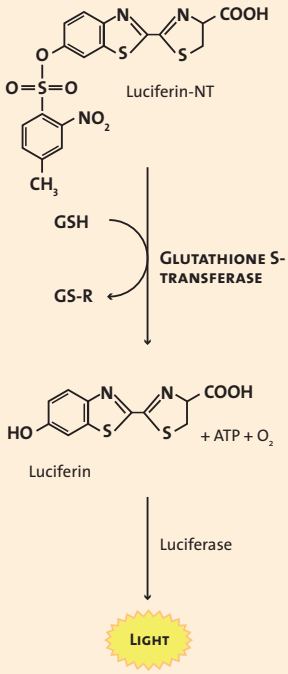
Assay principle

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

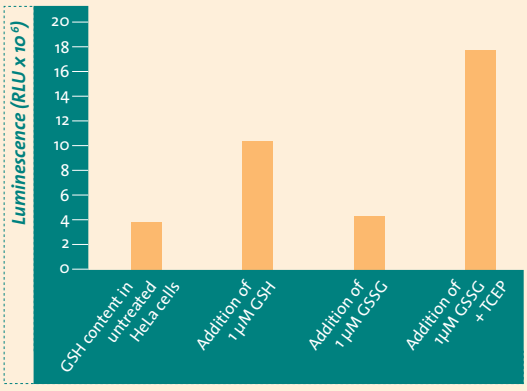
Schematic representation of the GSH-Glo™ Glutathione Assay



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.

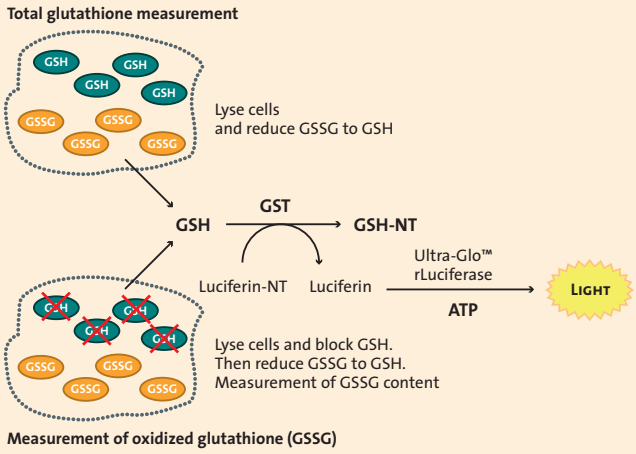
Assay principle

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

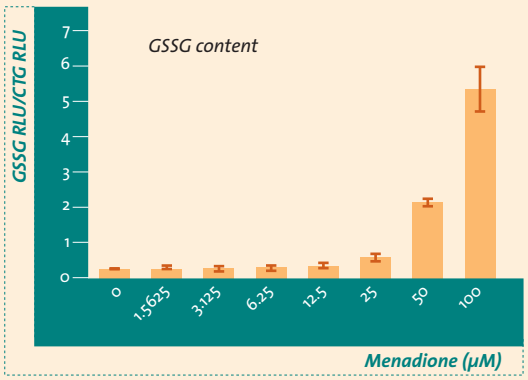
Total glutathione and GSSG are determined in two parallel reactions. The GSH level can be calculated by subtracting the GSSG level from the total glutathione level.

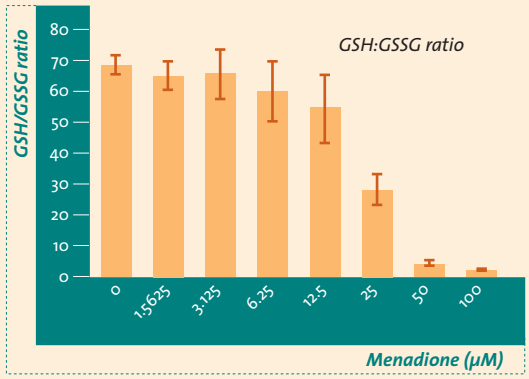
1. In the first mixture, after addition of the total glutathione reagent, total cellular glutathione (GSSG and GSH) is converted into GSH.
2. In the parallel second mixture, after the addition of the oxidized glutathione reagent, the cellular GSH is first blocked. The GSSG remains intact and is then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

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

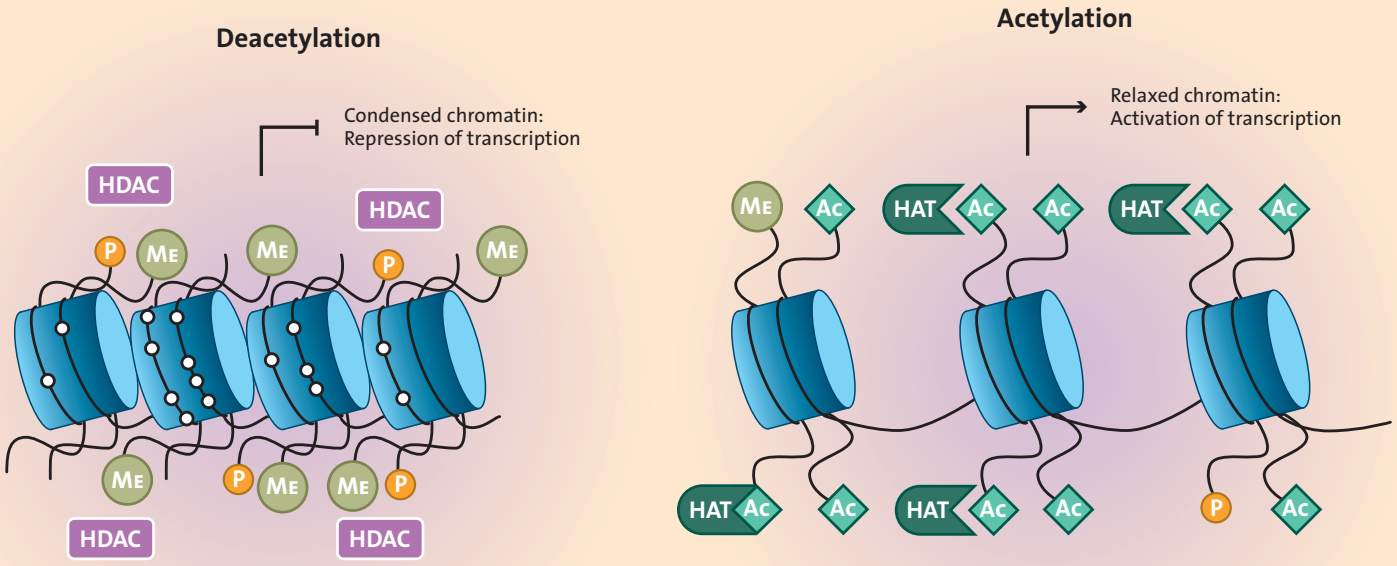
"Epigenetics" is the term used to refer to changes in gene expression caused by mechanisms which are not determined by the DNA sequence. These regulatory mechanisms can switch individual genes and/or gene segments on and off without any change in the DNA sequence. Higher-level expression patterns emerge, 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.

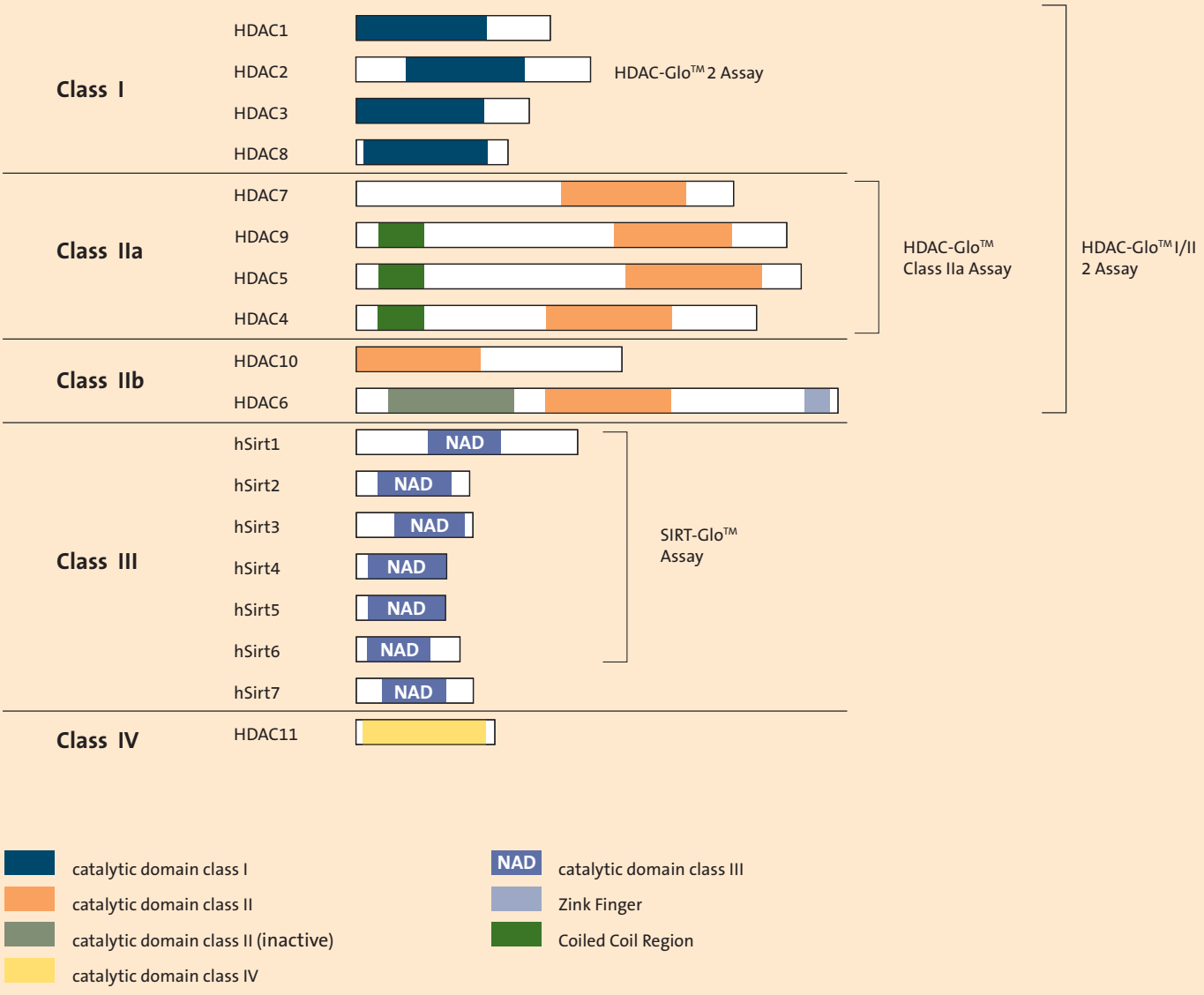
HDACs and the NAD^+-dependent sirtuins (class III histone-deacetylases) are histone-modifying enzymes which catalyze the deacetylation of lysine residues in histones and thereby influence their activity. They play an important role in numerous biological processes. As anti-cancer agents, HDAC inhibitors 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™ Class IIa Assay
HDAC-Glo™ I/II Assay & HDAC-Glo™ I/II Screening Systems
SIRT-Glo™ Assays and Screening Systems
UDP-Glo™ Glycosyltransferase 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

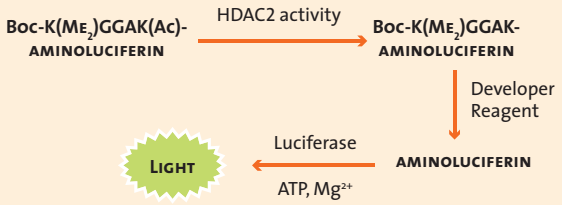
Assay principle

HDAC2 enzyme deacetylates the luminogenic substrate, Boc-K(Me_2)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.

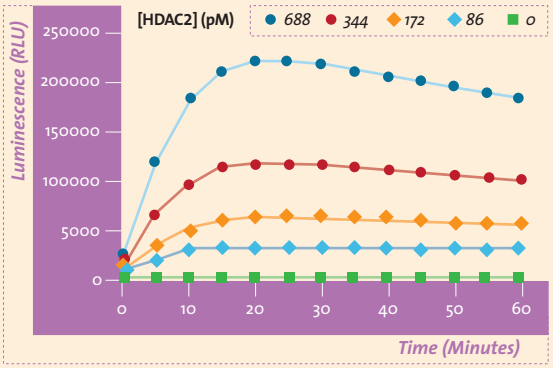
Assay features

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

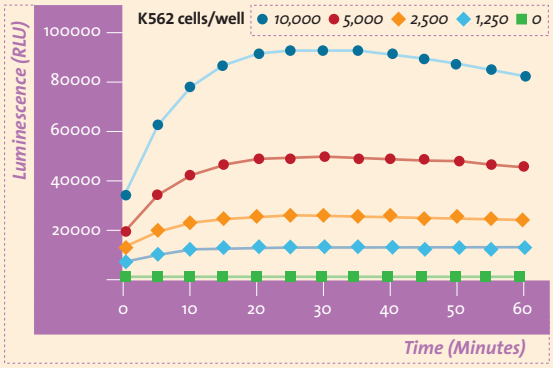
HDAC-Glo™ 2 Assay Principle



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.

HDAC-Glo™ Class IIa 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™ Class IIa Assay** is a single-reagent-addition, homogeneous, luminescence assay that selectively measures the relative activity of HDAC class IIa enzymes (HDAC4, 5, 7, 9) from cells, extracts or recombinant sources. The assay uses an isoenzyme-selective, acetylated, live-cell-permeant, luminogenic peptide substrate that can be deacetylated by HDAC Class IIa enzymes. 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.

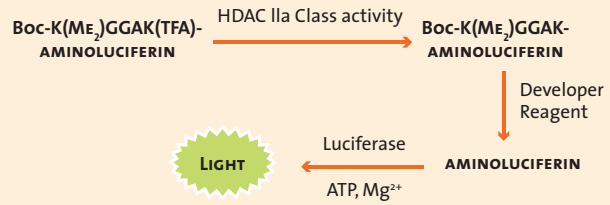
Assay principle

HDAC Class IIa enzymes deacetylates the luminogenic substrate, Boc-K(Me_2)GGAK(TFA)-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.

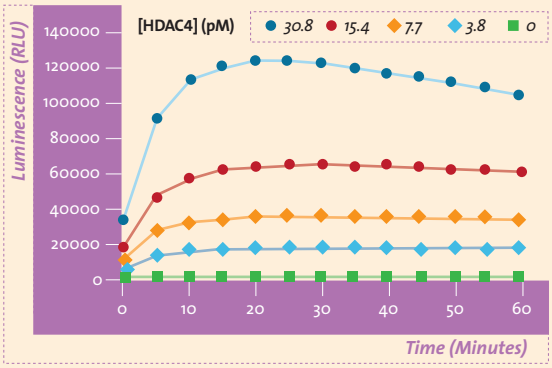
Assay features

Assay type	Luminescent (glow-type)
Markers	HDAC class IIa enzyme (HDAC4, 5, 7, 9)
Applications	Determination of HDAC inhibitor potency using purified enzymes, extracts or cells directly
Cell type/Sample	Cells or extracts, enzyme preparations
Implementation	Homogeneous, one-step assay
Time required	10–20 minutes
Robustness	Easily scalable from 96- to 384-well plate

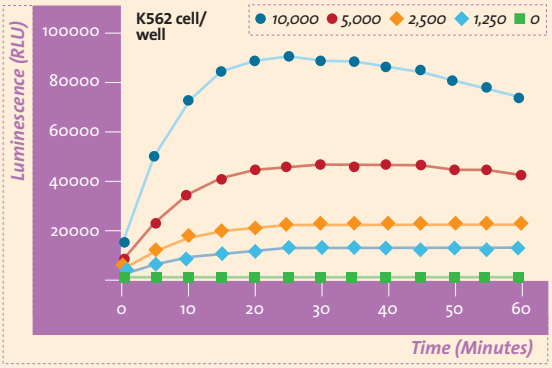
HDAC-Glo™ Class IIa Activity Assay Principle



A. Activity Measurement of Purified HDAC4 Enzyme



B. Measurement of HDAC Enzyme Activity in Cells



The HDAC-mediated luminescent signal is proportional to deacetylase activity and is persistent in (A.) in a biochemical assay and (B.) cell-based lytic assay. Enzymatic steady state (between deacetylase, developer enzyme and luciferase) is typically achieved within 10 minutes for biochemical assays and within 15–20 minutes for cell-based lytic assays, with a half-life of approximately 60–90 minutes after steady state is achieved.

HDAC-Glo™ I/II Assays & Screening Systems

Cell-based

Applications

Determination of HDAC class I and II enzyme activity; screening of HDAC inhibitors in living or lysed cells.

Assay description

The **HDAC-Glo™ I/II Assay** determines the activity of HDAC class I and II enzymes from cells, extracts or purified enzyme fractions. The assays are 10 to 100 times more sensitive than comparable fluorescent measurements and require fewer individual steps and only small quantities of enzymes for a very good signal-to-noise ratio. The luminescent HDAC assays can be combined with fluorescent cell-based assays in order to determine viability and cytotoxicity simultaneously.

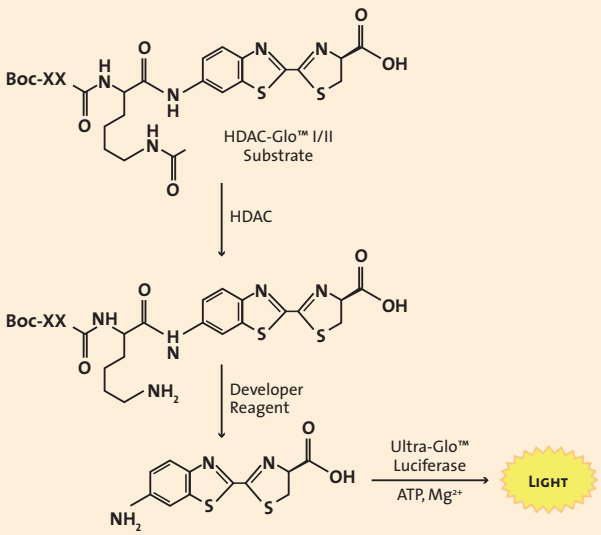
Assay principle

The assay is based on the enzymatic coupling of HDAC activity to the luciferase reaction. The reagents contain a cell-permeable peptide substrate which consists of an acetylated lysine peptide sequence derived from histone 4 and which is coupled to aminoluciferin. After deacetylation of the lysine residue by HDAC enzymes, the peptide is cleaved from aminoluciferin by a developer enzyme. The aminoluciferin serves as a substrate for the Ultra-Glo™ Luciferase. The HDAC-mediated luminescent signal is proportional to the deacetylase activity.

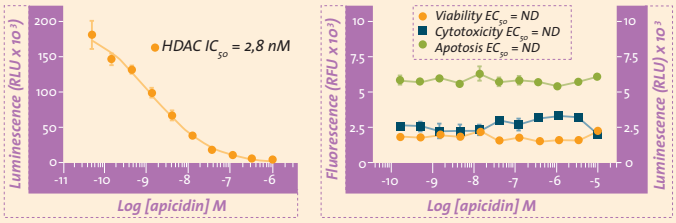
The cell permeability of the peptide substrate enables real-time measurement of HDAC inhibition and subsequent analyses of the mixture. A deacetylated substrate is available as a control.

Assay features

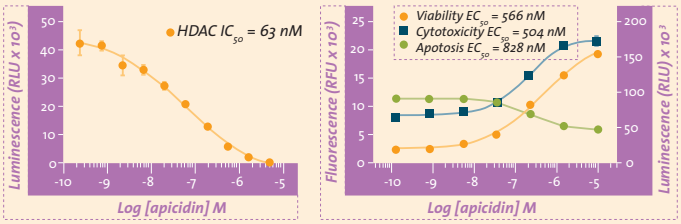
Assay type	Luminescent (glow-type)
Markers	HDAC enzyme activity
Applications	Determination of HDAC class I and II enzyme activities
Cell type	Lysed or non-lysed cell lines (primary, adherent or in suspension)
Implementation	Homogeneous, one-step assay, automatable
Time required	15–45 minutes



Primary cardiomyocytes



U937 monocytic lymphoma



Primary cardiomyocytes and U937 cells were seeded in 96-well plates. A serial dilution of apicidin was added and incubated for 24 hours at 37°C. In one half of the plate HDAC-Glo™ I/II Assay reagent was added, and in the other half ApoTox-Glo® Multiplex reagent. Fluorescence and luminescence were measured in a multireader.

This data shows that HDAC I/II can be inhibited in both cell types, but with differing consequences. Where HDAC inhibition occurs, differentiated cardiomyocytes are less susceptible in terms of their ability to survive, whereas cancer cells react very sensitively. HDAC inhibitors are therefore of interest as a target group for cancer drugs.

SIRT-Glo™ Assays and Screening Systems

Biochemical

Applications

Determination of sirtuin enzyme activity; screening of SIRT inhibitors.

Assay description

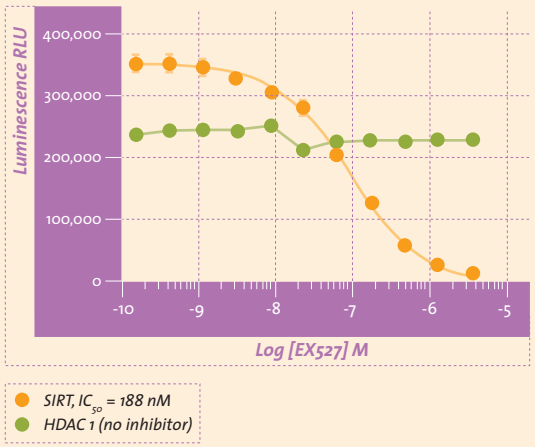
Determination of the enzyme activity of NAD^+-dependent (class III histone deacetylase) sirtuins (SIRT 1-6). This assay is available as a biochemical assay.

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



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 enyzmes 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.

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. The assay is fast, simple, and homogenous, it does not require antibodies, nor custom made substrates.

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 20 µM UDP. The assay is intended for use with purified or immunoprecipitated GTs.

Assay features

Assay type	Luminescent, 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
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

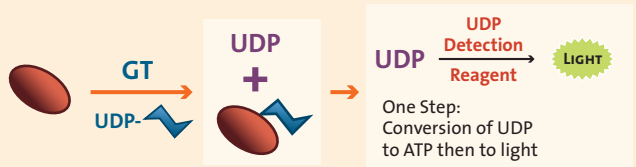
Simple "Add and Read":
No radioisotopes.
No product separation.
No HPLC

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-forming Reaction | Reaction Products | UDP Detection Assay

GT *Glycosyltransferase*
Clycosyltransferase substrate
UDP- *UDP-Sugar substrate*

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

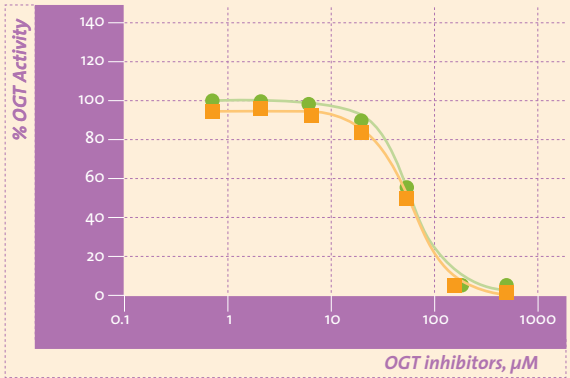
O-GlcNAc Transferase reaction (25 µl)

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

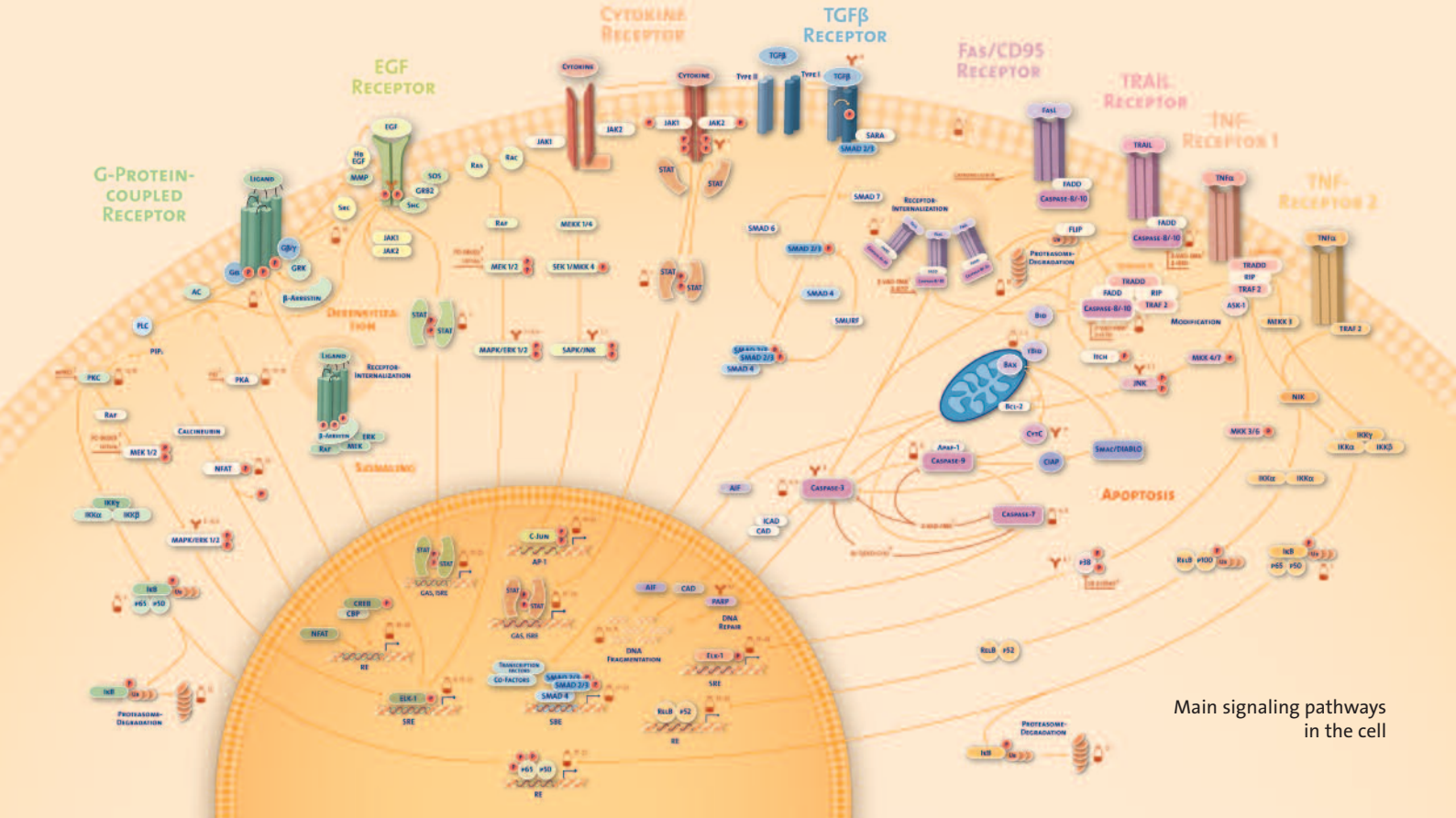
OGT-Inhibitors dose response



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 signaling 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™ MaxAssay
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.

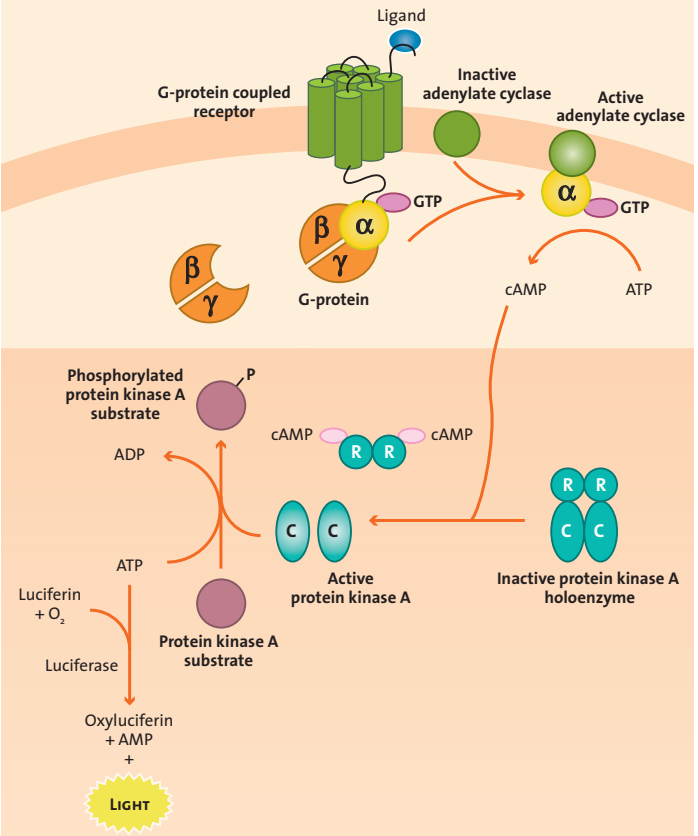
Assay principle

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.

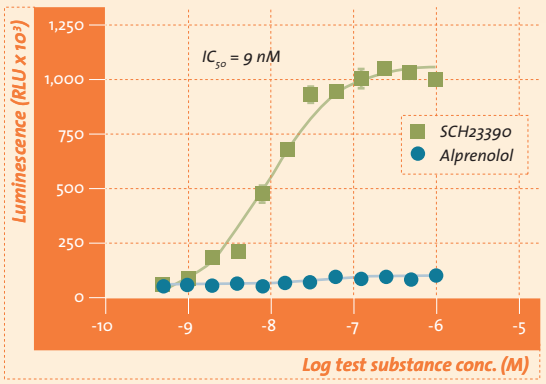
Assay features

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-, 1,536-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. IC_{50} 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.

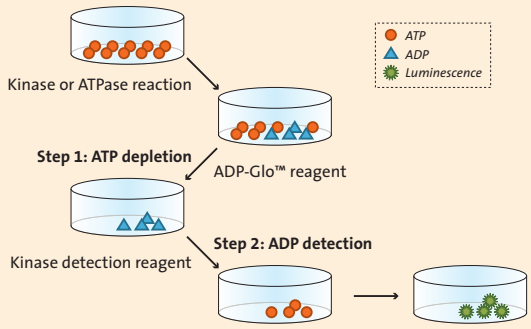
Assay principle

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.

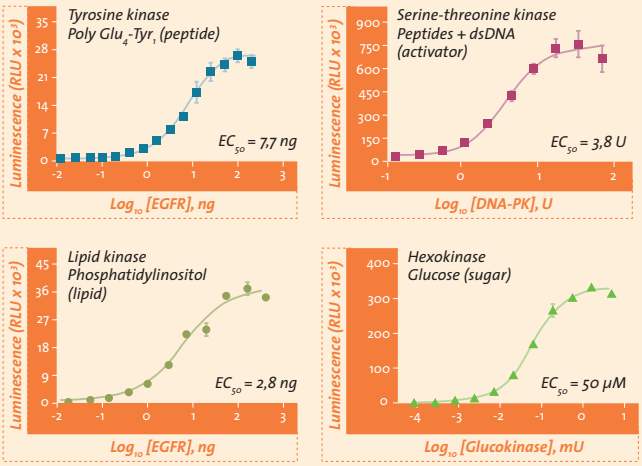
Assay features

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

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

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 µM ATP

Kinase-Glo® Plus Assay: 100 µM ATP

Kinase-Glo® Max Assay: 500 µM 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 µM ATP
Time required	10–15 minutes

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.

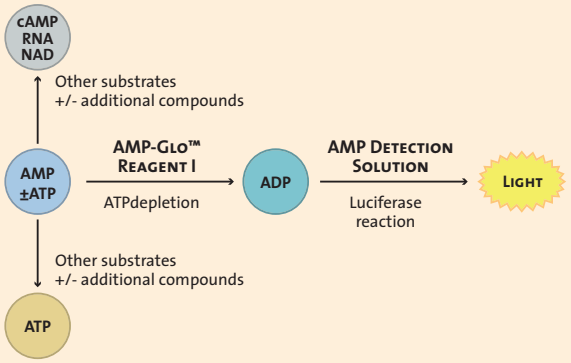
Assay principle

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.

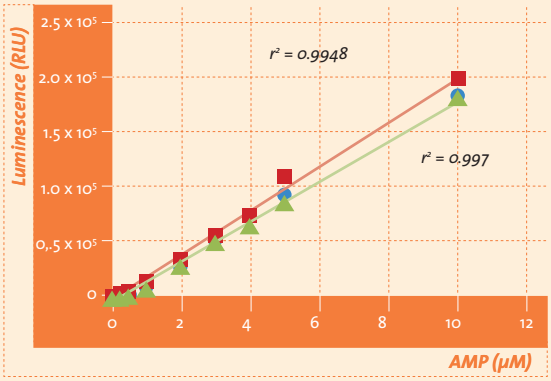
Assay features

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 Conversion	Determination of enzyme activity that more closely mimics physiological conditions—suited for low-activity enzymes
Robustness	Minimal false hits and Z´ values greater than 0.7, reactions are scalable in 96-, 384-, 1,536-well plates

Substrates for enzymes that do not use ATD.



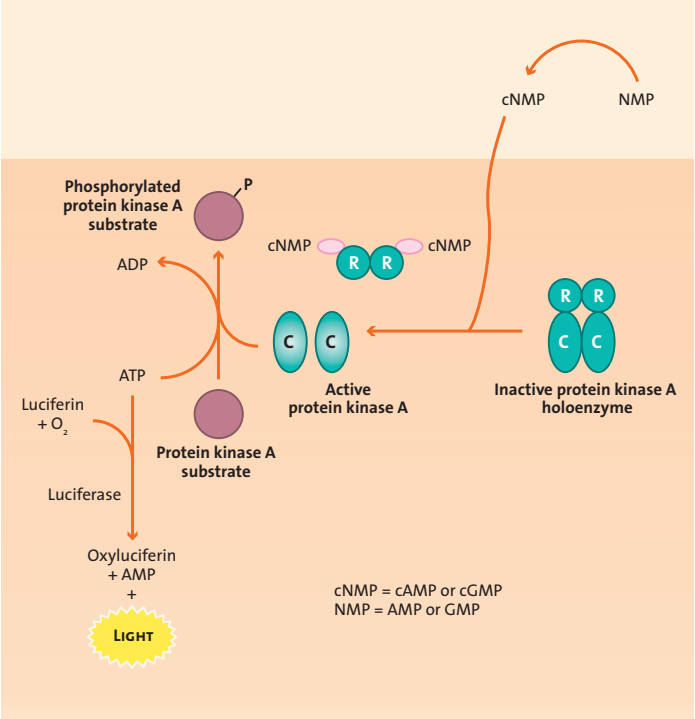
No Interference by ATP or cAMP



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 100µM ATP or with 10µM 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 phosphodiasterases. 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 phosphodieterases 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 GTP 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 1,536-well plates

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

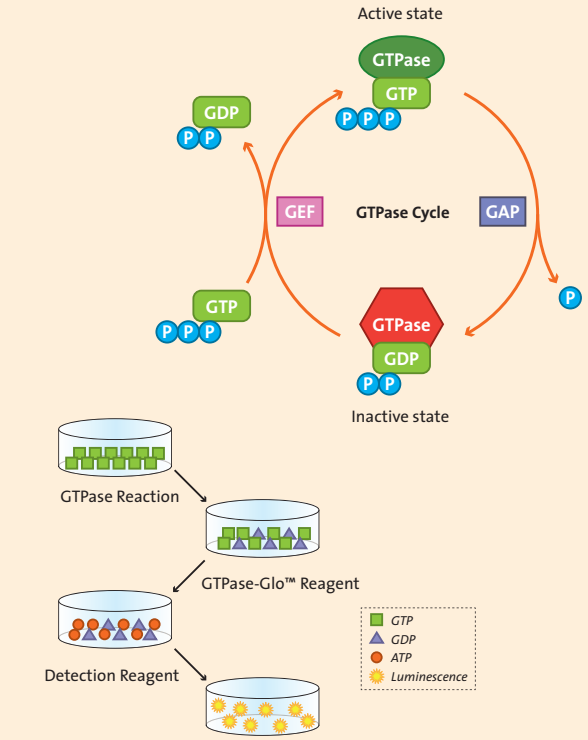
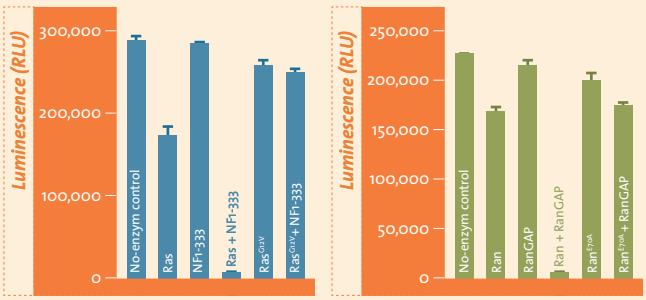


Diagram showing GTPase in active and inactive phases in relation to GDP, GTP. B. A diagram of the GTPase-Glo™ Assay. Following the GDP producing reaction, GTPase-Glo™ Reagent converts the remaining GTP to ATP. Then Detection Reagent is added, converting the ATP to a luminescent signal.

Stimulation of GTPase activity by GAP proteins



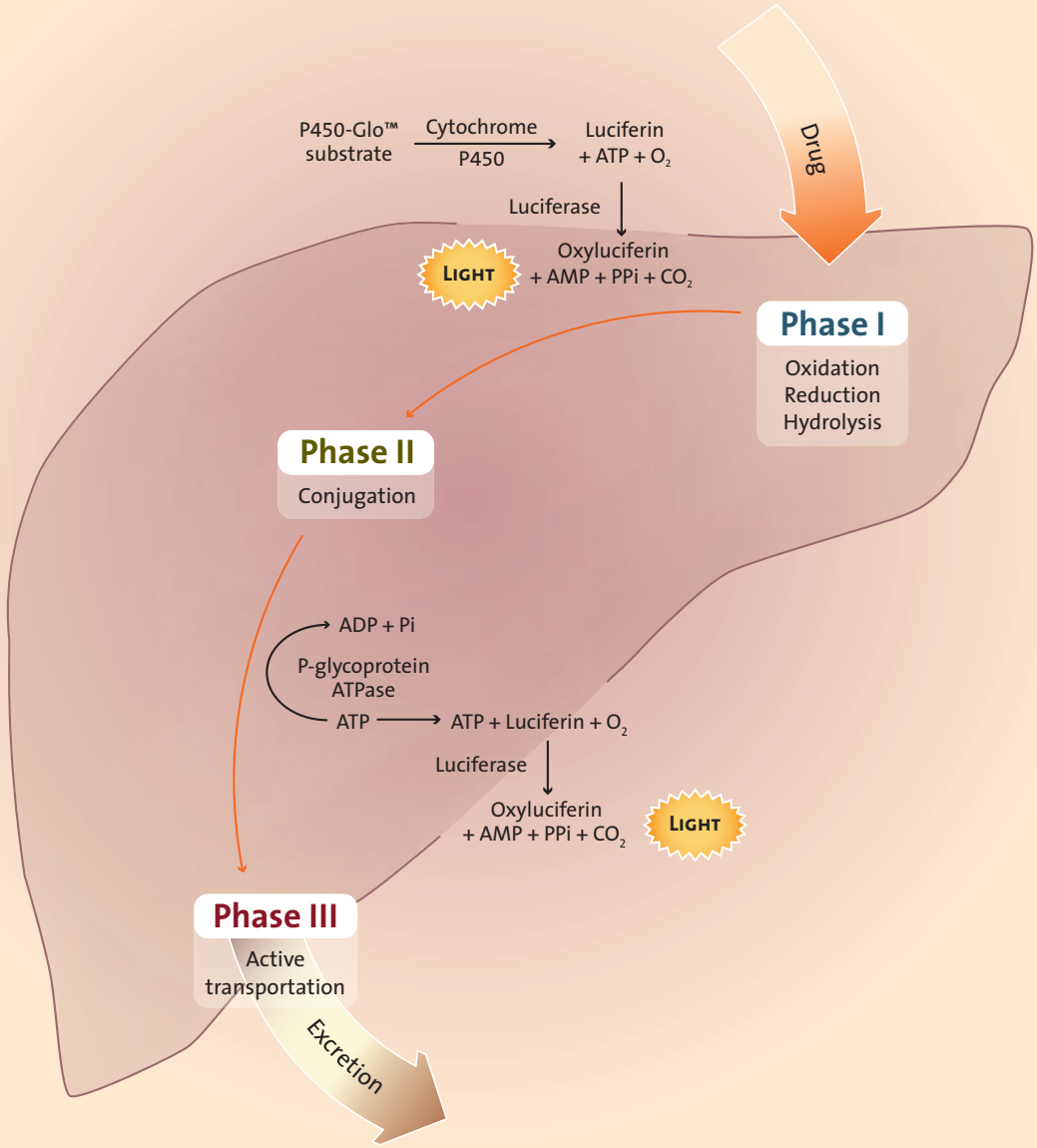
A. Reactions contain 2 µM of wildtype or mutant Ras (RasG12V) plus 1 µM of NF1-333 (GAP protein) in GTPase/GAP Buffer. **B.** Reactions contain 2 µM of wildtype or mutant Ran (RanE70A) plus 1 µM of RanGAP (GAP protein) in GTPase/GAP Buffer. Lower luminescent signals indicate higher GTPase activities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

VII Metabolism of drugs – ADME assays

Phase I and II enzymes

ADME/Tox stands for the absorption, distribution, metabolism and excretion of pharmaceutically active compounds in the human body. Metabolic enzymes can be readily detected in cell-based and biochemical assays, but the detection threshold can be limiting. Sensitivity of luminescence is therefore highly advantageous when detecting small enzyme quantities. For the measurement of ADME parameters, Promega offers a variety of highly-sensitive cell-based and biochemical assays which are based on the detection of cytochrome P450 activity, UDP glucuronosyltransferase activity, monoamine oxidase (MAO) activity or the plasma membrane protein P-glycoprotein (PgPp).

P450-Glo™ CYP450 Assay Systems
P450-Glo™ CYP450 Screening Systems
Pgp-Glo™ Assay
MAO-Glo™ Assay Systems
UGT-Glo™ Assay



Cytochrome P450 enzymes – Phase I

The cytochrome P450 system (CYP450) plays an important role in the metabolism of drugs. The enzymes of the cytochrome P450 superfamily are expressed mainly in the liver and function as monooxygenases. Based on homologies in the amino acid sequence, the superfamily of cytochrome P450 genes can be subdivided into 36 gene families, which in turn are divided into subfamilies. The CYP3A subfamily is the most important of these. At least 50 to 60 per cent of all therapeutically used drugs are CYP3A substrates. To determine the induction and inhibition of CY450 enzyme activities, a variety of methods are used. Luminescent assays have the advantage over known radioactive and LC/MS methods that, given comparable IC_{50} and EC_{50} values, they are quicker and easier to perform.

P450-Glo™ CYP450 Assay Systems

Biochemical & Cell-based

Applications

Determination of recombinant CYP450 activity in membrane fractions derived from heterologous expression systems, such as Sf9 cells and *E.coli*; determination of native CYP450 activity in microsomal fractions derived from tissues (e.g. HLMs: human liver microsomes); identification and characterization of CYP-inducing and -inhibiting drugs in cells.

Assay description

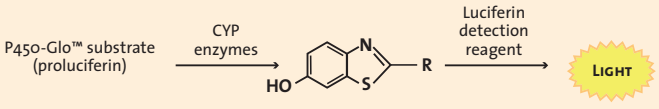
All CYP450 assays exhibit excellent sensitivity, low background-signal strength and a wide dynamic range. The assays can be used biochemically for cell-free CYP inhibition studies, and some can also be used for cell-based CYP induction. The advantage of the non-lytic assay is that it can be combined with other detection methods, e.g. the cells can be tested for viability using the CellTiter-Glo® Luminescent Cell Viability Assay.

Assay principle

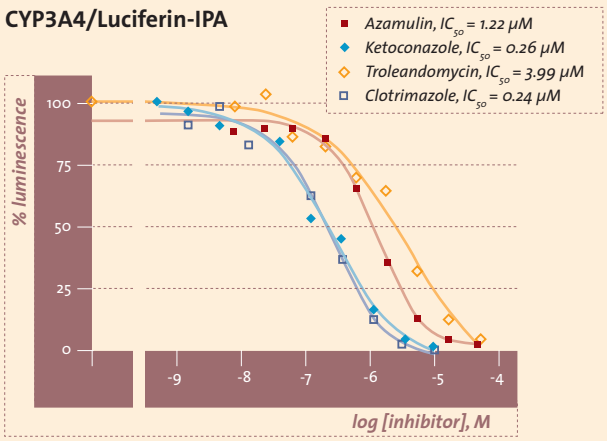
In the luminescent CYP450 assays, the luciferase substrate is modified so as to be specifically recognized and metabolized by the CYP450 isoenzymes. In this first step of the reaction, luciferin is released, and this is then converted in the luciferase reaction in the second step. The luminescence produced is proportional to the CYP450 activity. For a nonlytic assay format, the cell supernatant can be measured. Alternatively, a stable luminescence signal can be measured by adding the detection reagent directly to the cells (lytic assay).

Assay features

Assay type	Luminescent (glow-type)
Markers	CYP450 enzymes
Applications	Determination of cytochrome P450 enzyme activity
Sample material	Native and recombinant fractions, primary liver cells
Implementation	Homogeneous, two-step assay
Time required	20–60 minutes

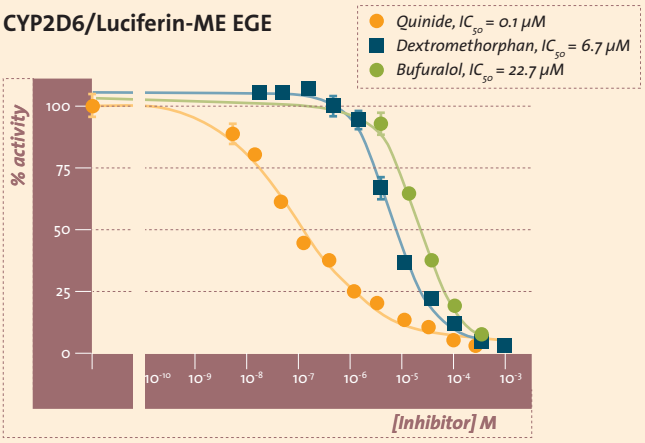


CYP3A4/Luciferin-IPA



Primary hepatocytes from a 40-year-old donor were treated with different CYP3A4 inhibitors, and enzyme activity determined by means of the cell-permeable CYP3A4 substrate luciferin-IPA. To this end, the cells were incubated for 15 minutes in a suspension culture with the aminoluciferin substrate.

CYP2D6/Luciferin-ME EGE



Recombinant CYP2D6 was used to investigate the effect of various substances on the activity of the liver enzyme. Active CYP2D6 converts its substrate luciferin-ME EGE, luciferin is produced and this serves in turn as a substrate for the firefly luciferase in the second step of the reaction. The light signal produced in this case is proportional to the CYP activity.

Cyp450 isoform	Assays	Applications*	Substrates
3A4	**P450-Glo™ CYP3A4 Assay with Luciferin-IPA**	BCA & CBA, inhibition and induction	Luc-IPA
1A2	**P450-Glo™ CYP1A2 Induction/Inhibition Assay**	BCA & CBA, inhibition and induction	Luc-1A2
2C9	**P450-Glo™ CYP2C9 Assay**	BCA & CBA, inhibition and induction	Luc-H
4A11 (4A1,2,3)	**Luciferin-4A and Luciferin Detection Reagent**	BCA & CBA, inhibition and induction	Luc-4A
2D6 (1A1, 1A2)	**P450-Glo™ CYP2D6 Assay**	BCA, inhibition	Luciferin-ME EGE
2C19 (1A1, 1A2)	**P450-Glo™ CYP2C19 Assay**	BCA, inhibition	Luciferin-H EGE
1A1, 1B1, 3A7	**P450-Glo™ CYP1A1 Assay**	BCA, inhibition	Luciferin-CEE
1A1, 1B1, 3A7	**P450-Glo™ CYP1B1 Assay**	BCA, inhibition	Luciferin-CEE
A2, 2C8/9, 2J2, 4A11, 4F3B, 19	**P450-Glo™ CYP2C8 Assay**	BCA, inhibition	Luciferin-ME
3A7	**Luciferin-3A7 and Luciferin Detection Reagent**	BCA, inhibition	Luciferin-3A7
4F2, 4F3	**Luciferin-4F2/3 and Luciferin Detection Reagent**	BCA, inhibition	Luciferin-4F2/3
4F12	**Luciferin-4F12 and Luciferin Detection Reagent**	BCA, inhibition	Luciferin-4F12
2J2, 4F12	**Luciferin-2J2/4F12 (Ester) and Luciferin Detection Reagent with Esterase**	BCA, inhibition	Luciferin-2J2/4F12 (Ester)
Many CYPs	**Luciferin-MultiCYP (Ester) and Luciferin Detection Reagent with Esterase**	BCA, inhibition	Luciferin-MultiCYP (Ester)

* BCA: Biochemical assay; CBA: Cell-based assay.

P450-Glo™ CYP450 Screening Systems

Biochemical

Applications

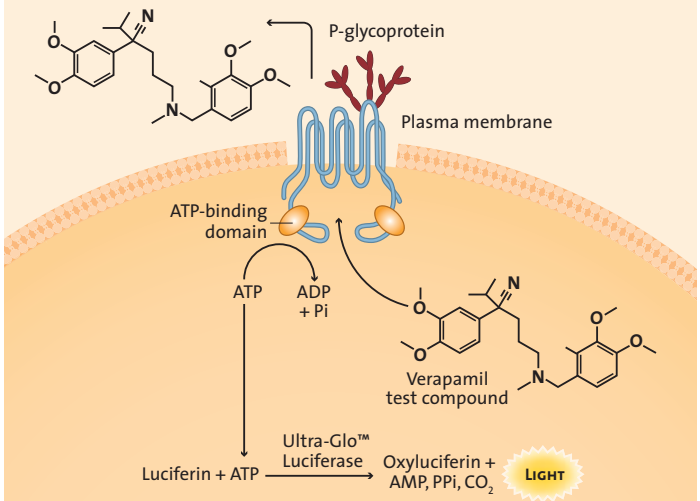
Screening of drugs to determine their capacity to regulate CYP450 activity.

Assay description

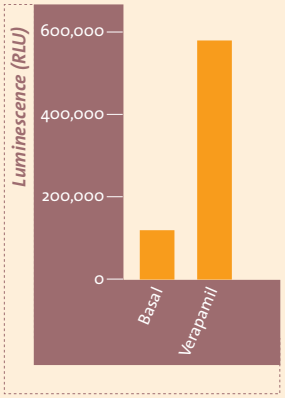
The **P450-Glo™ Screening Systems** include, in addition to the relevant cytochrome P450 assay, a membrane preparation containing recombinant human cytochrome P450 enzyme, P450 reductase (and, for CYP2C9 and CYP3A4, cytochrome b5), and, as a negative control, a membrane fraction devoid of P450 activity. They thus include all the components needed for a complete assay. Screening systems are available for the following human CYP450 isoforms: CYP1A2, CYP2C9, CYP2D6, CYP2C19 and CYP3A4.

P-glycoprotein ATPase – Phase I

P-Glycoprotein (Pgp) is a 170 kDa integral plasma membrane protein which, as an ATP-dependent efflux pump (ABC transporter), actively exports xenobiotic substances. Pgp, which is also known as MDR1 or ABCB1, plays an important role in multiple resistance to cytostatic agents (multi-drug resistance, MDR) and drug-drug interactions. Various compounds interact with Pgp and stimulate or inhibit its ATPase activity.



Stimulation of Pgp ATPase activity



The diagram shows the increase in Pgp activity after stimulation with Verapamil in comparison to basal activity.

Pgp-Glo™ Assay Systems

Biochemical

Applications

Screening of drugs for stimulating and inhibiting P-glycoprotein ATPase activity.

Assay description

The **Pgp-Glo™ Assay System** includes all the reagents required to perform the luminescent P-glycoprotein ATPase detection assay. The assay is used in drug screening and active substance screening and in the study of various factors affecting P-glycoprotein enzyme activity.

Assay principle

The assay is based on the ATP-dependent firefly luciferase reaction, which is used to measure the direct conversion of ATP. In the first step, a treated/untreated Pgp membrane fraction is incubated with the Pgp-Glo™ Assay buffer and a non-limiting concentration of ATP (5 mM).

In the second step, the Pgp ATPase reaction is terminated after addition of the ATP detection buffer and the remaining ATP is detected as a luciferase-dependent luminescent signal.

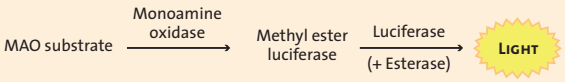
Reduction of the signal is proportional to Pgp activity. Substances which stimulate Pgp ATPase activity consequently generate significantly lower luminescence signals than samples which have no effect or an adverse effect on Pgp ATPase activity.

Assay features

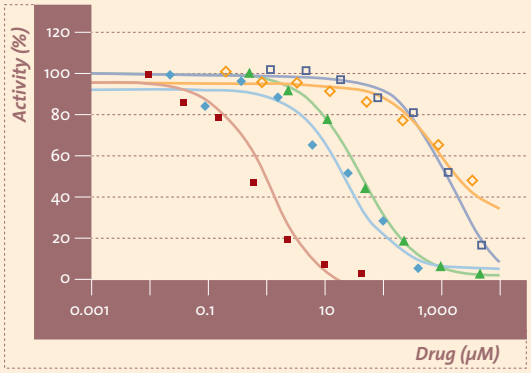
Assay type	Luminescent (glow-type)
Markers	Pgp activity
Applications	Determination of P-glycoprotein ATPase activity
Sample material	Native and recombinant membrane fractions
Implementation	Homogeneous, two-step assay
Time required	40–50 minutes

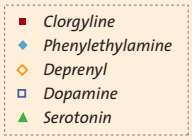
Monoamine oxidases – Phase I

After CYP450 enzymes, monoamine oxidases (MAOs) are the next most important phase I biotransformation enzymes. Monoamine oxidases (MAOs) catalyze the oxidative deamination of a large number of biogenic and xenobiotic amines. Direct measurement of MAO activity is essential for investigating the inhibition of monoamine oxidase by potential drugs. The MAO-Glo™ Assay offers an additional safety mechanism when selecting new active-substance candidates.



Measurement of MAO-B activity





Microsomes with MAO-B were incubated with various concentrations of known substrates and inhibitors for 1 hour. Following the addition of 50 µl luciferin detection reagent, readings were obtained for the samples using a GloMax™ 96 microplate luminometer. (See Technical Bulletin #TB345).

MAO-Glo™ Assay Systems

Cell-based & Biochemical

Applications

Determination of native and recombinant MAO activity; screening of drugs for regulating MAO activity in native and recombinant fractions.

Assay description

MAO-Glo™ Assay is a luminescence-based homogeneous method for quantifying monoamine oxidase (MAO) activity and can be used in place of time-consuming analytical methods such as HPLC. The very high level of sensitivity of the luminescent signal means that less MAO enzyme is needed than is the case with HPLC or fluorescence-based methods. Furthermore, the possibility of fluorescence interference is ruled out from the outset.

Assay principle

In the first step of the MAO™ Assay, the MAO enzyme and the test substance are incubated with the luminogenic MAO substrate. The substrate is a derivative of luciferin, which is converted by the selective MAO activity into methyl ester luciferin. Adding the luciferin detection reagent terminates the MAO reaction, and the converted methyl ester luciferin is converted into luciferin by the esterase contained in the reagent. The luminescent signal is directly proportional to the level of MAO activity.

The assay includes a luminogenic MAO substrate and two reaction buffers for determining monoamine oxidase A (MAO-A) and monoamine oxidase B (MAO-B)

The **MAO-Glo™ Assay** with MAO-A additionally contains human recombinant monoamine oxidase A enzyme for selectively determining activity levels.

Assay features

Assay type	Luminescent (glow-type)
Markers	MAO-A and MAO-B enzymes
Applications	Determination of MAO activity
Sample material	Native and recombinant fractions
Implementation	Homogeneous, two-step assay
Time required	40–50 minutes
Automatable	Very good Z' factor even in 384-well format.

UDP-glucuronosyltransferase – Phase II

UDP-glucuronosyltransferase (UGT) is an important member of the family of human phase II metabolizing enzymes and is found mainly in the liver. UGT transfers glucuronosyl groups from UDP glucoronic acid (UDPGA) to small and hydrophobic molecules. In this way, the UGT-mediated reaction converts xenobiotic and endogenous substances into more soluble and thus more easily excretable substances. UGTs modify many active pharmaceutical substances and may therefore be involved in drug-drug interactions.

UGT-Glo™ Assay

Biochemical

Applications

Screening of drugs for regulating UGT activity in native and recombinant fractions.

Assay description

The **UGT-Glo™ Assay** is used for detecting the activity of transferases from various sources, such as from UGT microsomes or UGT fractions derived from mammalian tissue. Suitable applications for the assay include investigating UGT inhibition by chemicals and pharmaceutically active substances. The **UGT-Glo™ Assay** contains two different pro-luciferin substrates. Firstly, the UGT multi-enzyme substrate, which is compatible with many UGTs, and secondly, the UGT1A4 substrate. The latter reacts specifically with the IGT isoform UGT1A4. The UGT-Glo™ screening systems additionally contain UGT1A4 or UGT2B7 microsomes and control membranes.

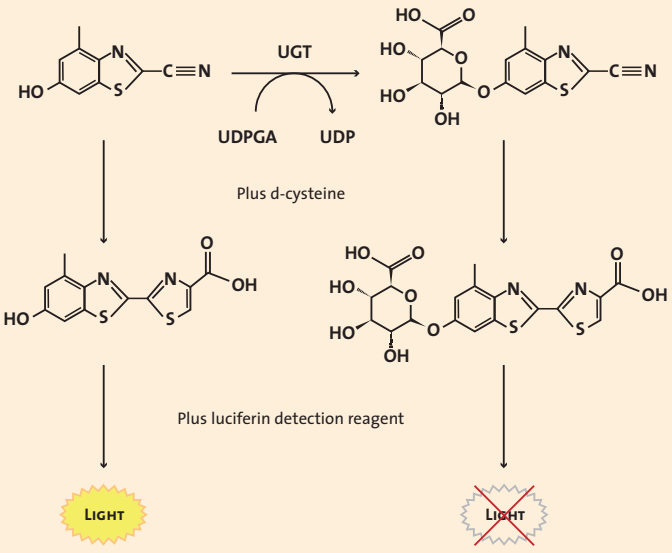
Assay principle

As a result of the UGT activity, a pro-luciferin substrate is conjugated with UDP. Upon addition of D-cysteine, residual unconjugated pro-luciferin is cyclized into luciferin, which is then converted by luciferase into light. UDT-conjugated pro-luciferin does not react with D-cysteine and therefore does not contribute to the luminescence. Higher levels of UGT activity consequently lead to a greater decrease in luminescence.

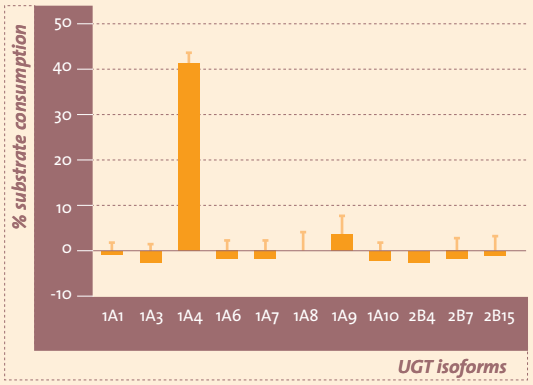
Assay features

Assay type	Luminescent (glow-type)
Markers	UGT enzyme
Applications	Determination of UGT activity
Sample material	Native and recombinant fractions
Implementation	Homogeneous, two-step assay
Time required	< 2 hours

Conversion of the UGT multi-enzyme substrate by the UGT enzyme



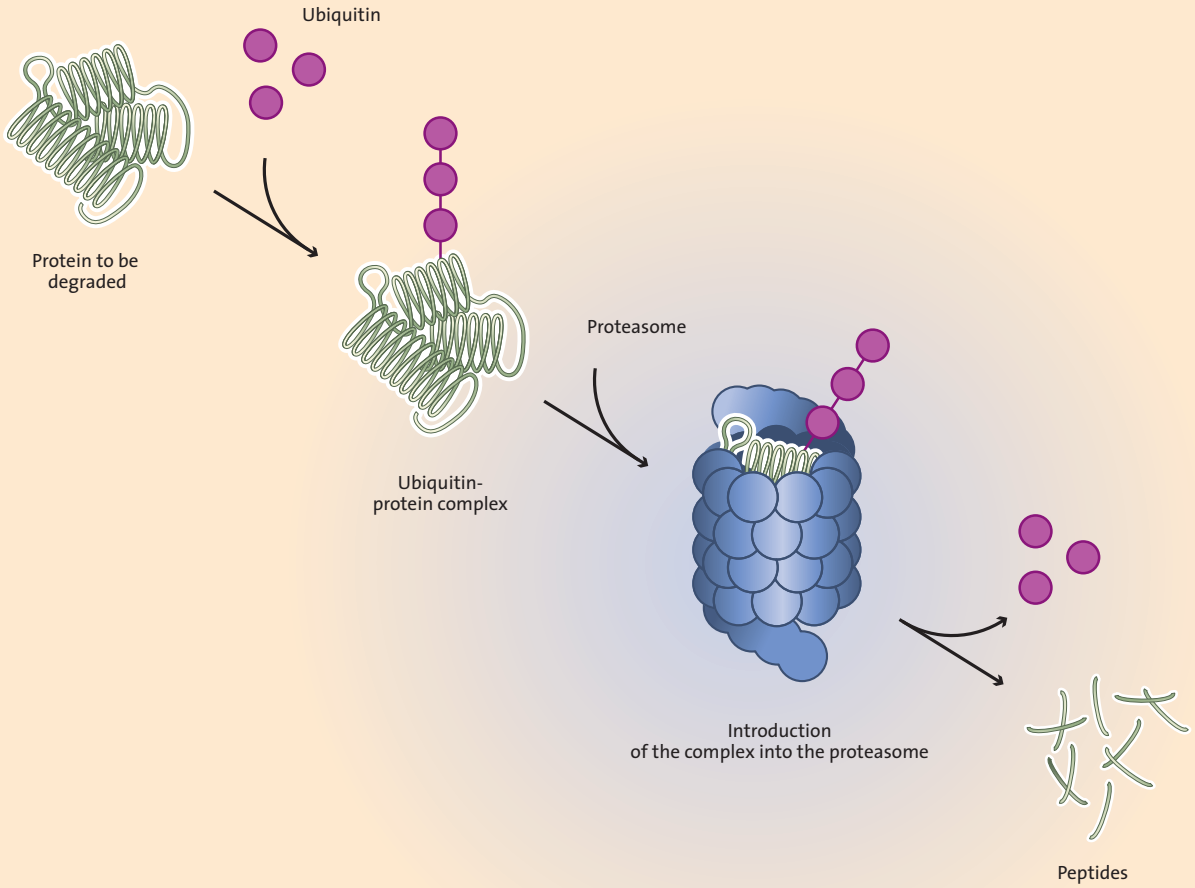
High specificity of UGT substrates



VIII Protease assays

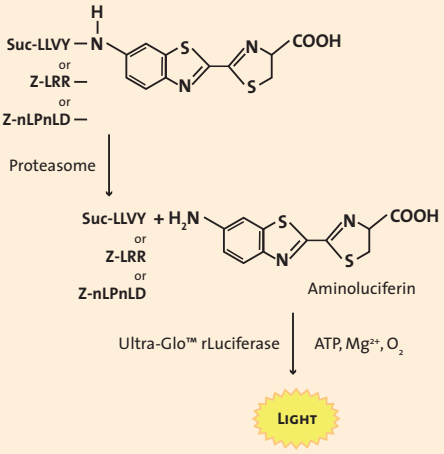
Proteases perform an extremely wide variety of functions in organisms. In the proteasome complex in eukaryotic cells, for example, they are responsible for degrading proteins that are defective or are temporarily no longer needed. The protease calpain is involved in many Ca^{2+}-dependent regulatory 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
DUB-Glo™ Protease Assay (DUB/SENP/NEDP)
DPPIV-Glo™ Protease Assay

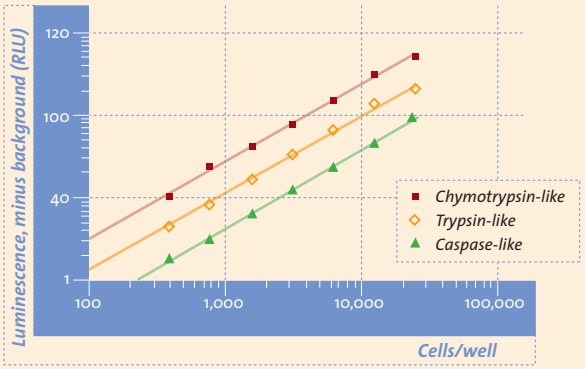


The proteasome

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.

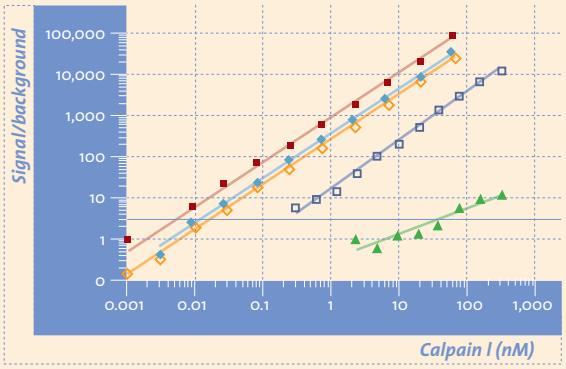
Assay features

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 Ca^{2+}-activated cysteine proteases. They modulate the biological activity of their substrates by targeted proteolysis and are involved in the regulation of numerous Ca^{2+}-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)-EVYGMMK(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.

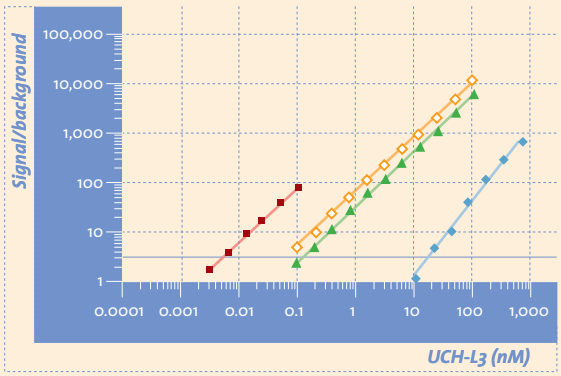
Assay features

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

DUB proteases

These proteases can reverse protein modifications by ubiquitin and ubiquitin-like proteases and are therefore an important component of the complex mechanism of post-translational protein regulation in eukaryotes.

DUB-Glo™ Protease Assay compared with fluorescent assays for UCH-L3



- *Ub-AMC, 30 minutes*
- *Z-RLRGG-aminoluciferin, 30 Minuten*
- *Z-RLRGG-aminoluciferin, 90 minutes*
- *Z-RLRGG-AMC, 90 minutes*

A titration of human recombinant UCH-L3 was performed in a 96-well plate. Either the DUB-Glo™ Protease Assay, a ubiquitin-AMC fluorescence substrate (250 nM) or Z-RLRGG-AMC fluorescence substrate (40 µM) was used. Luminescence or fluorescence was determined at various times after the reagent had been added. The results were shown as a signal-noise ratio. The detection limit was defined as a signal-to-noise ratio of > 3. The detection limit of the DUB-Glo™ Protease Assay lies between the ubiquitin-AMC substrate (more sensitive) and the Z-RLRGG-AMC substrate (lower sensitivity).

DUB-Glo™ Protease Assay (DUB/SENP/NEDP)

Biochemical

Applications

Screening of inhibitors of deubiquitinating (DUB) proteases; characterization of the ubiquitination/deubiquitination of proteins.

Assay description

The **DUB-Glo™ Protease Assay (DUB/SENP/NEDP)** is a luminescent, one-step assay which measures the activity of numerous deconjugating enzymes, including deubiquitinating (DUB), de-SUMOylating (SENP) and deneddylating (NEDP) proteases.

Assay principle

The DUB-Glo™ Protease Assay reagent contains a luminogenic Z-RLRGG-aminoluciferin substrate with the C-terminal pentapeptide of ubiquitin, RLRGG. This reagent is optimized for the enzyme-coupled measurement of protease and luciferase activity. After the DUB-Glo™ reagent has been added directly to the sample, the substrate Z-RLRGG is cleaved from aminoluciferin by active ubiqutin and ubiquitin-like proteases. In a subsequent reaction, the cleavage product luciferin is converted by the Ultra-Glo™ Luciferase into a stable light signal which is proportional to the DUB, SENP or NEDP1 activity.

Assay features

Assay type	Luminescent (glow-type)
Applications	Quantitative determination of DUB/SENP/NEDP activity
Sample material	Enzyme preparations
Implementation	Homogeneous, one-step assay
Linearity	Linear over 2–3 logs of deconjugating protease concentration
Time required	10–30 minutes

DPPIV-Glo™ Protease Assay

Biochemical

Applications

Determination of DPPIV enzyme activities;
kinetic studies of DPPIV inhibitors.

Assay description

The **DPPIV-Glo™ Protease Assay** is a luminescent one-step assay that measures the enzyme activity of the serine protease dipeptidyl peptidase IV (DPPIV). DPPIV cleaves N-terminal dipeptides from polypeptides with L-proline or L-alanine at the penultimate position. This robust and sensitive assay was developed for using with purified enzymes.

Assay principle

The **DPPIV-Glo™ Assay** contains the proluminescent DPPIV substrate, Gly-Pro-aminoluciferin, in a buffer system optimized for DPPIV and luciferase activity. After the DPPIV-Glo™ reagent is added, the peptide substrate is cleaved from aminoluciferin by active DPPIV proteases. The luciferin produced in the process is converted in a subsequent luciferase reaction into a stable light signal. The luminescent signal is directly proportional to the DPPIV activity.

Assay features

Assay type	Luminescent (glow-type)
Applications	Quantitative determination of DPPIV activity
Sample material	Enzyme preparations
Linearity	Linear over 3 logs of DPPIV activity
Implementation	Homogeneous, one-step assay
Time required	30 minutes

IX References

Cell viability/proliferation

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**

Automating Promega Cell-Based Assays in Multiwell Formats; Tracy Worzella and Brad Larson; *Promega Notes* ***85****, 25–27;* **2003**

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 Riss[1], Rich Moravec[1], Michael Beck[1], Rita Hannah[1], Karen Wilson[2] and Robert Swanson[3]; *Promega Notes* ***81****, 2–5;* **2002**

Peer-reviewed publications

Gupta, P.B., Onder, T.T., Jiang, G., Tao, K., Kuperwasser, C., Weinberg, R.A. and Lander, E.S.; **Identification of selective inhibitors of cancer stem by high-throughput screening.** *Cell* ***138****, 645–659;* **2009**
Notes: The authors of this study describe a proof-of-concept screen to use mammary epithelial cells that have been induced to undergo an epithelial to mesenchymal transition (EMT) as model cells to identify agents that may be selectively toxic against „epithelial cancer stem cells" (CSCs). They induced the transformed breast cancer cell line HMLER to undergo a mesenchymal transition using shRNA directed against the E-cadherin gene.

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**
Notes: The authors designed a high-throughput gene-expression screen to identify compounds that induce a neuroblastoma gene signature in BE(2)-C cells. They used the CellTiter-Glo® Luminescent Cell Viability Assay in a 96-well format to assess the effects of ATRA and a variety of inhibitors of histone deacetylase on BE(2)-C cell viability.

Lin, H., Lee, E., Hestir, K., Leo, C., Huang, M., Bosch, E., Halenbeck, R., Wu, G., Zhou, A., Behrens, D., Hollenboguh, 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**
Notes: The authors of this study created a cDNA library representative of the extracellular proteome (secreted proteins and the extracellular domains of transmembrane proteins). Each cDNA was individually transfected into 293T cells. The CellTiter-Glo® Assay was used to screen for secreted factors from the cell lines expressing the cDNA that affected viability of twelve cell lines.

CellTiter-Glo® 2.0 Cell Viability Assay

Promega articles

A Novel Luminescent Cell Viability Assay with Greatly Enhanced Storage Stability; Kevin Kershner, Michael P. Valley, Dan F. Lazar, James Unch, Kevin R. Kupcho, Andrew L. Niles, Poncho L. Meisenheimer, and James J. Cali; **2013**

CellTiter-Glo® 3D Viability Assay

Promega articles

Validating Performance of Cytotoxicity Assays Applied to 3D Cell Culture Models; Terry L. Riss, Michael P. Valley, Andrew L. Niles, Kevin R. Kupcho, Chad A. Zimprich, Matt B. Robers, James J. Cali, Jens M. Kelm, Wolfgang Moritz, and Dan F. Lazar; Promega Corporation, Madison, WI. and 2InSphero AG, Zürich, Switzerland; **2013**

A Bioluminescent Cell Viability Assay Optimized for 3D Microtissues; Michael P. Valley, Chad Zimprich, James J. Cali, and Dan F. Lazar; Promega Corporation **2013**

Design and Validation of Bioluminescent Assays for 3D Cell Culture Models; Terry L. Riss, Michael P. Valley, Chad A. Zimprich, Andrew L. Niles, Kevin R. Kupcho and Dan F. Lazar; Promega Corporation **2013**

CellTiter-96® AQ_{ueous} One Solution Cell Proliferation Assay

Promega articles

A HTS System for Screening Antiviral Compounds Using the CellTiter® 96 AQ_{ueous} One Solution System; Thomas Fletcher, III[1], Roger Ptak[1], Stacy Bartram[1], Susan Halliday[1], Robert Buckheit, Jr.[1], Rich Moravec[2] and Terry Riss[2]; *Promega Notes* ***75****, 13–16;* **2000**
Technically Speaking: Cell Viability Assays; Robert Deyes; *Promega Notes* ***81****, 32–33;* **2002**

Citation Note: HaloTag® Techology, P450-Glo® CYP2C8 Assay, and Beta-Glo® Assay; Terri Sundquist; *Cell Notes* ***21****, **17** and **27****;* **2008**

Monitoring Viability During Gene-Directed Enzyme Prodrug Therapy with the CellTiter-Glo Assay and Human Neural Progenitor Cells; Elizabeth E. Capowski and Clive N. Svendsen; *Cell Notes* ***20****, 6–8;* **2008**

The Predictive Nature of High-Throughput Toxicity Screening Using a Human Hepatocyte Cell Line; Norman L. Sussman[1], Monika Waltershield[1], Terolyn Bulter[1], James J. Cali[2], Terry Riss[2], and James H. Kelly[1]; *Cell Notes* ***3****, 7–10;* **2002**

Peer-reviewed publications

Tonello, F., Seweso, M., Marin, O., Mock, M., and Montecucco, C.; **Screening inhibitors of anthrax lethal factor.** *Nature* ***418****, 386;* **2002**
Notes: This brief communication discusses substrates of anthrax lethal factor that can be used for high-throughput screening of potenzial inhibitors. The CellTiter® Aqueous Cell Proliferation Assay was used to assess the effect of selected inhibitors on cytotoxicity of lethal factor in RAW264.7 cells.

CellTiter-Blue® Cell Viability Assay

Promega articles

Automating Promega Cell-Based Assays in Multiwell Formats; Tracy Worzella and Brad Larson; *Promega Notes* ***85****, 25–27;* **2003**

Introducing the CellTiter-Blue® Cell Viability Assay; Terry Riss and Rich Moravec; *Promega Notes* ***83****, 10–13;* **2003**

The CellTiter-Blue® Cell Viability Assay: Monitoring Cell Viability Using a Fluorescent Redox Indicator Dye; Rich Moravec and Terry Riss; *Cell Notes* ***5****, 12–14;* **2003**

Peer Reviewed Publikationen

Nakagawa, T., Shimizu, S., Watanabe, T., Yamaguchi, O., Otsu, K., Yamagata, H., Inohara, H., Kubo, T., Tsujimoto, Y. (2005) **Cyclophilin D-dependent mitochondrial permeability transition regulates some necrotic but not apoptotic cell death;** *Nature* ***434****, 652-658*
Notes: In this study, the role of Cyclophilin-D (CypD) in the mitochondrial permeability transition (mPT) response was investigated using CypD-deficient mice. The CellTiter™ Blue Cell Viability Assay was used to measure the viability of mouse embryonic fibroblasts (MEF) and hepatocytes isolated from normal and CypD-deficient mice after exposure to various apoptotic stimuli and H_2O_2.

Niles, A.L., Moravec, R.A. and Riss, T.L. (2009) **In vitro viability and cytotoxicity testing and same-well multi-parametric combinations for high-throughput screening;** *Current Chemical Genomics* ***3****, 33–41*
Notes: The authors review the use of in vitro cytotoxicity testing in drug discovery to characterize the toxic potenzial of new chemical entities (nce) at the earliest stages of profiling.

Cecchi, C., Pensalfini, A., Baglioni, S., Fiorillo, C., Caporale, R., Formigli, L., Liguri, G. and Stefani, M. (2006) **Differing molecular mechanisms appear to underlie early toxicity of prefibrillar HypF-N aggregates to different cell types.** *FEBS J.* ***273****, 2206–2222.*
Notes: The CellTiter-Blue® Cell Viability Assay was used to monitor viability of Hend murine endothelial cells and IMR90 fibroblasts in the presence of various concentrations (0.02, 0.2, 2.0 or 20 µm) of the N-terminal domain of the prokaryotic hydrogenase maturation factor (HypF-N).

CellTiter-Fluor™ Cell Viability Assay

Peer-reviewed publications aus Protokoll

Niles, A.L. *et al.* (2007) **A homogeneous assay to measure live and dead cells in the same sample by detecting different protease markers;** *Anal. Biochem.* ***366****, 197–206.*

Zhang, J-H. *et al.* (1999) **A simple statistical parameter for use in evaluation and validation of high-throughput screening assays;** *J. Biomol. Screen.* ***4****, 67–73.*

BacTiter-Glo™ Microbial Cell Viability Assay

Promega articles

BacTiter-Glo™ Assay for Antimicrobial Drug Discovery and General Microbiology; Frank Fan, Braeden Butler, Terry Riss and Keith Wood; *Promega Notes* ***89****, 25–27;* **2005**

Quantitate Microbial Cells Using a Rapid and Sensitive ATP-Based Luminescent Assay; Frank Fan, Braeden Butler, Terry Riss and Keith Wood; *Promega Notes* ***88****, 2–4;* **2004**

Determining Microbial Viability Using a Homogeneous Luminescent Assay; Frank Fan, Braeden Butler, Terry Riss, and Keith Wood; *Cell Notes* ***10****, 2–5;* **2004**

ATP Measurement as a Means for Directly Estimating Active Biomass; Silvana Velten, Frederik Hammes, Markus Boller and Thomas Egli; *Promega Notes* ***97****, 1–17;* **2007**

Application of the BacTiter-Glo™ Assay for Rapid Enumeration and Screening of Antimicrobial Compounds for Mycobacterium avium Complex Bacteria; Alice Yuroff[1], Frank Fan[2], Braeden Butler[2] and Michael Collins[1]; *Promega Notes* ***98****, 8–10;* **2008**

Peer-reviewed publications

Bosshard, F., Berney, M. and Scheifele, M. (2009) **Solar disinfection (SODIS) and subsequent dark storage of Salmonella typhimurium and Shigella flexneri;** *J. Microbiology* ***155****, 1310–17*
Notes: In this study, the effect of solar disinfection on Shigella flexneri and Salmonella typhimurium in drinking water samples was evaluated. A variety of viability indicators were used to investigate the effectiveness of the disinfection method, including measurement of cellular ATP levels. The BacTiter-Glo Assay was used for ATP detection.

Martinez, A., Bradley, A.S., Waldbauer, J.R., Summons, R.E. and Delong, E.F. (2007) **Proteorhodopsin photosystem gene expression enables photophosphorylation in a heterologous host;** *Proc. Natl. Acad. Sci. USA* ***104****, 5590–5595*
Notes: Photorhodopsins (PRs), retinal-binding membrane proteins that catalyze light-activated proton efflux across the cell membrane, are found in many marine bacteria. These authors screened a fosmid library of planktonic DNA, looking for PR-expressing clones. The BacTiter-Glo™ System was used to measure light-induced changes in ATP levels.

Berney, M., Weilenmann H.U., and Egli, T. (2006) **Flow-cytometric study of vital cellular functions in Escherichia coli during solar disinfection (SODIS);** *Microbiology* ***152****, 1719–1729*
Notes: The BacTiter-Glo™ Microbial Cell Viability Assay was used to assess total ATP levels in Escherichia Coli strain K-12 MG165 cultures.

Cytotoxicity

CellTox™ Green Cytotoxicity Assay

Promega articles

An Image-Based Method for Real-Time Monitoring of Cytotoxicity Using CellTox™ Green Dye and the Essen Bioscience IncuCyte™ FLR Live Content Imaging System; Tracy Worzella, Andrew Niles, Lyndsey Helley, Michael Conley and Terry Riss, **2013**

Events Using Multiplexed Assays; Alisha Truman and Brad Hook, *Promega Corporation,* **2013**

Gain More Informative Data by Multiplexing a Fluorescent Real-Time Cytotoxicity Assay with Luminescent, Fluorescent or Colorimetric Viability Assays; Brad Hook, Mark Bratz and Trista Schagat, **2013**

Real-Time Cytotoxicity Analysis; Tracy Worzella, Andrew Niles, Thomas Hengstl, Michael Fejtl, Christian Oberdanner and Jessica Merlino, **2013**

Measuring Cytotoxicity in Real Time with a Highly Stable Green Dye; Niles, A., Worzella, T., Zhou, M., McDougall, M. and Lazar, D., **2013**

Peer-reviewed publications

Kinetic measurement of cytotoxicity using CellTox™ Green Cytotoxicity Assay on the IncuCyteTM FLR or ZOOM; *Essen BioScience,* **2013**

CytoTox-Fluor™ Cytotoxicity Assay

Promega articles

High-Throughput Automation of Multiplexed Cell-Based Assays for Viability and Cytotoxicity; Tracy Worzella[1], Michael Busch[2] and Andrew Niles[1]; *Cell Notes* ***20****, 26–29;* **2008**
Using Protease Biomarkers to Measure Viability and Cytotoxicity; Andrew Niles, Michael Scurria, Laurent Bernad, Brian McNamara, Kay Rashka, Deborah Lange, Pam Guthmiller and Terry Riss; *Cell Notes* ***19****, 16–20;* **2007**

Peer-reviewed publications

Zhang *et al.* (1999) **A simple statistical parameter for use in evaluation and validation of high-throughput screening assays;** *J. Bio. Mol. Screen.* ***4****, 67.73*

CytoTox-Glo™ Cytotoxicity Assay

Peer-reviewed publications

Niles, A.L. *et al.* (2007) **A homogeneous assay to measure live and dead cells in the same sample by detecting different protease markers;** *Anal. Biochem.* ***366****, 197–206*

Zhang, J.H., Chung, T.D. and Oldenburg, K. (1999) **A simple statistical parameter for use in evaluation and validation of high-throughput screening assays;** *J. Bio. Mol. Screen.* ***4****, 67–73*

CytoTox-ONE™ Homogeneous Membrane Integrity Assay

Promega articles

Automating Promega Cell-Based Assays in Multiwell Formats; Tracy Worzella and Brad Larson; *Promega Notes* ***85****, 25–27;* **2003**

Introducing the CytoTox-ONE™ Homogeneous Membrane Integrity Assay; Terry Riss and Rich Moravec; *Promega Notes* ***82****, 15–18;* **2002**

Frequently Asked Questions: CytoTox-ONE™ Homogeneous Membrane Integrity Assay; Abigail Farfan and Trista Schagat; *Cell Notes* ***6****, 19–20;* **2003**

Introducing the CytoTox-ONE™ Homogeneous Membrane Integrity Assay; Terry Riss and Rich Moravec; *Cell Notes* ***4****, 6–9;* **2002**

CytoTox-ONE™ Homogeneous Membrane Integrity Assay: A Tool for Automated Cytotoxicity Research; Randy Hoffman; *Cell Notes* ***4****, 10–11;* **2002**

Peer-reviewed publications

Chen, J., Douglas, G.C., Thirkill, T.L., Lohstroh, P.N., Bielmeier, S.R., Narotsky, M.G., Best, D.S., Harrison, R.A., Natarajan, K., Pegram, R.A., Overstreet, J.W. and Lasley, B.L. (2003) **Effect of bromodichloromethane on chorionic gonadotrophin secretion by human placental trophoblast cultures;** *Toxicol. Sci.* ***76****, 75–82*

Notes: The effects of bromodichloromethane (BDCM), a byproduct of water disinfection processes, on trophoblast cells isolated from term human placentas were investigated. The CytoTox-ONE™ Homogeneous Membrane Integrity Assay was used to assess cell membrane integrity and LDH release after trophoblasts were exposed to BDCM for 24 hours.

Cox, S., Cole, M., Mankarious, S., and Tawil, N. (2003) **Effect of tranexamic acid incorporated in fibrin sealant clots on the cell behavior of neuronal and nonneuronal cells.** *J. Neurosci. Res.* ***72(6)****, 734–746*
Notes: The CytoTox-ONE™ Homogeneous Membrane Integrity Assay was used to assess the cytotoxic effects of tranexamic acid (t-AMCHA) on neural human normal progenitor (NHNP) or normal human dermal fibroblasts (NHDF) cells.

Kato, A., Okaya, T., and Lentsch, A.B. (2003) Endogenous IL-13 protects hepatocytes and vascular endothelial cells during ischemia/reperfusion injury; *Hepatology* ***37(2)****, 304-312*
Notes: Mouse hepatocytes (AML-12 cell line) were assayed for cytotoxicity to 1 mmol/L hydrogen peroxide with and without 20ng/mL IL-13 using the CytoTox-ONE™ Homogeneous Membrane Integrity Assay.
assays; *J. Bio. Mol. Screen.* ***4****, 67–73*

ViralTox-Glo™ Assay

Promega articles

Determine Viral-Induced Cytopathic Effect Using a Luminescent Assay; Andrew Niles, Jim Noah, Lynn Rasmussen and Dan Lazar, **2013**

Apoptosis

Caspase-Glo® 3/7 Assay

Promega articles

Technically Speaking: Cell-Based Caspase Assays Analyzing the Data; Martha O'Brien; *Promega Notes* ***87****, 33–36;* **2004**

Correlation of Caspase Activity and ChemoResponse in Epithelial Ovarian Cancer Cell Lines; Ayesha B. Alvero and Gil Mor; *Promega Notes* ***87****, 15–17;* 2004

Detecting UV Irradiation-Induced Apoptosis with the Caspase-Glo® 3/7 Assay; Ronald Lai**;** *Promega Notes* ***85****, 19–20;* **2003**

Automating Promega Cell-Based Assays in Multiwell Formats; Tracy Worzella and Brad Larson; *Promega Notes* ***85****, 25–27;* **2003**

Citation Note: HaloTag® Techology, P450-Glo® CYP2C8 Assay and Beta-Glo® Assay; Terri Sundquist; *Cell Notes* ***21, 17 and 27****;* **2008**

Peer-reviewed publications

Straszewshi-Chavez, A., Visintin, I.P., Karassina, N., Los, G., Liston, P., Halaban, R., Fadiel, A. and Mor, G. (2007) **XAF1 mediates tumor necrosis factor-α-induced apoptosis and X-linked inhibitor of apoptosis cleavage by acting through the mitochondrial pathway;** *Journal of Biological Chemistry* ***282****, 13059–13072.*
Notes: The authors sought to determine the mechanism by which first-trimester trophoblasts resist FAS ligand-induced apoptosis but remain sensitive to TNFα-mediated apoptosis. First trimester trophoblasts express XAF1 [X-linked inhibitor of apoptosis (XIAP)-associated factor 1], which may be involved in regulating their response to proapoptotic signals. The authors created HaloTag™-XAF1 fusion constructs and transiently transfected the first trimester trophoblast cell line (3A). Cells were labeled with the HaloTag™ TMR ligand, and XAF1 was shown to localize to the cytoplasm. 3A cells transiently transfected with the fusion construct were also separated into cytoplasmic and mitochondrial fractions. The fractions

were labeled with HaloTag™ TMR ligand. Expression of the fusion peaked at 48 hours after transfection in both mitochondrial and cytoplasmic fractions. TNFα-treatment of 3A cells induced translocation of endogenous XAF1 to the mitochondria. The authors used the Caspase-Glo® Assays to demonstrate activation of caspase-3 and caspase-9 in response to expression of XAF-1.

Apo-ONE® Homogeneous Caspase-3/7 Assay

Promega articles

Apo-ONE™ Homogeneous Caspase 3/7 Assay: Rapid Apoptosis Detection in High-Throughput Applications; Jean Humpal-Winter, Andrew Niles and Michael Bjerke; *Promega Notes* ***79****, 33–35;* **2001**

Automate the Apo-ONE® Homogeneous Caspase-3/7 Assay on the Eppendorf ep*Motion*® 5075 TMX; Dagmar Bracht and Sylvia Baranowski; Eppendorf Instrumente GmbH, Hamburg, Germany; **2009**

Automating Promega Cell-Based Assays in Multiwell Formats; Tracy Worzella and Brad Larson; **2003**

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

Technically Speaking: Cell Viability Assays; Robert Deyes; *Promega Notes* ***81****, 32–33;* **2002**

Peer-Reviewed Artikel

Niles, A.L., Moravec, R.A. and Riss, T.L. (2009) **In vitro viability and cytotoxicity testing and same-well multi-parametric combinations for high-throughput screening;** *Current Chemical Genomics* ***3****, 33–41*
Notes: The authors review the use of in vitro cytotoxicity testing in drug discovery to characterize the, D.C. (2004) **Argininosuccinate synthase expression is required to maintain nitric oxide production and cell viability in aortic endothelial cells;** *J. Biol. Chem.* ***279(18)****, 18353–18360*
Notes: In this study, the effect of arginosuccinate synthase (AS) on nitric oxide (NO) signaling was explored in bovine aortic endothelial cells. Expression of arginosuccinate synthase was knocked down using in-vitro-transcribed siRNAs. Using the CytoTox-ONE™ Homogeneous Membrane Integrity Assay and the Apo-ONE® Homogeneous Caspase-3/7 Assay, it was found that reduction of AS leads to the induction of apoptosis.

Bruno, I.G., Jin, W., Cote, G.J. (2004) **Correction of aberrant FGFR1 alternative RNA splicing through targeting of intronic regulatory elements;** *Hum. Mol. Genet.* ***13(20)****, 2409–20*
Notes: Human U251 glioblastoma cell lines treated with antisense morpholino oligonucleotides were assessed for viability and apoptosis by multiplexing the CellTiter-Blue® Cell Viability and Apo-ONE® Homogeneous Caspase-3/7 Assays on single cell cultures. Cell viability was measured 4 hours after the addition of the CellTiter-Blue® Cell Viability Reagent to the cultures. Next, apoptosis measurements were performed on the same cell cultures by adding Apo-ONE® Homogeneous Caspase-3/7 Assay reagent to the cultures. Caspase-3/7 activity was then measured 12 hours later.

CaspACE™ FITC-VAD-FMK in situ Marker

Promega articles

CaspACE™ FITC-VAD-FMK In Situ Marker as a Probe for Flow Cytometry Detection of Apoptotic Cells; Francis Belloc, Olivier Garnier, Catherine Boyer and Francis Lacombe; *Promega Notes* ***76****, 10–13;* **2000**

CaspACE™ FITC-VAD-FMK In Situ Marker for Apoptosis: Applications for Flow Cytometry; Matt Sylte[1], Martha O'Brien[2], Thomas J. Inzana[3] and Chuck Czuprynski[1]; *Promega Notes* ***75****, 20–23;* **2000**

CaspACE™ FITC-VAD-FMK In Situ Marker as a Probe for Flow Cytometry Detection of Apoptotic Cells; Francis Belloc, Olivier Garnier, Catherine Boyer and Francis Lacombe; **2000**

A Herpes Simplex Virus Type 2 Protein (ICP10 PK) Inhibits Caspase-3 Activation in Hippocampal Neurons; D. Perkins, E.F.R. Pereira and L. Aurelian; *Cell Notes* ***2****, 7–8;* **2001**

Akt-Mediated Survival of Oligodendrocytes Induced by Neuregulins; Ana I. Flores and Wendy B. Macklin; **2001**

Peer-reviewed publications

Rouet-Benzineb, P., Rouyer-Fessard, C., Jarry, A., Avondo, V., Pouzet, C., Yanagisawa, M., Laboisse, C., Laburthe, M. and Voisin, T. (2004) **Orexins acting at native OX1 receptor in colon cancer and neuroblastoma cells or at recombinant OX1 receptor suppress cell growth by inducing apoptosis;** *J. Biol. Chem.* ***279****, 45875–45886*
Notes: Human colon cancer (HT29-D4) cells were analyzed for activated caspases using the CaspACE™ FITC-VAD-FMK In Situ Marker. HT29-D4 cells (7 x 105) were cultured in the presence or absence of 1µM orexins, peptide growth inhibitors. Cells were washed, and bound CaspACE™ FITC-VAD-FMK In Situ Marker was visualized by confocal microscopy.

Qi, H., Li, T-K., Kuo, D., Nur-E-Kamal, A., Liu, L.F. (2003) **Inactivation of Cdc13p triggers MEC-1-dependent apoptotic signals in yeast;** *J. Biol. Chem.* ***278****, 15136–15141*
Notes: Apoptosis in yeast cells was detected using the CaspACE™ FITC-VAD-FMK In Situ marker. Yeast cells were stained with the marker at room temperature, washed and resuspended. FACS analysis of cells was performed with excitation at 488nm and emission of 520–550nm.

Vaudry, D., Rousselle, C., Basille, M., Falluel-Morel, A., Pamantung, T.F., Fontaine, M., Fournier, A., Vaudry, H., and Gonzalez, B.J. (2002) **Pituitary adenylate cyclase-activating polypeptide protects rat cerebellar granule neurons against ethanol-induced apoptotic cell death;** *Proc. Natl. Acad. Sci. USA* ***99****, 6398*
Notes: The effects of pituitary adenylate cyclase-activating polypeptide (PACP) on ethanol induced caspace activation in cultured granule cells was measured using the CaspACE™ FITC-VAD-FMK In Situ Marker.

Multiplexing

Multitox-Fluor Multiplex Cytotoxicity Assay

Promega articles

Multiplexing Cell-Based Assays: Get More Biologically Relevant Data; Kyle Hooper**; 2011**

Using Protease Biomarkers to Measure Viability and Cytotoxicity; Andrew Niles, Michael Scurria, Laurent Bernad, Brian McNamara, Kay Rashka, Deborah Lange, Pam Guthmiller and Terry Riss; *Cell Notes* ***19****, 16–20;* **2007**

Multiplexed Viability Cytotoxicity And Apoptosis Assays For Cell-Based Screening; Andrew Niles[1], Tracy Worzella[1], Michael Scurria[2], William Daily[2], Laurent Bernad[2], Pam Guthmiller[1], Brian McNamara[1], Kay Rashka[1], Deborah Lagne[1] and Terry L. Riss[1]; *Cell Notes* ***16****, 12–15;* **2006**

MultiTox-Fluor Multiplex Cytotoxicity Assay Technology; Andrew L. Niles[1], Richard A. Moravec[1], Michael Scurria[2], William Daily[2], Laurent Bernad[2], Brian McNamara[1], Anissa Moraes[1], Kay Rashka[1], Deborah Lange[1] and Terry L. Riss[1]; *Cell Notes* ***15****, 11–15;* **2006**

Monitor the Ratio of Live and Dead Cells Within a Population MultiTox Fluor Multiplex Cytotoxicity Assay; Andrew L. Niles, Richard A. Moravec, Michael Scurria, William Daily, Laurent Bernad, Brian McNamara, Pam Guthmiller, Kay Rashka Deborah Lange, Michele Arduengo and Terry L. Riss; *Promega Notes* ***94****, 22–26;* **2006**

Peer-reviewed publications

Niles, A.L., Moravec, R.A. and Riss, T.L. (2009) **In vitro viability and cytotoxicity testing and same-well multi-parametric combinations for high-throughput screening;** *Current Chemical Genomics **3**, 33–41*
Notes: The authors review the use of in vitro cytotoxicity testing in drug discovery to characterize the toxic potenzial of new chemical entities (nce) at the earliest stages of profiling.

Grenier, A.L., Abu-Ihweij, K., Zhang, G., Ruppert, S.M., Boohaker, R., Slepkov, E.R., Pridemore, K., Ren, J.J., Fliegel, L. and Khaled, A.R. (2008) **Apoptosis-induced alkalinization by the Na+/H+ exchanger isoform 1 is mediated through phosphorylation of amino;** *Am. J. Physiol. Cell Physiol. **295**, C883–C896*
Notes: The authors wanted to examine the role of plasma membrane protein Na+/H+ exchanger isoform 1 (NHE1) in apoptosis. API cells, a NHE1-deficient Chinese hamster ovary cell line, was cotransfected with wild-type NHE1 or mutant NHE1 constructs and destabilized yellow fluorescent protein (YFP). Cells were plated at a density of 1 x 10^4 cells/well in a 96-well plate with or without FBS. To induce apoptosis in the cells, serum was withdrawn for 24 hours. The ratio of dead-to-live cells was measured using the MultiTox-Fluor Multiplex Cytotoxicity Assay. Cell death was also determined by examining the loss of YFP fluorescence under a microscope.

Niles, A.L., Moravec, R.A., Hesselberth, P.E., Scurria, M.A., Daily, W.J. and Riss, T.L. (2007) **A homogeneous assay to measure live and dead cells in the same sample by detecting different protease markers;** *Anal. Biochem. **366**, 197–206*
Notes: The authors of this paper describe an assay that uses protease biomarkers to assess cell viability and cell death simultaneously in a population of cells. The assay detects an ubiquitous protease activity that is associated with live cells and a second protease activity that is associated with cells that have lost membrane integrity. The readouts are either fluorescent or fluorescent and luminescent. The assay can be performed in multiplex with other assays, such as caspase assays, to gain additional information on the cell population, and it is amenable to high-throughput screening.

Multitox-Glo Multiplex Cytotoxicity Assay

Promega articles

High-Throughput Automation of Multiplexed Cell-Based Assays for Viability and Cytotoxicity; Tracy Worzella, Michael Busch and Andrew Niles; *Cell Notes **20**, 26–29;* **2008**

Using Protease Biomarkers to Measure Viability and Cytotoxicity; Andrew Niles, Michael Scurria, Laurent Bernad, Brian McNamara, Kay Rashka, Deborah Lange, Pam Guthmiller and Terry Riss; *Cell Notes **19**, 16–20;* **2007**

Measure Relative Numbers of Live and Dead Cells and Normalize Assay Data to Cell Number; Andrew Niles, Michael Scurria, Laurent Bernad, Brian McNamara, Kay Rashka, Deborah Lange, Pam Guthmiller, Tracy Worzella and Terry Riss; *Cell Notes **18**, 15–20;* **2007**

Measuring Cell Health and Viability Sequentially by Same-Well Multiplexing Using the GloMax®-Multi Detection System; Halina Zakowicz, Trista Schagat, David Yoder and Andrew Niles; *Promega Notes **99**, 25–28;* **2008**

Luminogenic Enzyme Substrates: The Basis for a New Paradigm in Assay Design; Poncho L. Meisenheimer[1], Martha A. O'Brien[2] and James J. Cali[2]; *Promega Notes **100**, 22–26;* **2008**

Peer-reviewed publications

Niles, A.L., Moravec, R.A. and Riss, T.L. (2009) **In vitro viability and cytotoxicity testing and same-well multi-parametric combinations for high-throughput screening**; *Current Chemical Genomics **3**, 33–41*
Notes: The authors review the use of in vitro cytotoxicity testing in drug discovery to characterize the toxic potenzial of new chemical entities (nce) at the earliest stages of profiling.

Niles, A.L., Moravec, R.A., Hesselberth, P.E., Scurria, M.A., Daily, W.J. and Riss, T.L. (2007) **A homogeneous assay to measure live and dead cells in the same sample by detecting different protease markers;** *Anal. Biochem. **366**, 197–206*
Notes: The authors of this paper describe an assay that uses protease biomarkers to assess cell viability and cell death simultaneously in a population of cells. The assay detects an ubiquitous protease activity that is associated with live cells and a second protease activity that is associated with cells that have lost membrane integrity. The readouts are either fluorescent or fluorescent and luminescent. The assay can be performed in multiplex with other assays, such as caspase assays, to gain additional information on the cell population, and it is amenable to high-throughput screening.

ApoLive-Glo™ Multiplex Assay

Promega articles

Zakowicz, H. *et al.* (2008) **Measuring cell health and viability sequentially by samewell multiplexing using the GloMax®-Multi Detection System;** *Promega Notes **99**, 25–8*

Worzella, T., Busch, M. and Niles, A.L. (2008) **High-throughput automation of multiplexed cell-based methods for viability and cytotoxicity;** *Cell Notes **20**, 26–9*

Niles, A.L. *et al.* (2007) **Using protease biomarkers to measure viability and cytotoxicity;** *Cell Notes **19**, 16–20*

Niles, A.L. *et al.* (2007) **Measure relative numbers of live and dead cells and normalize assay data to cell number;** *Cell Notes **18**, 15–20*

Peer-reviewed publications

Niles, A.L. *et al.* (2007) **A homogeneous assay to measure live and dead cells in the same sample by detecting different protease markers;** *Anal. Biochem. **366**, 197–206*

Inglese, J. *et al.* (2006) **Quantitative high-throughput screening: A titration-based approach that efficiently identifies biological activities in large chemical libraries;** *Proc. Natl. Acad. Sci. USA **103**, 11473–8*

Apotox-Glo™ Triplex Assay

Promega articles

Multiplexing Cell-Based Assays: Get More Biologically Relevant Data; Kyle Hooper; **2011**

The Versatility of the GloMax®-Multi+ Detection System with Instinct Software; Doug Wieczorek, Amanda Bychinski, Simon Allard and Trista Schagat; **2010**

Determining the Predictive Mechanism of Toxicity Using a Single-Well Multiplexed Assay; Sarah Shultz; **2009**

Peer-reviewed publications

Niles, A.L. *et al.* (2007) **A homogeneous assay to measure live and dead cells in the same sample by detecting different protease markers;** *Anal. Biochem.* ***366****, 197–206*

O'Brien, M.A. *et al.* (2005) **Homogeneous, bioluminescent protease assays: Caspase-3 as a model;** *J. Biomol. Screen.* ***10****, 137–48*

Inglese, J. *et al.* (2006) **Quantitative high-throughput screening: A titration-based approach that efficiently identifies biological activities in large chemical libraries;** *Proc. Natl. Acad. Sci. USA* ***103****, 11473–8*

Niles, A.L., Moravec, R.A. and Riss, T.L. (2008) **Update on in vitro cytotoxicity assays for drug development;** *Expert Opin. Drug Discovery* ***3****, 655–69*

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

Promega articles

Zakowicz, H. *et al.* (2008) **Measuring cell health and viability sequentially by samewell multiplexing using the GloMax®-Multi Detection System;** *Promega Notes* ***99****, 25–8*

Schagat, T. and Kopish, K. **Optimize Transfection of Cultured Cells.** 2009. Available from: http://www.promega.com/pubs/tpub_020.htm

Peer-reviewed publications

Niles, A.L. *et al.* (2007) **A homogeneous assay to measure live and dead cells in the same sample by detecting different protease markers;** *Anal. Biochem.* ***366****, 197–206*

Mitochondrial ToxGlo™ Assay

Peer-reviewed publications

Niles, A.L. *et al.* (2007) **A homogeneous assay to measure live and dead cells in the same sample by detecting different protease biomarkers;** *Anal Biochem.* ***366****, 197–206*

Marroquin, L. D. *et al.* (2007) **Circumventing the Crabtree effect: Replacing media glucose with galactose increases susceptibility of HepG2 cells to mitochondrial toxicants;** *Toxicol. Sci.* ***97****, 539–47*

Rossignol, R. *et al.* (2004) **Energy substrate modulates mitochondrial structure and oxidative capacity in cancer cells;** *Cancer Res.* ***64****, 985–93*

Rodriguez-Enriquez, S. *et al.* (2001) **Multisite control of the Crabtree effect in ascites hepatoma cells;** *Eur. J. Biochem.* ***268****, 2512–9*

Cell metabolism

NAD(P)/NAD(P)H-Glo™ Assays

Promega articles

A Novel Bioluminescent HTS Method for Rapid NAD(P)/NAD(P)H Detection; Jolanta Vidugiriene, Donna Leippe, Mary Sobol, Wenhui Zhou, Gediminas Vidugiris, Troy Good, Laurent Bernad, Poncho Meisenheimer and James J. Cali; Promega Corporation **2013**

Novel Bioluminescent Cell Metabolism Assays Integration with HP D300 Digital Dispenser and Tecan Gas Controlled Module Equipped Infinite M200 Pro Reader; Gediminas Vidugiris, Donna Leippe, Mary Sobol, Sarah Duellman, Wenhui Zhou, Jolanta Vidugiriene, Jessica Merlino, Michael Reitman, Thomas Hengstl, Poncho Meisenheimer, Cristopher Cowan, James Cali; Promega Corporation, Madison, WI; Promega Biosciences, LLC, San Luis Obispo, CA; Tecan Schweiz, Männedorf, Switzerland; Tecan US, Morrisville, NC; Tecan Austria GmbH, Groedig; Austria, **2013**

Oxidative stress – glutathione assays

GSH-Glo™ Glutathione Assay

Promega articles

Homogeneous Luminescence-Based Assay for Quantifying the Glutathione Content in Mammalian Cells; Christiane Scherer, Silvia Christofanon, Mario Dicato, and Marc Diederich; *Cell Notes* ***22****, 7–9; 2008*

Detecting Toxicological Responses in Cells with the Bioluminescent GSH-Glo™ Glutathione Assay; Nancy Murphy, John Schultz, Wenhui Zhou and Keith V. Wood; *Cell Notes* ***20****, 15–17; 2008*

Peer-reviewed publications

Tang, Y., Scheef, E.A., Wang, S., Sorenson, C.M., Marcus, C.B., Jefcoate, C.R. and Sheibani, N. (2009) **CYP1B1 expression promotes the proangiogenic phenotype of endothelium through decreased intracellular oxidative stress and thrombospondin-2 expression;** *Blood* ***113****, 744–754*
Notes: The authors tested if CYP1B1 removed cellular oxygenation products that induce oxidative stress and promote the release of antiangiogenic factors. The P450-Glo™ CYP1B1 Assay was used to determine CYP1B1 activity. The presence of glutathione was assessed using either 104 retinal endothelial cells or 50µl of mouse retinal extracts dispensed into each well of a 96-well plate with the GSH-Glo™ Glutathione Assay.

Reisman, S.A., Yeager, R.L., Yamamoto, M. and Klaassen, C.D. (2009) **Increased Nrf2 activation in livers from Keap1-knockdown mice increases expression of cytoprotective genes that detoxify electrophiles more than those that detoxify reactive oxygen species;** *Toxicol. Sci.* ***108****, 35–47*
Notes: In this study, the researchers wanted to determine the role of kelch-like ECH associated protein 1 knockdown (Keap1-kd) mice protein products, which are thought to protect against oxidative and electrophilic stress, and compare the hepatic phenotype with that of transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2)-null and wild-type mice. Microsomal suspensions from liver homogenates were prepared, and bile was collected from wild-type, Nrf2-null, and Keap1-kd mice. Reduced GSH was quantified using the GSH-Glo™ Glutathione Assay.

Jamaluddin, M.S., Wang, X., Wang, H., Rafael, C., Yao, Q. and Chen, C. (2009) **Eotaxin increases monolayer permeability of human coronary artery endothelial cells. Arterioscler;** *Thromb. Vasc. Biol. Sept 24*
Notes: Glutathione levels were assessed in human coronary artery endothelial cells (HCAECs) as a measure of oxidative stress. HCAECs were treated with either 100ng/ml eotaxin, a newly discovered chemokine, or pretreated with 2µmol/l MnTBAP for 30 minutes followed by eotaxin treatment for 45 minutes. Positive controls were treatment with 10µg/ml antimycin A and 2ng/ml TNF-α. Cellular glutathione was measured using the GSH-Glo™ Glutathione Assay.

GSH/GSSG-Glo™ Assay

Promega articles

Detecting Ozone-Induced Changes in Cellular Redox Balance via GSH/GSSG-Glo™ Assay; Madeleine Chalfant and Karen Bernd; **2011**

Peer-reviewed publications

Pompella, A. *et al.* (2003) **The changing faces of glutathione, a cellular protagonist;** *Biochem. Pharmacol.* ***66****, 1499–1503*

Ballatori, N. *et al.* (2009) **Glutathione dysregulation and the etiology and progression of human diseases;** *Biol. Chem.* ***390****, 191–214*

Rebrin, I. and Sohal, R.S. (2008) **Pro-oxidant shift in glutathione redox state during aging;** *Adv. Drug Deliv. Rev.* ***60****, 1545–52*

ROS-Glo™ H_2O_2 Assay

Promega articles

A New Luminescent Assay for Detection of Reactive Oxygen Species; Sarah Duellman, John Shultz, Gediminas Vidugiris, and James Cali, 2013 *www.promega.de/resources/scientific_posters/posters/ros-glo-h2o2-assay-a-luminescent-assay-for-detection-of-reactive-oxygen-species-poster/*

Cell-Based Bioluminescent Hydrogen Peroxide Assay: Effects of Inducers and Generators with Cells; Hui Wang, Jean Osterman, Wenhui Zhou, Poncho Meisenheimer, John Shultz, Sarah Duellman, Jolanta Vidugiriene, Gediminas Vidugiris and James Cali, **2013** *www.promega.de/resources/scientific_posters/posters/cell-based-bioluminescent-hydrogen-peroxide-assay-effects-of-inducers-and-generators-poster/*

Epigenetic assays

HDAC-Glo™ I/II Assay

Peer-reviewed publications

Smith, E.R. *et al.* (2000) **The Drosophila MSL complex acetylates histone H4 at lysine 16, a chromatin modification linked to dosage compensation;** *Mol. Cell. Biol.* ***20****, 312–8*

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

Auld, D. S. *et al.* (2008) **Characterization of chemical libraries for luciferase inhibitory activity;** *J. Med. Chem.* ***51****, 2372–86*

HDAC-Glo™ I/II Screening Systems

Siehe HDAC-Glo™ I/II Assay

SIRT-Glo™ Assays and Screening Systems

Peer-reviewed publications

Abraham, J. *et al.* (2000) **Post-translational modification of p53 protein in response to ionizing radiation analyzed by mass spectrometry;** *J. Mol. Biol.* ***295****, 853–64*

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

Auld, D. S. *et al.* (2008) **Characterization of chemical libraries for luciferase inhibitory activity;** *J. Med. Chem.* ***51****, 2372–86*

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**

Monitoring the Activity of GPCR Modulated by Lipid or Free Fatty Acid Agonists; Said Goueli and Kevin Hsaio; *Cell Notes* ***23****, 13–16;* **2009**

Peer-reviewed publications

Kumar, M., Hsiao, K., Vidugiriene, J. and Goueli, S.A. (2007) **A bioluminescent-based, HTS-compatible assay to monitor G-protein-coupled receptor modulation of cellular cyclic AMP;** *ASSAY and Drug Development Technologies* ***5****, 237–245*
Notes: The authors of this paper introduce a luminescent assay to monitor changes in cellular cAMP concentration. The assay can be used to study the activity of G-protein coupled receptors that modulate adenylate cyclase activity. The assay is compatible with high-throughput screening in 96-, 384- and 1536-well formats.

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**

Peer-reviewed publications aus Protokoll

Auld, D.S. *et al.* (2009) **A basis for reduced chemical library inhibition of firefly luciferase obtained from directed evolution;** *J. Med. Chem.* ***52****, 1450–8*

Zhang, J.H. *et al.* (1999) **A simple statistical parameter for use in evaluation and validation of high throughput screening assays;** *J. Biomol. Screen.* ***4****, 67–73*

Kinase-Glo® Luminescent Kinase Assay

Promega articles

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

Screen for Kinase Modulators in a High-Throughput Format with Promega Kinase Reagents; Michael Curtin; *Cell Notes* ***20****, 21–24, Correction published in Cell Notes* ***22*** *(PDF file);* **2008**

The Biology of Chemical Space; John Watson; *Cell Notes* ***13****, 3–4;* **2005**

Choosing the Best Kinase Assay to Meet Your Research Needs; Michael Curtin; *Cell Notes* ***13****, 11–15;* **2005**

Assay Virtually Any Kinase with Kinase-Glo® Plus Luminescent Kinase Assay: A Homogeneous High-Throughput Assay; Said Goueli and Kevin Hsaio; *Cell Notes* ***12****, 8–12;* **2005**

Peer-reviewed publications

Kannan, S., Audet, A., Huang, H., Chen, L-J. and Wu, M. (2008) **Cholesterol-rich membrane rafts and Lyn are involved in phagocytosis during Pseudomonas aeruginosa infection;** *J. Immunology* ***180****, 2396–2408*
Notes: The authors of this study investigated the role of Lyn, a Src-family tyrosine kinase, in regulating the formation of the phagosome in alveolar macrophages in response to Psuedomonas aeruginosa (PA) infection. The Kinase-Glo® Assay was used to assess Lyn activity, using acid-denatured enolase as the substrate. The authors found that Lyn kinase activity was increased following infection with PA.

Wierenga, K.J., Lai, K., Buchwald, P. and Tang, M. (2008) **High-throughput screening for human galactokinase inhibitors;** *J. Biomol. Screen.* ***13*** *, 415–423*
Notes: Thees authors searched for small-molecule inhibitors of galactokinase (GALK). They developed an HTS assay using the Kinase-Glo® Assay System. The HTS assay used 15 µM ATP and α-D-galactose as the substrate and was performed in 384-well plates against 50,000 small molecules.

Two hundred compounds were identified from the primary screen as GALK inhibitors.

Baki, A., Bielik, A., Molnár, L., Szendrei, G. and Keserü, G.M. (2007) **A high throughput luminescent assay for glycogen synthase kinase-3β inhibitors;** *Assay and Drug Development Technologies* ***5****, 75–83*
Notes: These authors used the Kinase-Glo® Luminescent Kinase Assay to perform a high-throughput screening assay for inhibitors of glycogen synthase kinase-3β in 96-well plates.

PDE-Glo™ Phosphodiesterase Assay

Peer-reviewed publications

Barad, M. *et al.* (1998) **Rolipram, a type IV-specific phosphodiesterase inhibitor, facilitates the establishment of long-lasting long-term potentiation and improves memory;** *Proc. Natl. Acad Sci. USA* ***95****, 15020–5*

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

Metabolism of drugs – ADME assays

P450-Glo™ CYP450 Assay Systems

Promega articles

Luminogenic Enzyme Substrates: The Basis for a New Paradigm in Assay Design; Poncho L. Meisenheimer, Martha A. O'Brien, and James J. Cali; *Cell Notes* ***22****, 10–14;* **2008**

Citation Note: HaloTag® Techology, P450-Glo® CYP2C8 Assay, and Beta-Glo® Assay; Terri Sundquist; *Cell Notes* ***21****, 17 and 27;* **2008**

Custom Enzyme Substrates for Luciferase-Based Assays; Neal Cosby[1], Mike Scurria[2], William Daily[2] and Tim Ugo[2]; *Cell Notes* ***18****, 9–11;* **2007**

In Vitro Compound Profiling in 384- and 1536-Well Formats Using Bioluminescent ADME Assays to Understand In Vivo Biology; Tracy Worzella[1], James Cali[1], Neal Cosby[1], Jean Shieh[2], Aoife Gallagher[3], Kim Titus4, Gerlinde Zerza-Schnitzhofer[5]; *Cell Notes* ***16****, 4–8;* **2006**

P450Glo® CYP2C19 and CYP2D6 Assays and Screening Systems: The Method of Choice for in vitro P450 Assays; James J. Cali, Dongping MA, Mary Sobol, Troy Good and David Liu; *Cell Notes* ***14****, 20–24;* **2006**

Peer-reviewed publications

Tang, Y., Scheef, E.A., Wang, S., Sorenson, C.M., Marcus, C.B., Jefcoate, C.R. and Sheibani, N. (2009) **CYP1B1 expression promotes the proangiogenic phenotype of endothelium through decreased intracellular oxidative stress and thrombospondin-2 expression;** *Blood* ***113****, 744–754*
Notes: The authors tested if CYP1B1 removed cellular oxygenation products that induce oxidative stress and promote the release of antiangiogenic factors. The P450-Glo™ CYP1B1 Assay was used to determine CYP1B1 activity. The presence of glutathione was assessed using either 104 retinal endothelial cells or 50µl of mouse retinal extracts dispensed into each well of a 96-well plate with the GSH-Glo™ Glutathione Assay.

Zhang, W., Chen, M., West, D.B. and Purchio, A.F. (2005) **Visualizing Drug Efficacy In Vivo;** *Molecular Imaging* ***4****, 88–90*
Notes: The authors of this paper present proof-of-concept experiments showing that drug metabolism enzyme activity can be measured in whole animals (in vivo) in real time. Using a mouse that expresses a luciferase transgene at constitutively high levels in the liver, the authors evaluated CYP3A4 and CYP3A7 activity using a CYP3A P450 substrate (proluciferin substrate) that is converted into a luciferase substrate by CYP34 activity. The luciferase substrate produced by the P450 activity is then used by luciferase in a reaction that produces light. An increase in luminescence correlates with an increase in enzyme activity in this assay.

P450-Glo™ CYP450 Screening Systems

Promega articles

Luminogenic Enzyme Substrates: The Basis for a New Paradigm in Assay Design; Poncho L. Meisenheimer, Martha A. O'Brien, and James J. Cali; *Cell Notes* ***22****, 10–14;* **2008**

Rat Hepatocyte Culture Physiology Shows Enhanced Cytochrome P450 Activity on a Synthetic Extracellular Matrix; Mark E. Rothenberg[1], James J. Cali[2], Mary Sobol[2], Michael W. Briggs[1] and Todd Upton[1]; *Cell Notes* ***20****, 18–20;* **2008**

Screen for Cytochrome P450 Activity Using a Luminescent Assay; **James Cali, Mary Sobol, Dongping Ma;** *Cell Notes* ***13****, 8–10;* **2005**

Selective Cytochrome P450 4A11 Enzyme Assay Using a Novel Bioluminescent Probe Substrate; Mary Sobol, Dongping Ma and James J. Cali; **2008**

Cytochrome P450 4F12 Enzyme Assay Using a Novel Bioluminescent Probe Substrate; Mary Sobol, Dongping Ma, Carolyn C. Woodroofe and James J. Cali

Peer-reviewed publications

Cali, J.S. *et al.* (2006) **Luminogenic cytochrome P450 assays;** *Expert Opin. Drug Metab. Toxicol.* ***2****, 629–45*

Pgp-Glo™ Assay

Promega articles

The Biology of Chemical Space; John Watson; *Cell Notes* ***13****, 3–4;* **2005**

High-Throughput Compound Profiling Using Tecan Instrumentation; Tracy Worzella and Brad Larson; *Promega Notes* ***92****, 4–6;* **2006**

Identify P-glycoprotein Substrates and Inhibitors with the Rapid HTS Pgp-Glo™ Assay System; Dongping Ma and James J. Cali; *Promega Notes* ***96****, 11–14;* **2007**

Peer-reviewed publications

Ambudkar, S.V. *et al.* (1999) **Biochemical, cellular and pharmacological aspects of the multidrug transporter;** *Annu. Rev. Pharmacol. Toxicol.* ***39****, 361–98*

Litman, T. *et al.* (2003) **Pumping of drugs by P-glycoprotein: A two step process?** *J. Pharm. Exp. Ther.* ***3F07(3)****, 846–853*

Boulton, D.W. *et al.* (2002) **In vitro P-glycoprotein affinity for a typical and conventional antipsychotics;** *Life Sciences* ***71****, 163–169*

MAO-Glo™ Assay Systems

Promega articles

In Vitro Compound Profiling in 384- and 1536-Well Formats Using Bioluminescent ADME Assays to Understand In Vivo Biology; Tracy Worzella[1], James Cali[1], Neal Cosby[1], Jean Shieh[2], Aoife Gallagher[3], Kim Titus[4], Gerlinde Zerza-Schnitzhofer[5]; *Cell Notes* ***16****, 4–8;* **2006**

The MAO-Glo™ Assay: A Bioluminescent, Coupled Assay for Monoamine Oxidase Activity; Michael P. Valley, Erika M. Hawkins, John Shultz, Terry L. Riss, Keith V. Wood, Wenhui Zhou, Laurent Bernad, Troy Good, Dave Good and Dieter H. Klaubert; *Cell Notes* ***14****, 4–7;* **2006**

High-Throughput Compound Profiling Using Tecan Instrumentation; Tracy Worzella and Brad Larson; *Promega Notes* ***92****, 4–6;* **2006**

The MAO-Glo® Assay: A Bioluminescent, Coupled Assay for Monoamine Oxidase Activity; Michael P. Valley[1], Erika M. Hawkins[1], John Shultz[1], Terry L. Riss[1], Keith V. Wood[1], Wenhui Zhou[2], Laurent Bernad[2], Troy Good[2], Dave Good[2] and Dieter H. Klaubert[2]; *Promega Notes* ***93****, 11–14;* **2006**

Peer-reviewed publications

Fan, F. and Wood, K.V. (2007) **Bioluminescent assays for high-throughput screening;** *Assay Drug Dev. Technol.* ***5****, 127–136*
Notes: The authors of this paper review bioluminescent assay technologies, discussing HTS reporter, cell-based and luciferase biosensor assays. They divide luminescent assays into three basic categories: assays that measure ATP concentration (cell viability and kinase assays), assays that measure changes in luciferase levels (reporter assays, GPCR assays), and assays that measure changes in luciferin levels (protease [including caspase], P450 and MAO assays).

UGT-Glo™ Assay

Referencecs Protocol

Zhang, D. *et al.* (2005) **In vitro inhibition of UDP glucuronosyltransferases by atazanavir and other HIV protease inhibitors and the relationship of this property to in vivo bilirubin glucuronidation;** *Drug Metab. Disp.* ***33****, 1729–39*

Sakaguchi, K. *et al.* (2004) **Glucuronidation of carboxylic acid containing compounds by UDP-glucuronosyltransferase isoforms;** *Archives Biochem. Biophys.* ***424****, 219–25*

Uchaipichat, V. *et al.* (2004) **Human UDP-glucuronosyltransferases: Isoform selectivity and kinetics of 4-methylumbelliferone and 1-naphthol glucuronidation, effects of organic solvents, and inhibition by diclofenac and probenicid;** *Drug Metab. Dispos.* ***32****, 413–23*

Protease assays

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

Promega articles Cell-based

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 Moravec[1], Martha O'Brien[1], Bill Daily[2], Mike Scurria[2], Laurent Bernad[2], Brad Larson[1], Tracy Worzella[1], Kay Rashka[1], Jeri Culp[1], Brian McNamara[1] and Terry Riss[1]; *Cell Notes* ***15****, 4–7;* **2006**

Peer-reviewed publications Cell-Based

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*
Notes: The authors of this study investigated the mechanism of action of syringolin A (SylA), which is secreted by virulent strains of the plant athogen Pseudomonas syringae. They show that SylA inhibits all three activities of the proteasome in vitro. They also used the Proteasome-Glo™ Chymotrypsin-Like Cell-Based Assay to show that SylA inhibits the chymotrypsin-like activity of the proteasome in SK-N-HS neuroblastoma cells.

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*
Notes: Endosomal sorting complexes required for transport (ESCRTs) are necessary for sorting membrane proteins into the intralumenal vesicles of the multivesicular body for eventual degradation by the lysosome/vacuole. Mutations in at least one subunit of the ESCRTs are associated with frontotemporal dementia and ALS. In this study, the authors demonstrate that ESCRTs are required for autophagy and prevention of protein aggregation. They address the question of whether loss of ESCRTs might interfere with proteasome activity. Using the Proteasome-Glo™ Chymotrypsin-Like Cell-Based Assay, they show that proteasome activity is minimally affected in ESCRT-depleted cells.

Promega articles Proteasome GLO

Functional Proteomics Techniques to Isolate and Characterize the Human Proteasome; Brad Hook and Trista Schagat; **2011**

Luminescence Based Assay for Proteasome Activity in Tissue Extracts; 2010

Measurement of Three Proteasome Proteolytic Activities Using Luminescent Assays; Martha O'Brien[1], Mike Scurria[2], Laurent Bernad[2], William Daily[2], James Unch[2], Kay Rashka[1], Sandra Hagen[1], Jeri Culp[1], Rich Moravec[1], Brian McNamara[1], and Terry Riss[1]; *Promega Notes* ***94****, 19–21;* **2006**

Calpain-Glo™ Protease Assay

Promega articles

Screen for Calpain Inhibitors Using a Cell-Based, High-Throughput Assay; Katheleen Seyb[1,2], Jake Ni[1], Mickey Huang[1], Eli Schuman[1], Mary L. Michaelis[2] and Marcie A. Glicksman[1]; *Cell Notes* ***18****, 6–8;* **2007**

A Bioluminescent Assay for Calpain Activity; Martha O'Brien[1], Mike Scurria[2], Kay Rashka[1], Bill Daily[2] and Terry Riss[1]; *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 sreening 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*
Notes: Taxol-induced peripheral neuropathy is a common side-effect of treatment that has been associated with disturbed intracellular calcium homeostasis in neuronal cells. These authors investigated whether prolonged exposure to Taxol caused alterations in calcium signaling in human neuroblastoma and rat dorsal root ganglia. They found that expression of the inositol 1,4,5-triphosphate receptor modulator NCS-1 was reduced in Taxol-treated neuronal cells.

DUB-Glo™ Protease Assay (DUB/SENP/NEDP)

Promega articles

Detecting the Deubiquitin Activity of SARS-CoV PLpro: Turning on the Light with the DUB-Glo™ Protease Assay; Yahira M. Baez-Santos[1], Andrew D. Mesecar[1] and Martha A. O'Brien[2]; **2009**

Peer-reviewed publications aus Protokoll

Love, K.R. *et al.* (2007) **Mechanisms, biology and inhibitors of deubiquitinating enzymes;** *Nature Chem. Biol.* ***3****, 697-705*

Hochstrasser, M. (2009) **Origin and function of ubiquitin-like proteins;** *Nature* ***458****, 422*

DPPIV-Glo™ Protease Assay

Promega articles

Detection of Dipeptidyl Peptidase Activity with DPPIV-Glo™ Assay; Joseph Alex Davis, Vamshi Krishna Tulasi Abhijit Ray and Pradip Bhatnagar; *Cell Notes* ***23****, 6–9;* **2009**

DPPIV-GLO™ Protease Assay: A More Sensitive Method For Measuring GLY-PRO Cleaving Activity In Serum; Martha O'Brien; *Cell Notes* ***16****, 9–11;* **2006**

Assay for DPPIV Activity Using a Homogeneous Luminescent Method; Martha O'Brien[1], Bill Daily[2], Mike Schurria[1], and Terry Riss[1]; *Cell Notes* ***11****, 8–11;* **2005**

Peer-reviewed publications

Marguet, D. *et al.* (2000) **Enhanced insulin secretion and improved glucose tolerance in mice lacking CD26;** *Proc. Natl. Acad. Sci. USA* ***97****, 6874–9*

Engel, M. *et al.* (2003) **The crystal structure of dipeptidyl peptidase IV (CD26) reveals its functional regulation and enzymatic mechanism;** *Proc. Natl. Acad. Sci. USA* ***100****, 5063–8*

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® AQ_{ueous} 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.
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.
Caspase-Glo® 2 Assay	10 ml	G0940
	50 ml	G0941
Caspase-Glo® 3/7 Assay	2.5 ml	G8090
	10 ml	G8091
	10 x 10 ml	G8093
	100 ml	G8092
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

Inflammasome

Product	Quantity	Catalog No.
Caspase-Glo® 1 Inflammasome Assay	10 ml	G9951
	5 x 10 ml	G9952

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
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_2O_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™ Class IIa Assay	10 ml	G9560
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™ Assays	10 ml	G6450
	5 x 10 ml	G6451
	100 ml	G6452
SIRT-Glo™ Screening System	10 ml	G6470
	5 x 10 ml	G6471
SIRT-Glo™ Control Substrate	35 µl	G6460
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
Ultra Pure UDP-GlcNAc, 100mM	50 µl	V7071
	250 µl	V7072
Ultra Pure UDP-GalNAc, 100mM	50 µl	V7081
UDP-GalNAc, 100mM	250 µl	V7082
Ultra Pure UDP-Glucose, 100mM	50 µl	V7091
	250 µl	V7092
Ultra Pure UDP-Galactose, 100mM	50 µl	V7171
	250 µl	V7172
HeLa Nuclear Extract	10 µl	G6570
Nicotinamide	30 µl	G6540

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
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

Metabolism of drugs – ADME assays

Product	Quantity	Catalog No.
P450-Glo™ CYP3A4 Assay with Luciferin-IPA	50 ml	V9002
P450-Glo™ CYP2C9 Screening System	1,000 assays	V9790
P450-Glo™ CYP2C9 Assay	50 ml	V8792
P450-Glo™ CYP3A4 Screening System with Luciferin-PPXE	1,000 assays	V9910
P450-Glo™ CYP1A2 Induction/ Inhibition Assay	50 ml	V8422
P450-Glo™ CYP2C8 Assay	50 ml	V8782
P450-Glo™ CYP2C19 Assay	50 ml	V8882
P450-Glo™ CYP2D6 Assay	50 ml	V8892
P450-Glo™ CYP3A4 Screening System	1,000 assays	V9800
P450-Glo™ CYP1A2 Screening System	1,000 assays	V9770
P450-Glo™ CYP1A1 Assay	50 ml	V8752
P450-Glo™ CYP1B1 Assay	50 ml	V8762
P450-Glo™ CYP1A2 Assay	50 ml	V8772
P450-Glo™ CYP3A4 Assay	50 ml	V8802
P450-Glo™ CYP3A7 Assay	50 ml	V8812

Metabolism of drugs – ADME assays (continued)

Product	Quantity	Catalog No.
P450-Glo™ CYP3A4 Assay (Luc -PFBE) Cell-Based/Bio	50 ml	V8902
P450-Glo™ CYP3A4 Assay with Luciferin PPXE	50 ml	V8912
P450-Glo™ CYP3A4 Screening System with Luciferin-IPA	1,000 assays	V9920
P450-Glo™ CYP2D6 Screening System	1,000 assays	V9890
P450-Glo™ CYP3A4 Assay with Luciferin-IPA	10 ml	V9001
P450-Glo™ CYP2C9 Assay	10 ml	V8791
P450-Glo™ CYP3A7 Assay	10 ml	V8811
P450-Glo™ CYP1A1 Assay	10 ml	V8751
P450-Glo™ CYP1B1 Assay	10 ml	V8761
P450-Glo™ CYP1A2 Assay	10 ml	V8771
P450-Glo™ CYP3A4 Assay (Luc -PFBE) Cell-Based/Bio	10 ml	V8901
P450-Glo™ CYP2C19 Screening System	1,000 assays	V9880
MAO-Glo™ Assay	200 assays	V1401
UGT-Glo™ Assay	200 assays	V2081
UGT-Glo™ Assay	1,000 assays	V2082
Pgp-Glo™ Assay System with P-glycoprotein	10 ml	V3601

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
DUB-Glo™ Protease Assay (DUB/SENP/NEDP)	10 ml	G6260
	50 ml	G6261
DPPIV-Glo™ Protease Assay	10 ml	G8350
	50 ml	G8351

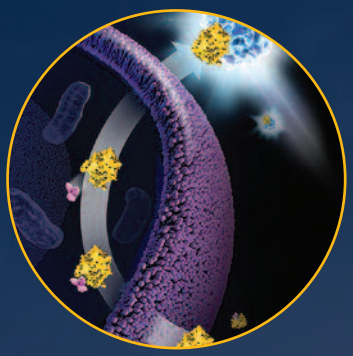
Promega

Product Portfolio

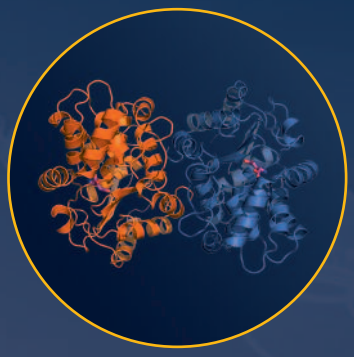
Genomic Essentials

Nucleic acid purification, enzymes, RNase inhibitors, reverse transcription, PCR, real-time PCR, markers, cloning systems, transfection

Cellular & Biochemical Assays



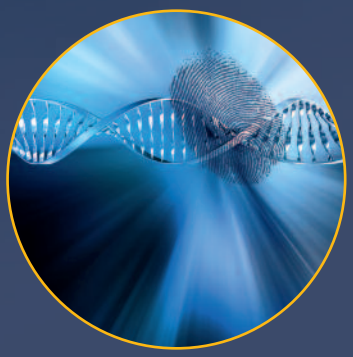
Viability, apoptosis, cytotoxicity, oxidative stress, cell signaling, kinases, epigenetics, real-time analysis, 3D-culture assays, cell metabolism, drug discovery, reporter gene assays



Protein Analysis

Protein expression and purification, live cell labeling and imaging, protein interaction assays, antibody labeling and purification, antibody fragmentation, Western Blotting, ELISA, reagents for mass spectrometry

Genetic Identity



Preprocessing and differential extraction, STR amplification and analysis, DNA isolation, human-specific DNA quantitation, automation for genetic identity

Instruments

- Nucleic Acid Extraction Instruments Maxwell® for molecular diagnostic, research or forensic
- GloMax® Discover and Explorer Multimode Systems for detection of luminescence, fluorescence, absorbance, BRET and FRET
- Nucleic acid and protein quantitation using the Quantus™ Fluorometer

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