

Assays for Drug Discovery & Development



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GloMax® Discover is an advanced multimode plate reader designed to provide optimal performance for Promega reagents with high-performance luminescence, fluorescence, UV-visible absorbance, BRET and FRET, and filtered luminescence. GloMax® Discover can be used as a standalone plate reading instrument or integrated into high-throughput automated workflows. Results are easy to interpret using integrated data analysis software.

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- Cell viability, cytotoxicity and apoptosis assays
- Kinetic measurements
- Cellular metabolism assays
- ELISA
- BRET/FRET analysis

GloMax DISCOVER

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



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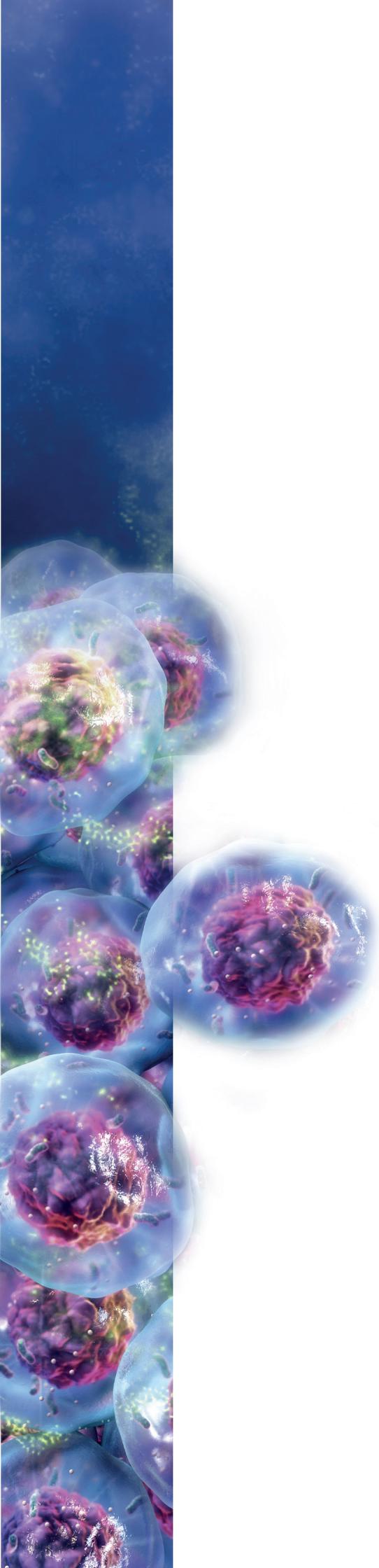


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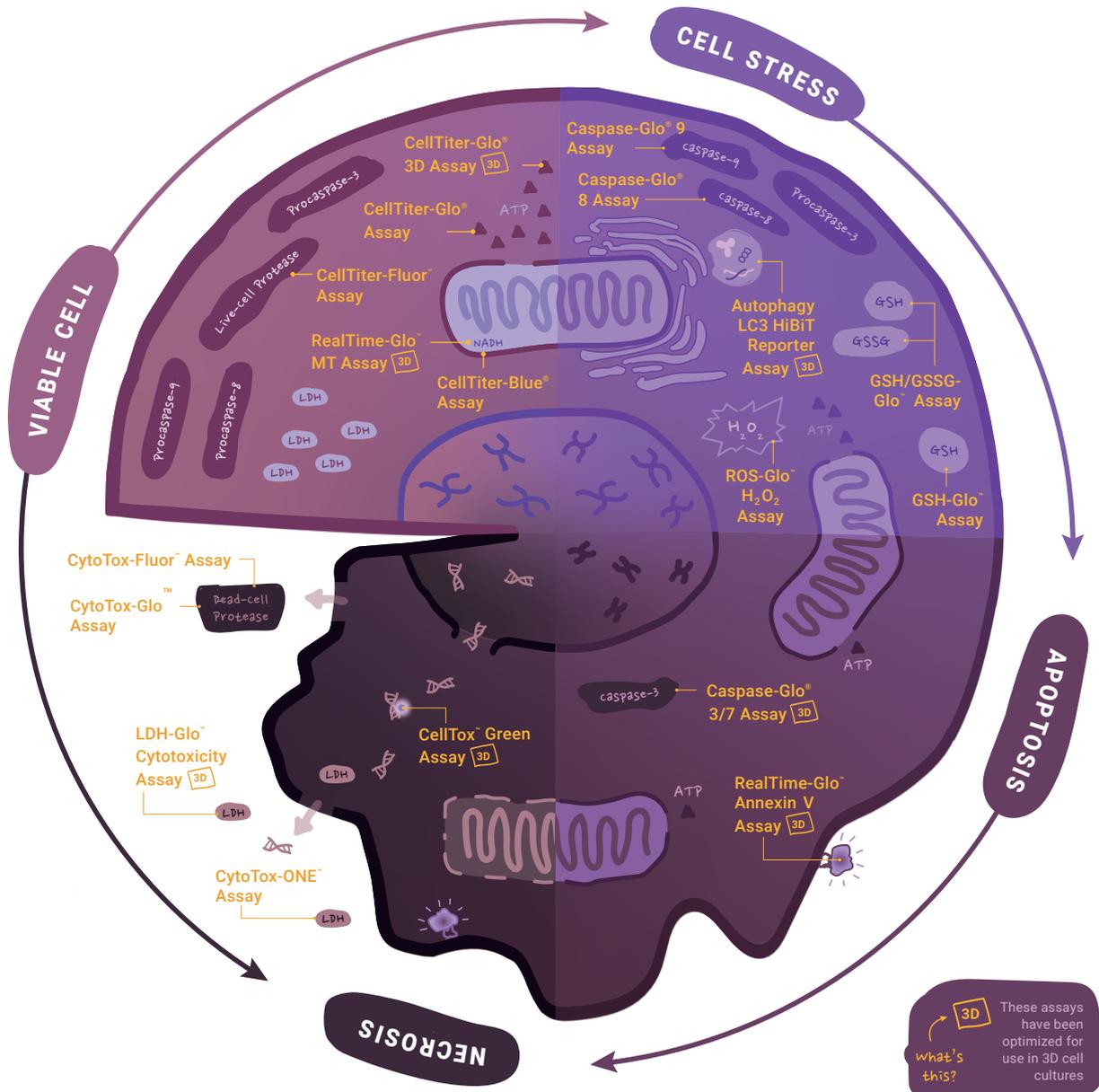
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1. Cell Health

Cell-Based Assays for Measuring Cell Viability, Stress, Apoptosis, and Death

Cell-based assays can be used to measure the number of **live** cells, **dead** cells, and cells undergoing **apoptosis**, **autophagy**, or **oxidative stress**. Our portfolio of cell-based assays uses **luminescence** or **fluorescence** detection to provide you with valuable data about the viability of your cells. Depending on your experimental needs, we offer **endpoint** assays, live-cell **kinetic** assays that allow you to monitor the cell health status in real-time, or assays optimized for **3D** cell cultures.

Explore Our Complete Range of Cell Health Assays:



Cell Health Assays at a Glance

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

CellTiter-Glo[®] Luminescent Cell Viability Assay

Applications

Monitoring of cell viability, proliferation, and cytotoxicity.

For pricing and more information about the product, visit:



Description

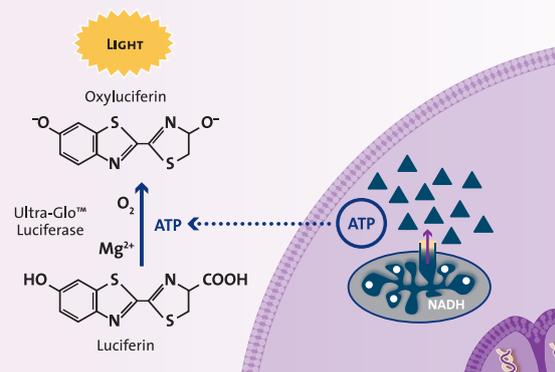
CellTiter-Glo[®] Assay is the most sensitive cell-based assay for detecting cell viability. It is therefore particularly suitable for use in studies on primary cells. Notable features of the assay include the ease and speed with which it can be performed and the reproducibility of the data (Z' factor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

Principle

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

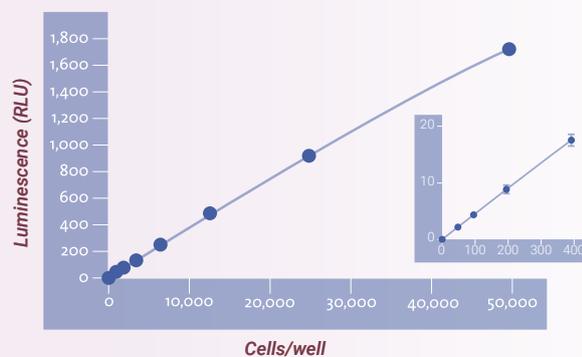
Features

- ✔ **Assay Type:** Luminescent (glow-type; $T_{1/2} > 5$ h)
- ✔ **Marker:** ATP
- ✔ **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)
- ✔ **Robustness:** High Z' factor, reactions are scalable in 96-, 384- and 1536-well formats



CellTiter-Glo[®] Assay Principle

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

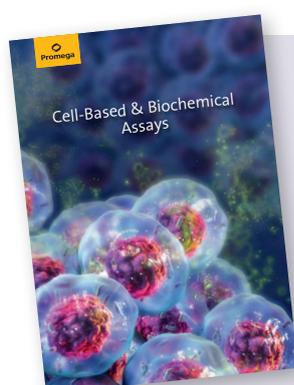


Excellent Linearity and Sensitivity

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 ($R = 0.99$).

Catalog Information

Product	Quantity	Cat. #
CellTiter-Glo® Luminescent Cell Viability Assay	10 ml	G7570
	10x10 ml	G7571
	100 ml	G7572
	10x100 ml	G7573
Related Products		
CellTiter-Glo® Cell Viability 2.0 Assay	10 ml	G9241
	100 ml	G9242
	500 ml	G9243
CellTiter-Glo® 3D Viability Assay	10 ml	G9681
	10x10 ml	G9682
	100 ml	G9683



For more information download our brochure:

The brochure **Cell-based & Biochemical Assays** provides an overview of our comprehensive portfolio for the analysis of complex cellular and biochemical processes.

www.promega.com/CellbasedAssays



Peer-Reviewed Publications

Gupta PB, Onder TT, Jiang G, *et al.* **Identification of selective inhibitors of cancer stem cells by high-throughput screening.** *Cell.* 2009;138(4):645–659. doi:10.1016/j.cell.2009.06.034.

Hahn CK, Ross KN, Warrington IM, *et al.* **Expression-based screening identifies the combination of histone deacetylase inhibitors and retinoids for neuroblastoma differentiation.** *Proc. Natl. Acad. Sci. USA* 2008;105 (28):9751–9756. doi: 10.1073/pnas.0710413105.

Lin H, Lee E, Hestir K, *et al.* **Discovery of a cytokine and its receptor by functional screening of the extracellular proteome.** *Science.* 2008;320(5877):807–811. doi:10.1126/science.1154370.

Cellular Metabolism Assays at a Glance

Assay	Biomarkers	Time required	96-well sensitivity	Plate format	Instrument
Glucose-Glo™ Assay	Glucose	60 min	5 nM with linear range up to 50 μ M, S/B max > 1000	96/384	Luminometer
Lactate-Glo™ Assay	Lactate	60 min	100 nM with linear range up to 200 μ M, S/B max > 200	96/384	Luminometer
Glutamate-Glo™ Assay	Glutamate	60 min	5 nM with linear range up to 50 μ M, S/B max > 100	96/384	Luminometer
Glutamine/ Glutamate-Glo™ Assay	Glutamine	60 min	5 nM with linear range up to 50 μ M, S/B max > 300	96/384	Luminometer
Glycogen-Glo™ Assay	Glycogen	60 min	< 1 μ g with linear range up to 20 μ g, S/B max > 200	96/384	Luminometer
Glucose Uptake-Glo™ Assay	2-deoxyglucose (2DG)	0.5 – 5 h	0.5 to 30 μ M 2DG6P and generates a signal-to-background ratio > 3 with as few as 5,000 cells	96/384	Luminometer
Triglyceride-Glo™ Assay	Triglycerides	< 2 h	1 – 5 pmole/sample with broad linear range 0.1 – 80 μ M	96/384	Luminometer
Glycerol-Glo™ Assay	Glycerol	< 2 h	1 – 5 pmole/sample with broad linear range 0.1 – 80 μ M	96/384	Luminometer
Cholesterol/Cholesterol Ester-Glo™ Assay	Cholesterol and cholesterol esters	< 2 h	1 – 5 pmole/sample with broad linear range 0.1 – 80 μ M	96/384	Luminometer
NAD(P)H-Glo™ Detection System	NADH, NADPH	40 – 60 min	Broad linear range (25 nM – 50 μ M) , S/B max ~ 400	96/384/1536	Luminometer
NAD ⁺ /NADH-Glo™ Assay	NAD ⁺ and NADH	30 – 60 min	Linear range of 10 to 400 nM, S/B max ~ 250	96/384/1536	Luminometer
NADP ⁺ /NADPH-Glo™ Assay	NADP ⁺ /NADPH	30 – 60 min	Linear range of 10 to 400 nM, S/B max ~ 250	96/384/1536	Luminometer
ROS-Glo™ H ₂ O ₂ Assay	H ₂ O ₂	2 h		96/384	Luminometer
GSH/GSSG-Glo™ Assay	Total glutathione and GSSG	45 min		96/384	Luminometer

Glucose Uptake-Glo™ Assay

For pricing and more information about the product, visit:



Applications

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

Description

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

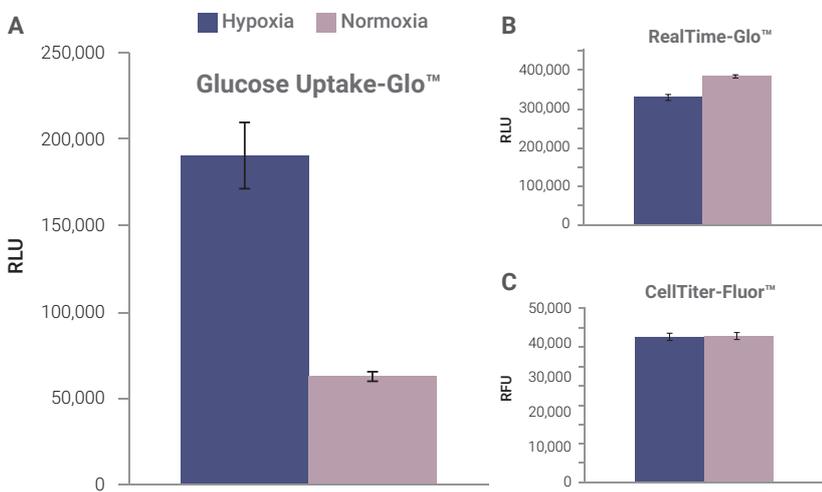
Principle

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



Glucose Uptake-Glo™ Assay Principle

2DG = 2-deoxyglucose / 2DG6P = 2-deoxyglucose-6-phosphate / G6PDH = glucose-6-phosphate-dehydrogenase



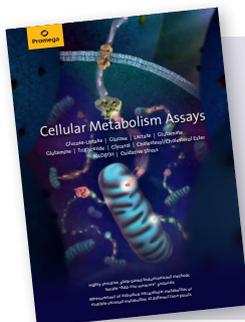
Cancer Model: Glucose Uptake in Hypoxia. When cells are oxygen-starved, the hypoxic conditions shift cellular metabolism from oxidative phosphorylation to glycolysis. This results in increased glucose uptake. MCF7 cells grown under hypoxia (1% oxygen) show an increase in glucose uptake (**Panel A**),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

Features

- ✔ **Assay Type:** Luminescent
- ✔ **Cell Type:** Mammalian cells
- ✔ **Implementation:** Homogeneous, after addition of 2DG, there are no wash steps – all steps are additions
- ✔ **Time Required:** 0.5 – 5 hours
- ✔ **Sensitivity:** 0.5 to 30 μM 2DG6P and generates a signal-to-background ratio > 3 with as few as 5,000 cells
- ✔ **Robustness:** Z' factors > 0.5, scalable for use in 96- and 384-well plates

Catalog Information

Product	Quantity	Cat.#
Glucose Uptake-Glo™ Assay	5 ml	J1341
	10 ml	J1342
	50 ml	J1343
Related Products		
Glucose-Glo™ Assay	5 ml	J6021
	50 ml	J6022
Lactate-Glo™ Assay	5 ml	J5021
	50 ml	J5022
Glutamate-Glo™ Assay	5 ml	J7021
	50 ml	J7022
Glutamine/Glutamate-Glo™ Assay	5 ml	J8021
	50 ml	J8022



For more information download our brochure:

The brochure **Cellular Metabolism Assays** gives you an overview of new non-radioactive technologies that can be used to measure important metabolites.

www.promega.com/CellularMetabolismAssays



Peer-Reviewed Publications

Deshmukh A, Arfuso F, Newsholme P, Dharmarajan A. **Regulation of cancer stem cell metabolism by secreted frizzled-related protein 4 (sFRP4)**. *Cancers*. 2018; 10(2):40. doi:10.3390/cancers10020040.

Tucker DF, Sullivan JT, Mattia KA, *et al.* **Isolation of state-dependent monoclonal antibodies against the 12-transmembrane domain glucose transporter 4 using virus-like particles**. *Proc. Natl. Acad. Sci.* 2018; 115 (22) E4990-E4999. doi:10.1073/pnas.1716788115.

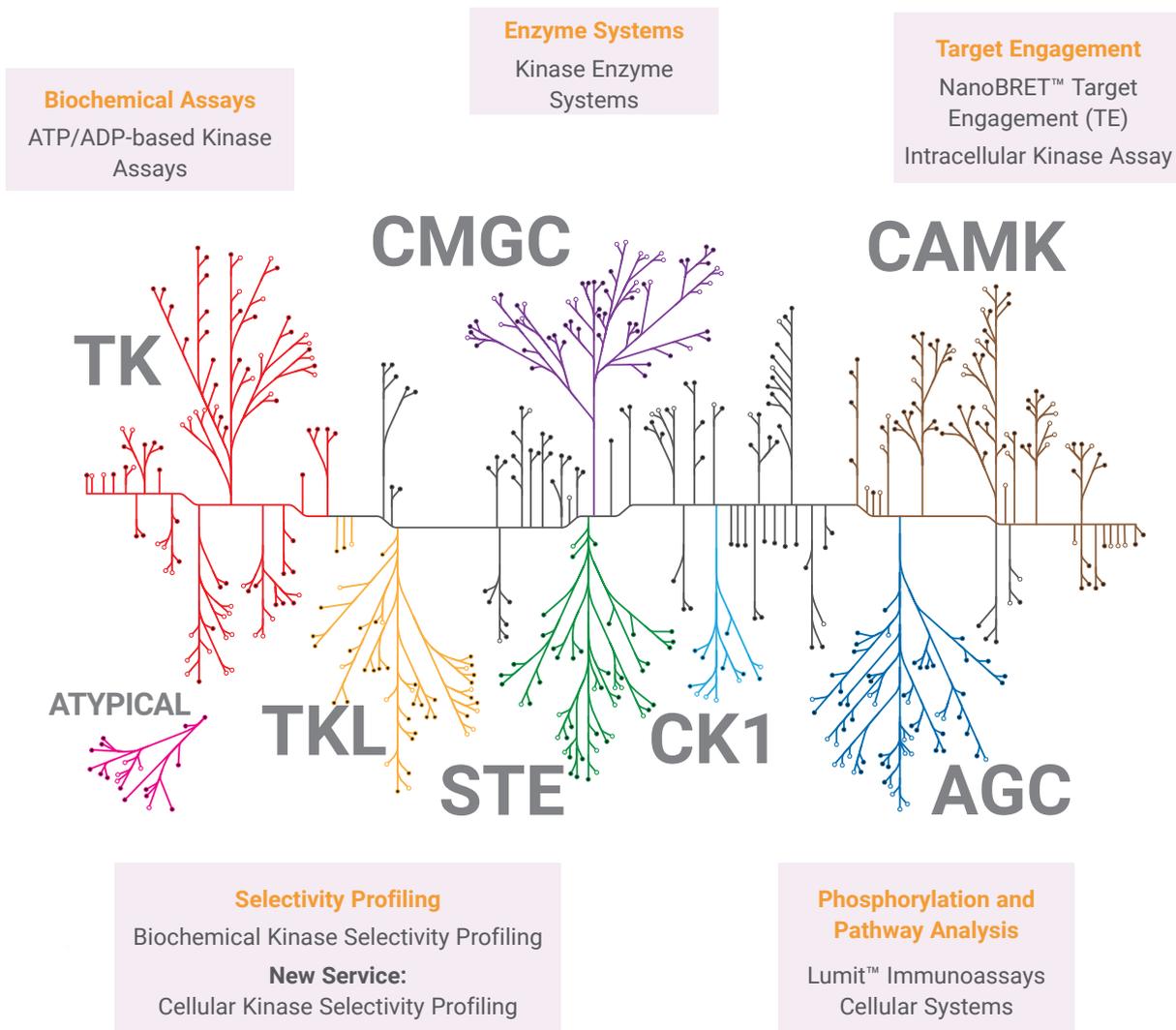
Shimobayashi M, Albert V, Woelnerhanssen B, *et al.* **Insulin resistance causes inflammation in adipose tissue**. *J. Clin. Invest.* 2018; 128(4):1538–1550. doi:10.1172/JCI96139.

3. Kinase Biology

High-Throughput Biochemical and Cellular Assays to Study Kinase Activation and Inhibition

Protein and lipid kinases are involved in a wide variety of pathological conditions. Accordingly, kinase biology constitutes a critical field for drug discovery research. Several **bioluminescent** assays are available to query the ability of compounds to **bind** or **inhibit** kinases in **biochemical** and **cellular** formats, amenable to **high-throughput** or **selectivity profiling**.

Explore Our Complete Range of Kinase Assays:



Assays to Study Kinases at a Glance

Product	Description	Targets	Features
ADP-Glo™ Kinase Assay	Luminescent ADP Accumulation Assay: quantitates ADP from a kinase reaction starting from 0 – 1 mM ATP	Universal use for any kinase or kinase-substrate combination (peptides, proteins, lipids, sugars)	384-well format; high sensitivity: detects 0.2 pmol ADP; highly stable signal for batch plate processing; Z' factor values > 0.7
ADP-Glo™ Max Assay	Luminescent ADP Accumulation Assay: quantitates ADP from a kinase reaction starting from 0 – 5 mM ATP (important for enzymes with high K_m values for ATP and for mode of action studies)	Universal use for any ADP-producing enzyme; ideal for examining non-ATP binding site kinase inhibitors and low-activity ATPases such as drug membrane transporters and heat shock proteins	384-well format; high sensitivity: detect low amount of ADP produced using high ATP concentrations as substrate; highly stable signal for batch plate processing; Z' factor values > 0.7
Kinase-Glo® Luminescent Kinase Assays	Luminescent ATP Depletion Assay: quantitates ATP remaining in solution following a kinase reaction. Linear to 10 μM (Kinase-Glo®); 100 μM (Kinase-Glo® Max), or 500 μM (Kinase-Glo® Plus) ATP	Universal use for any kinase or kinase-substrate combination (peptides, proteins, lipids, sugars)	96-/384-well format; highly stable signal for batch plate processing; Z'-factor values > 0.7
NanoBRET™ Target Engagement (TE) Intracellular Kinase Assay	Quantitates compound binding to full-length kinases in live cells	> 340 kinases	96-/394-well format; modular assay; uses full-length kinases; monitor in-cell residence time
Kinase Enzyme Systems	Easily screen and profile kinase inhibitors	379 kinases and > 100 mutants	Complete Systems: include a recombinant kinase enzyme, a substrate appropriate for the enzyme, a reaction buffer and supplemental reagents
Biochemical Kinase Selectivity Profiling	Fast and simple kinase inhibitor profiling	Kinases from single kinase families are either grouped together or are available as a general panel of kinases representative of the human kinome, or create your own custom Kinase Selectivity Profiling System	Fast turnaround time in a matter of hours; simple reaction assembly and automation-friendly protocol
Cellular Kinase Selectivity Profiling	Quantitates compound binding to kinases in live cells	Broad spectrum of kinases or kinase subfamilies. 192- or 234- full length kinase panel, transiently expressed in HEK293 cells	Percent occupancy of test compound at a single concentration at two technical replicates against each kinase in the panel
Lumit™ Immunoassays Cellular Systems (see also pp. 36)	Sensitive luminescent antibody detection method to examine phosphorylation and study inhibitors that target specific nodes of the major kinase signaling pathways	Can be adapted to detect any phosphoprotein, total protein or small molecule of interest, provided that appropriate primary antibodies are available	Highly sensitive; no washing, or immunoprecipitation; high-throughput compatible

Not sure which product best suits your experiment?

Contact us to submit a question or to request our kinase profiling services.

ADP-Glo™ Kinase Assay

For pricing and more information about the product, visit:



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.

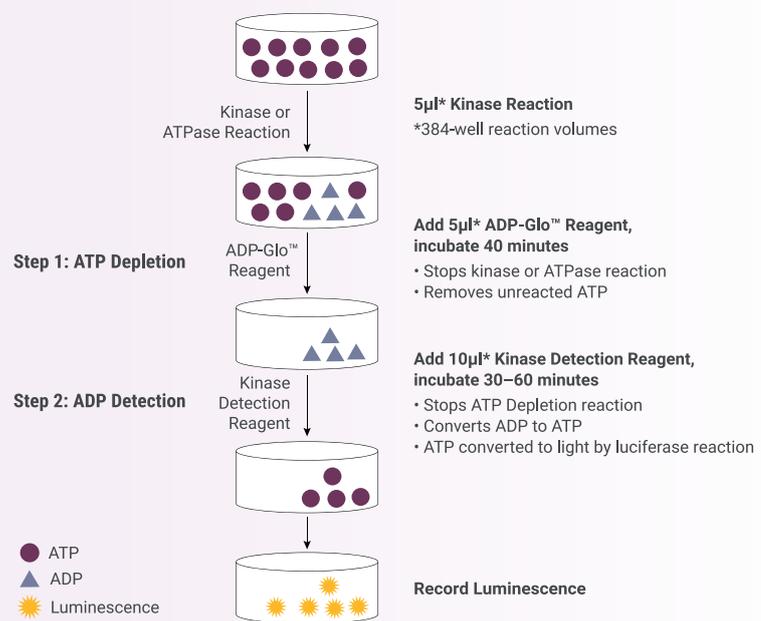
Description

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 non-competitive 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.

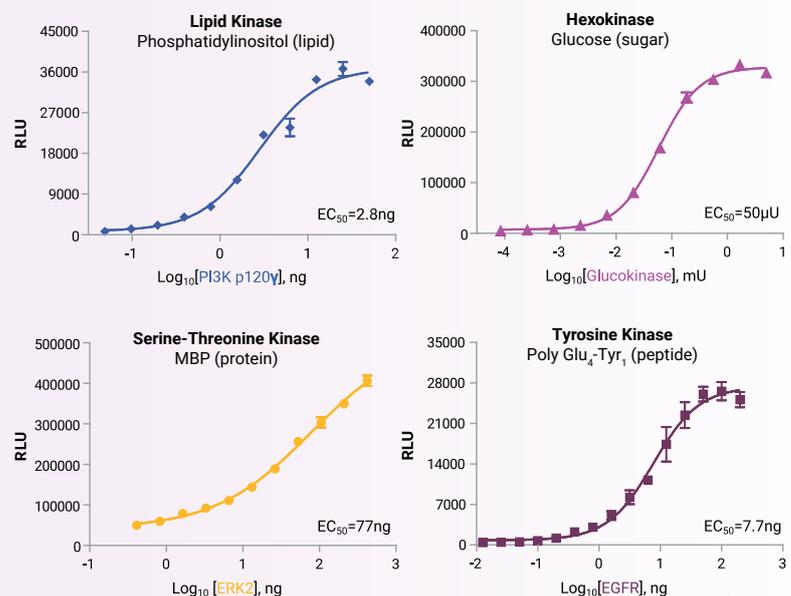
Principle

The ADP-Glo™ Assay is performed in two steps. In the first step, the 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.

Simple Kinase Assay Protocol



Detects Activity of a Range of Kinases



Features

- ✔ **Assay Type:** Luminescent (glow-type)
- ✔ **Sample Material:** Purified kinases
- ✔ **Implementation:** Homogeneous, two-step assay
- ✔ **Time Required:** 60 – 90 minutes
- ✔ **Sensitivity:** Detection even at very low enzyme concentrations: 0.2 pmol ADP
- ✔ **Robustness:** Reactions are scalable in 96-, 384-, 1536-well plates

Catalog Information

Product	Quantity	Cat.#
ADP-Glo™ Kinase Assay	400 assays	V6930
	1,000 assays	V9101
	10,000 assays	V9102
	10 x 10,000 assays	V9103
	100,000 assays	V9104
Related Products		
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® Plus Luminescent Kinase Assay	10 ml	V3771
	10 x 10 ml	V3772
	100 ml	V3773
	10 x 100 ml	V3774
Kinase-Glo® Max Luminescent Kinase Assay	10 ml	V6071
	10 x 10 ml	V6072
	100 ml	V6073
	10 x 100 ml	V6074

Peer-Reviewed Publications

Patel PR, Sun H, Li SQ, *et al.* **Identification of potent Yes1 kinase inhibitors using a library screening approach.** *Bioorg. Med. Chem. Lett.* 2013;23(15):4398-403. doi: 10.1016/j.bmcl.2013.05.072.

Auld DS, Zhang YQ, Southall NT, *et al.* **A basis for reduced chemical library inhibition of firefly luciferase obtained from directed evolution.** *J. Med. Chem.* 2009;52 (5):1450–1458. doi:10.1021/jm8014525.

Zhang JH, Chung TD, Oldenburg KR. **A simple statistical parameter for use in evaluation and validation of high throughput screening assays.** *J. Biomol. Screen.* 1999;4(2):67-73. doi:10.1177/108705719900400206.

NanoBRET™ Target Engagement (TE) Intracellular Kinase Assays

Applications

Measure compound binding at kinase targets in intact cells; target validation; compound optimization.

For pricing and more information about the product, visit:

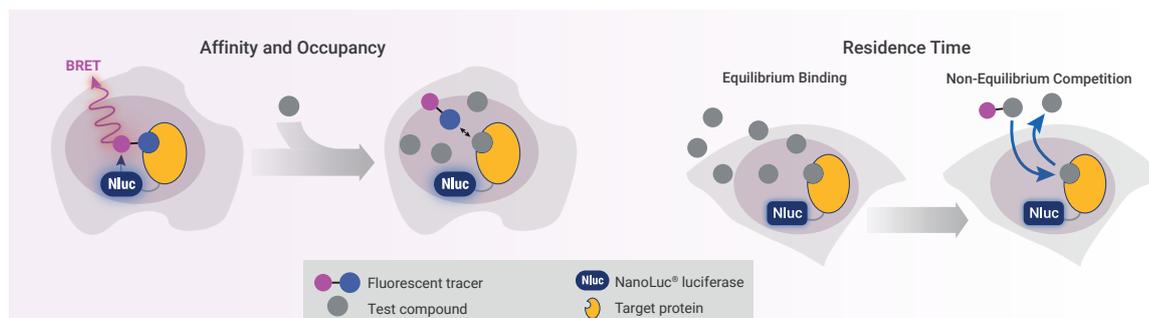


Description

The NanoBRET™ Target Engagement (TE) Intracellular Kinase Assay measures compound binding at select target proteins within intact cells. This target engagement assay is based on the NanoBRET™ System, an energy transfer technique designed to measure molecular proximity in living cells. The NanoBRET™ TE Assays measure the apparent affinity of test compounds by competitive displacement of the NanoBRET™ tracer, reversibly bound to a NanoLuc® luciferase-kinase fusion expressed in cells.

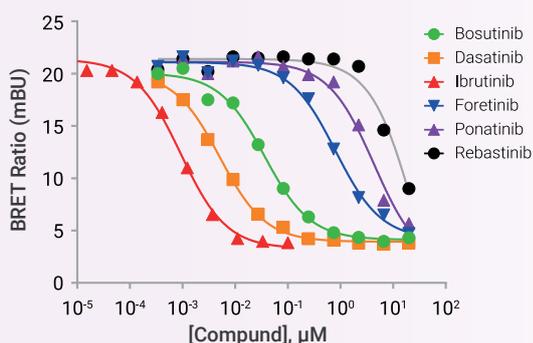
Principle

Using the NanoBRET™ TE Intracellular Kinase Assays, a fixed concentration of tracer is added to cells expressing the desired kinase-NanoLuc® fusion to generate a BRET signal. Kinase-NanoLuc® fusion protein expression is achieved by introducing into cells a mammalian expression vector for the kinase-NanoLuc® fusion. The introduction of competing compounds to cells results in a dose-dependent decrease in NanoBRET™ signal, which allows quantitation of the intracellular affinity of the target protein for the test compound.



Applications of NanoBRET™ TE Kinase Assays

The assays can measure test compound affinity under equilibrium conditions, or residence time under non-equilibrium conditions.



To determine test compound affinity, cells are titrated with varying concentrations of the test compound in the presence of a fixed concentration of NanoBRET™ tracer. Binding of the test compound results in a loss of NanoBRET™ signal between the target protein and the tracer in intact cells.

Features

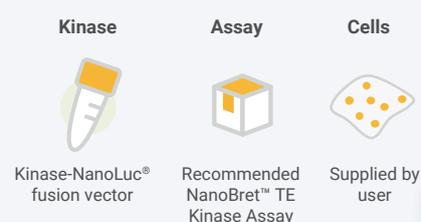
- ✔ **Kinase Target Engagement in Live Cells:** Quantify compound affinity & fractional occupancy for multiple types of kinase inhibitors (I–IV)
- ✔ **Assays for Over 340 Kinases:** Ready-to-use assays span the kinome, readily enabling selectivity analysis. Data for each kinase is provided
- ✔ **Use Full-Length Kinase:** Assays use full-length wild-type kinases. Select mutant kinase or domain-specific kinase assays are available
- ✔ **Multi-Well Plate Format:** Ready-to-use, kinase-specific assays offer a simple workflow and high-throughput compatibility scalable from 96-well to 384-well plates
- ✔ **Assess Residence Time:** Determine the duration of test compound binding to target kinase in live cells

Catalog Information

Product	Quantity	Cat.#
NanoBRET™ TE Intracellular Kinase Assay, K-4	100 assays	N2520
	1,000 assays	N2521
	10,000 assays	N2540
Related Products		
NanoBRET™ TE Intracellular Kinase Assay, K-5	100 assays	N2500
	1,000 assays	N2501
	10,000 assays	N2530
NanoBRET™ TE Intracellular Kinase Assay, K-10	100 assays	N2640
	1,000 assays	N2641
	10,000 assays	N2840
NanoBRET™ TE Intracellular Kinase Assay, K-11	100 assays	N2650
	1,000 assays	N2651
	10,000 assays	N2850

What do you need for a NanoBRET™ TE Kinase Assay?

Promega supplies the individual kinase-NanoLuc® fusion vector and the appropriate NanoBRET™ TE Kinase Assay. You will need to supply the cells and cell culture reagents. The tracers and substrate/inhibitor combinations are also available as standalone products.



For a list of currently available kinase-NanoLuc® fusion vectors or for further advice, please contact us.

Peer-Reviewed Publications

Comess KM, *et al.* **Emerging approaches for the identification of protein targets of small molecules – a practitioners' perspective.** *J. Med. Chem.* 2018;61(19):8504–8535. doi:10.1021/acs.jmedchem.7b01921.

Ferguson FM, Gray NS. **Kinase inhibitors: the road ahead.** *Nat. Rev. Drug. Discov.* 2018;17(5):353–377. doi:10.1038/nrd.2018.21.

Vasta JD, *et al.* **Quantitative, wide-spectrum kinase profiling in live cells for assessing the effect of cellular ATP on target engagement.** *Cell Chem. Biol.* 2018 Feb 15;25(2):206–214.e11. doi:10.1016/j.chembiol.2017.10.010.

4. Biologics

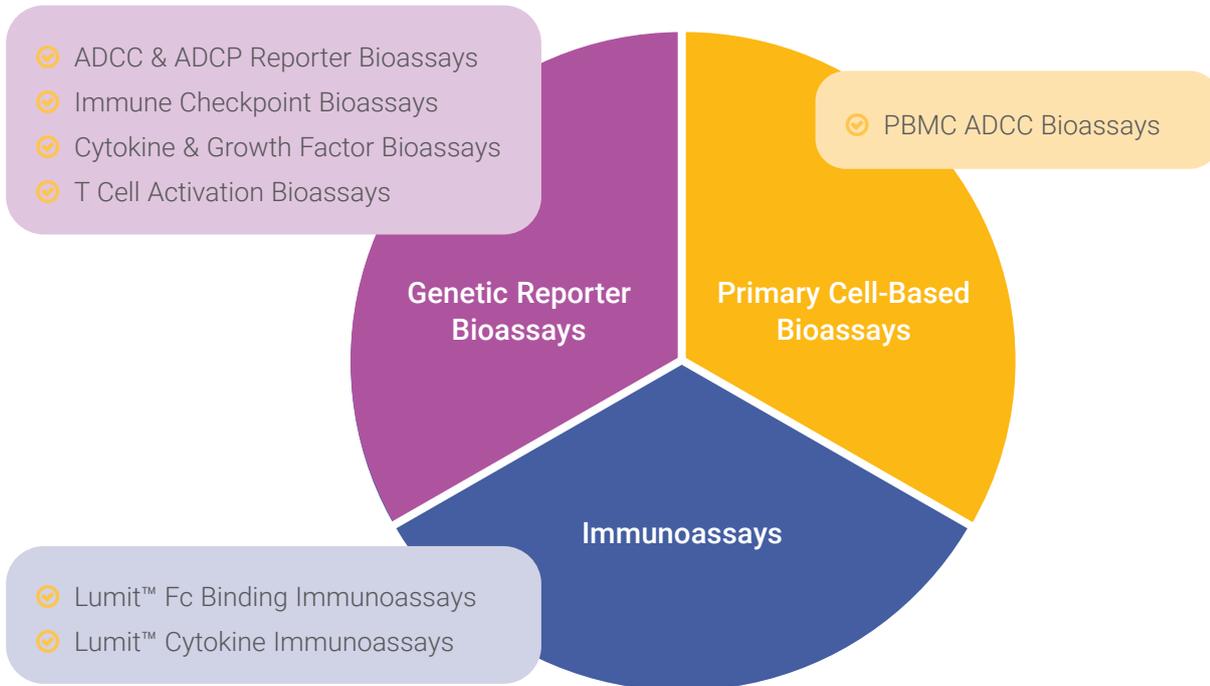
Functional Bioassays for Biologics

Due to their high degree of complexity, the development of biologics drugs requires a comprehensive set of quantitative, accurate, and precise bioanalytical tools. We offer an extensive toolbox of bioassays for the development and characterization of novel **monoclonal antibody-based therapeutics** or other **biologics**. These assays are useful for interrogating a range of biological functions, including **Fc effector activity, immune checkpoint modulation, T-cell activation, and cytokine and growth factor signaling**.

Promega Biologics Benefits:

- ✔ Mechanism of action (MOA)-based bioassays
- ✔ Prequalified according to ICH guidelines
- ✔ Accurate, precise, and reproducible
- ✔ Ideal for QC and lot release of biologics
- ✔ Amenable to high-throughput formats
- ✔ "Thaw-and-use" cell format reduces variability

Explore Our Complete Range of Bioassays:



Genetic Reporter Bioassays

ADCC & ADCP Reporter Bioassays: Measure the potency and stability of antibodies and other biologics that specifically bind and activate Fcγ receptors.

- ✔ Human ADCC Reporter Bioassays for FcγRIIIa (V158 and F158 variants)
- ✔ Human ADCP Reporter Bioassays for FcγRIIa (H131 and R131 variants), FcγRI
- ✔ Mouse ADCC Reporter Bioassays for FcγRIII and FcγRIV

Immune Checkpoint Bioassays: Measure the potency and stability of antibodies and other biologics designed to block the interaction between immune checkpoint receptors and their ligands.

- ✔ Co-inhibitory Receptor Bioassays for PD-1, CTLA-4, TIGIT, TIM-3, VISTA, HVEM, CD226 and LAG-3/MHC-II
- ✔ Co-stimulatory Receptor Bioassays for GITR, OX40, CD40, 4-1BB, CD27, CD28, and ICOS
- ✔ Combination Receptor Bioassays for PD-1+TIGIT, PD-1+CTLA-4, PD-1+LAG-3, PD-1+4-1BB

Cytokine & Growth Factor Bioassays: Quantify and monitor the activity of ligands, as well as antibody-mediated blockade of ligand-receptor binding.

- ✔ VEGF, RANKL, TNFα, TGFβ, IFN-α, IFN-β, and IFN-γ
- ✔ IL-2, IL-6, IL-12, IL-15, IL-17, and IL-23

T Cell Activation Bioassays: Measure the potency and stability of biologics designed to affect T cell responses, such as bispecific antibodies and CAR-T cell therapies.

- ✔ TCR/CD3 (NFAT), or TCR/CD3 (IL2) effector cells
- ✔ TCRαβ-KO (CD4+), TCRαβ-KO (CD8+), and TCRαβ-KO (CD4+, CD8+) cell lines

Primary Cell-Based Bioassays

PBMC ADCC Bioassays: Detect target-cell killing, e.g., triggered by CD8+ T cell activation or macrophage-mediated cell lysis, using ADCC-qualified peripheral blood mononuclear cells (PBMCs) and engineered target cells, stably expressing HiBiT fusion proteins.

Immunoassays

Lumit™ Fc Binding Immunoassays: Measure the interaction of antibody therapeutics with a set of Fc receptors in a simple no-wash competition assay.

- ✔ FcRn, FcγRI, FcγRIIA (H131), FcγRIIA (R131), FcγRIIIA (V158), FcγRIIIA (F158)

Lumit™ Cytokine Immunoassays: Quantitatively measure released cytokines in cell culture samples with a simple no-wash protocol.

- ✔ IL-2, IL-1β, IL-6, IL-4, IL-10, IFN-γ, TNFα

See the **full Lumit™ Immunoassays portfolio** for simple and precise analyte detection at *pp.* 34.

Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC) Assays

Applications

Bioluminescent cell-based bioassays to measure ADCC for therapeutic antibody development.

For pricing and more information about the product, visit:



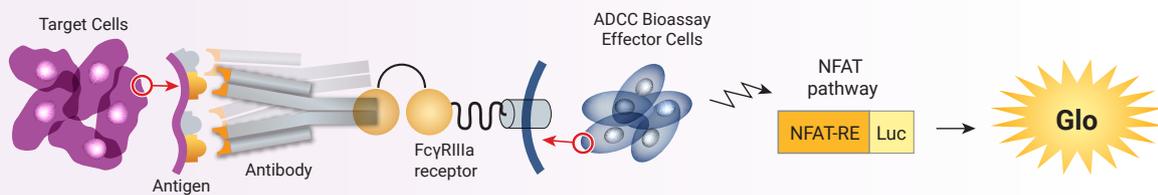
Description

ADCC Reporter Bioassays are biologically relevant, MOA based assays that can be used to measure the potency and stability of antibodies and other biologics that specifically bind and activate Fc receptors. The bioassays overcome the limitations of more labor-intensive and highly variable primary cell assays. The workflow is simple, compatible with 96-well and 384-well plate formats. They are accurate, precise, and stability-indicating and prequalified according to ICH guidelines, making them suitable for all stages of biologic research and development.

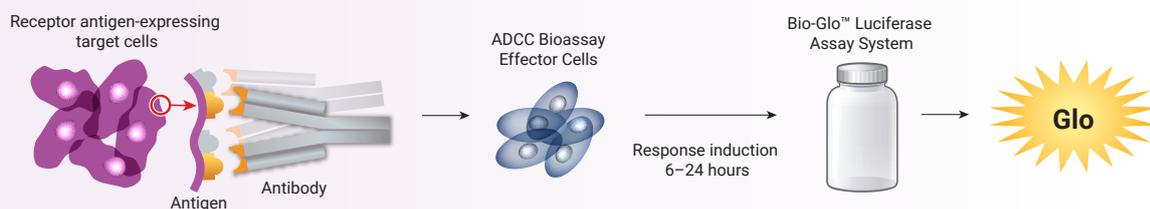
Principle

The ADCC bioassays utilize genetically engineered stable Jurkat Effector Cells to express a specific human or mouse/murine Fc receptor and an NFAT (Nuclear Factor of Activated T cells) response element that drives expression of luciferase. Following engagement with the Fc domain of a relevant antibody bound to target cells, signaling through the specific Fc receptor induces luciferase activity that is easily detected and quantified. By using engineered effector cells instead of primary cells, the assay reproducibility is greatly increased, and the variability is significantly reduced while retaining the ability to discriminate antibodies with varying degrees of Fc effector function.

ADCC Reporter Bioassay Principle



Schematic Protocol



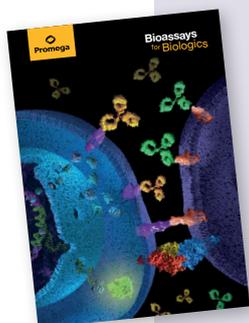
Features

- ✓ Accurate, precise and stability-indicating
- ✓ Prequalified according to ICH guidelines
- ✓ Supplied in convenient “thaw-and-use” format
- ✓ Correlation with primary cell-based ADCC assays
- ✓ Discriminates levels of glycosylation and fucosylation of antibodies

Catalog Information

Product	Components	Cat.#
ADCC Reporter Bioassay, V Variant Complete Kit (Raji)	ADCC Bioassay Effector Cells (1 vial), ADCC Bioassay Target Cells (Raji) (1 vial), Control antibody (5 µg), Low IgG Serum (4 ml), RPMI 1640 Medium (36 ml), Bio-Glo™ Luciferase Assay Buffer (10 ml), Bio-Glo™ Luciferase Assay Substrate (500 µl)	G7015
ADCC Reporter Bioassay, V Variant Core Kit <i>Used with customer-defined Ab and target cells</i>	ADCC Bioassay Effector Cells (1 vial), RPMI 1640 Medium (36 ml), Bio-Glo™ Luciferase Assay Buffer (10 ml), Bio-Glo™ Luciferase Assay Substrate (500 µl)	G7010
ADCC Reporter Bioassay, F Variant Core Kit <i>Used with customer-defined Ab and target cells</i>	ADCC Bioassay Effector Cells (1 vial), RPMI 1640 Medium (36 ml), Bio-Glo™ Luciferase Assay Buffer (10 ml), Bio-Glo™ Luciferase Assay Substrate (500 µl)	G9790

Also available: Mouse ADCC Reporter Bioassays for FcγRIII and FcγRIV.



For more information download our brochure:



The brochure **Bioassays for Biologics** guides you in finding the right bioassay of your biologics depending on its mechanism of action (MOA).

www.promega.com/BioassaysForBiologics

Use **Lumit™ Fc Binding Immunoassays** to complement ADCC Reporter Bioassays for a more complete picture of the MOA of your biologics:

- ✓ Lumit™ FcRn Binding Immunoassay
- ✓ Lumit™ Fcγ RI Binding Immunoassay
- ✓ Lumit™ Fcγ RIIA (H131) Binding Immunoassay
- ✓ Lumit™ Fcγ RIIA (R131) Binding Immunoassay
- ✓ Lumit™ Fcγ RIIIA (V158) Binding Immunoassay
- ✓ Lumit™ Fcγ RIIIA (F158) Binding Immunoassay

For more information see *pp. 34*.

Peer-Reviewed Publications

Xie, I. *et al.* (2020) **Demonstrating analytical similarity of Trastuzumab biosimilar HLX02 to Herceptin® with a panel of sensitive and orthogonal methods including a novel FcγRIIIa affinity chromatography technology.** *BioDrugs* 34, 363–379. doi:10.1007/s40259-020-00407-0.

Kvarnhammar, A.M. *et al.* (2019) **The CTLA-4 x OX40 bispecific antibody ATOR-1015 induces anti-tumor effects through tumor-directed immune activation.** *J. Immunother. Cancer* 7, 103. doi:10.1186/s40425-019-0570-8.

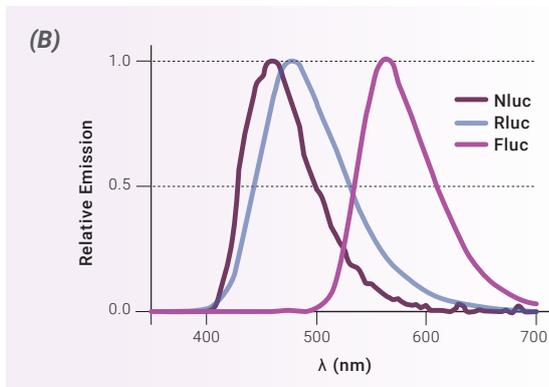
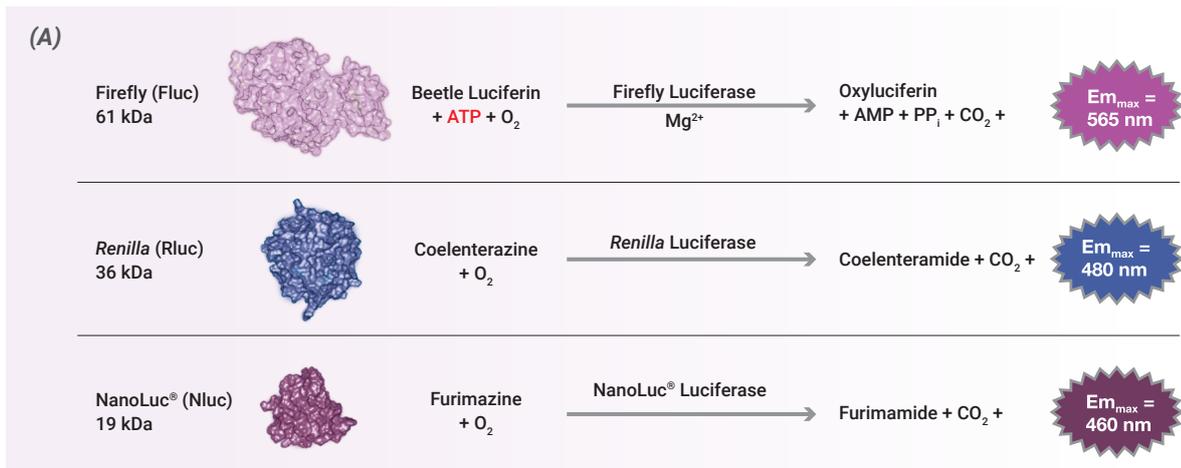
Cerutti, M.L. *et al.* (2019) **Physicochemical and biological characterization of RTX83, a new Rituximab biosimilar.** *BioDrugs* 33, 307–319. doi:10.1007/s40259-019-00349-2.

5. Reporter

Bioluminescent Genetic Reporter Assays

Bioluminescent reporter assays are indispensable tools to study cellular signaling pathways and gene expression levels. They consist of both a luciferase reporter enzyme and a detection reagent that provides the enzyme substrate. When the reporter enzyme and detection reagent are combined, the light emitted is proportional to reporter gene expression levels and is detected using a luminometer. We offer bioluminescent reagents for detecting **firefly**, **Renilla**, and **NanoLuc® luciferases** with multiple detection reagent options for each enzyme to create a reporter assay optimized for your specific experimental goals. The detection reagents have varying signal brightness and stability, include both **lytic or live-cell options**, and can provide either **single- or dual-reporter detection**.

Explore Our Range of Luciferases:



(A) Promega offers three genetically optimized luciferases differing in size, substrate, cofactors, light emission, sensitivity, and linear range.

(B) Emission spectra of NanoLuc® (Nluc), Renilla (Rluc), and firefly (Fluc) luciferases. Emission maxima: Nluc 460 nm, Rluc 480 nm, Fluc 565 nm.

Promega offers a collection of empty reporter vectors for cloning the target of your choice and of prebuilt reporter vectors for studying variations in signaling pathways and cellular stress response.

Luciferase Detection Systems at a Glance

Product	Reporter	Glo/ Flash	Format	Substrate	Half-Life	Signal Stability	Intensity
Single Reporter Systems (lytic, endpoint measurement of single luciferases)							
Nano-Glo® Luciferase Assay System	NanoLuc	Glo	Homogeneous	Furimazine	≥ 2 h	4+	6+
Luciferase Assay System	Firefly	Flash	Requires cell lysate	Beetle luciferin	~ 5–10 min	1+	5+
Bright-Glo™ Luciferase Assay System	Firefly	Glo	Homogeneous	Beetle luciferin	~ 30 min	2+	4+
ONE-Glo™ Luciferase Assay System	Firefly	Glo	Homogeneous	5' - Fluoroluciferin	≥ 45 min	2+	3+
ONE-Glo™ EX Luciferase Assay System	Firefly	Glo	Homogeneous	5' - Fluoroluciferin	≥ 2 h	4+	2+
Steady-Glo® Luciferase Assay System	Firefly	Glo	Homogeneous	Beetle luciferin	> 5 h	5+	1+
Renilla Luciferase Assay System	Renilla	Flash	Requires cell lysate	Coelenterazine	~ 5–10 min	1+	4+
Renilla-Glo™ Luciferase Assay System	Renilla	Glo	Homogeneous	Coelenterazine-h	> 60 min	3+	2+
Dual Reporter Systems (lytic, endpoint measurement of two luciferases per sample)							
Nano-Glo® Dual-Luciferase® Reporter Assay System	NanoLuc®/ Firefly	Glo	Homogeneous	Furimazine/ Beetle luciferin	Nluc: ~ 2 h, Fluc: ~ 2 h	4+	6+/3+
Dual-Luciferase® Reporter Assay System	Firefly/ Renilla	Flash	Requires cell lysate	Beetle luciferin/ Coelenterazine	FLuc: ~ 8 min, RLuc: ~ 8 min	1+	4+/4+
Dual-Glo® Luciferase Assay System	Firefly/ Renilla	Glo	Homogeneous	Beetle luciferin/ Coelenterazine	Fluc: ~ 2 h, Rluc: ~ 2 h	4+	2+/2+
Live-Cell Substrates (non-lytic, kinetic live-cell measurement)							
Nano-Glo® Live Cell Assay System	NanoLuc®, NanoBiT®	Glo	Add-only	Furimazine	~ 2 h	4+	6+
Nano-Glo® Endurazine™ Live Cell Substrate	NanoLuc®, NanoBiT®	Glo	Add-only	Endurazine	~ 40 h	5+	5+
Nano-Glo® Vivazine™ Live Cell Substrate	NanoLuc®, NanoBiT®	Glo	Add-only	Vivazine	~ 20 h	5+	5+
NanoBRET™ Nano-Glo® Detection System	NanoLuc®, NanoBRET™	Glo	Add-only	Furimazine	~ 2 h	4+	6+

Luciferase detection systems are mostly **homogeneous assays**, which are based on the simple “**add-mix-measure**” format. In this format, the detection reagent is directly added to the cell culture medium.

The incubation time between reagent addition and signal detection largely depends on the half-life of the luminescent signal. In the case of **flash-type assays**, the sample is directly measured upon addition of the detection reagent since the signal half-life is in the range of minutes. Flash-type assays require the use of automated injectors.

In contrast, **glow-type assays** (Glo™ Assays) exhibit a prolonged signal half-life – in some cases several hours – which is particularly beneficial for high-throughput applications. Glo™ Assays do not require an injection system.

ONE-Glo™ Luciferase Assay System

Applications

Determination of firefly luciferase activity in studies on gene expression, signal transduction, and mRNA processing.

For pricing and more information about the product, visit:

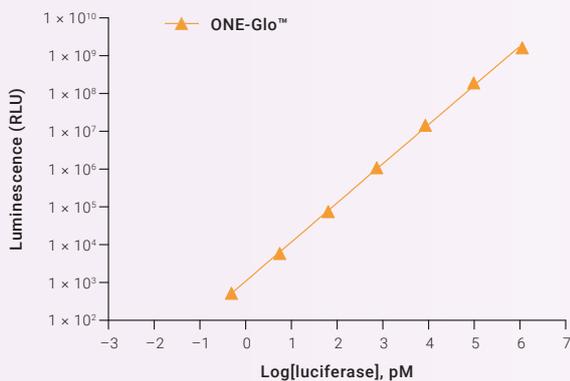


Description

The ONE-Glo™ Luciferase Assay System is based on a modified firefly luciferase substrate conferring an optimized signal intensity and prolonged signal stability when compared to the Bright-Glo™ Luciferase Assay System. Additionally, it exhibits improved tolerance to cell culture media supplements and compounds. The ONE-Glo™ Luciferase Assay System is ideally suited to detect firefly expression within mammalian cells and it is easily adaptable to HT screening and ultra-HT screening approaches.

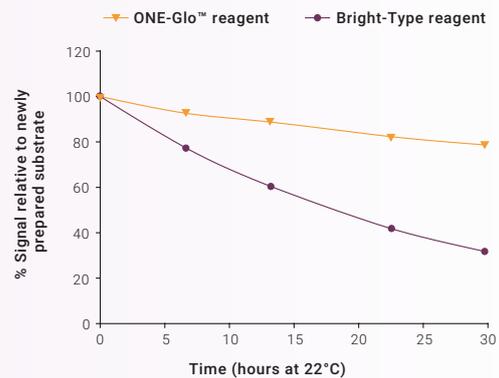
Features

- ✔ **Luciferase:** Firefly: luc, luc+, luc2
- ✔ **Assay Type:** Single assay (glow-type)
- ✔ **Sample Material:** Mammalian cells
- ✔ **Implementation:** Homogeneous, one-step assay
- ✔ **Substrate:** 5'-Fluoroluciferin
- ✔ **Signal Half-Life:** ≥ 45 min
- ✔ **Robustness:** Reactions are scalable in 96-, 384-, 1536-well plates



High Signal Linearity

The ONE-Glo™ Luciferase Assay System yields robust signals over a broad concentration range (~ 7 orders of magnitude).



Improved Reagent Stability

The reagent stability of ONE-Glo™ is considerably higher than that of bright-type reagents of other manufacturers.

Catalog Information

Product	Quantity	Cat.#
ONE-Glo™ Luciferase System	10 ml	E6110
	100 ml	E6120
	1 l	E6130
Related Products		
ONE-Glo™ EX Luciferase System	10 ml	E8110
	100 ml	E8120
	10 x 10 ml	E8130
	10 x 100 ml	E8150
Bright-Glo™ Luciferase Assay System	10 ml	E2610
	100 ml	E2620
	1 l	E2630
Steady-Glo® Luciferase Assay System	10 ml	E2510
	100 ml	E2520
	1 l	E2530



For more information download our brochure:

The brochure **Bioluminescent Reporter Assay** provides an overview of bioluminescent reporter assays and various application examples.

www.promega.com/BioluminescentReporterAssays



Peer-Reviewed Publications

Tada M, Suzuki T, Ishii-Watabe A. **Development and characterization of an anti-rituximab monoclonal antibody panel.** MAbs. 2018;10(3):370–379. doi: 10.1080/19420862.2018.1424610.

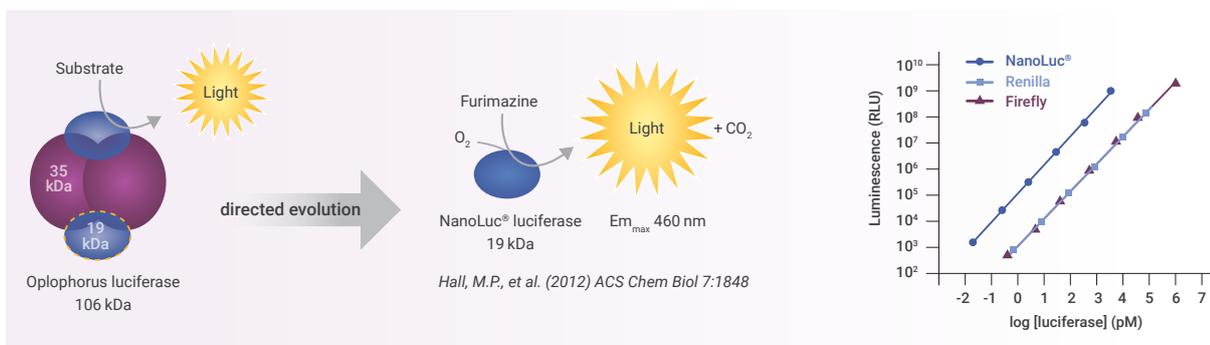
Futami M, Sato K, Miyazaki K, *et al.* **Efficacy and safety of doubly-regulated vaccinia virus in a mouse xenograft model of multiple myeloma.** Mol. Ther. Oncolytics. 2017;6:57–68. doi:10.1016/j.omto.2017.07.001.

Emonet SF, Garidou L, McGavern DB, de la Torre JC. **Generation of recombinant lymphocytic choriomeningitis viruses with trisegmented genomes stably expressing two additional genes of interest.** Proc. Natl. Acad. Sci. USA. 2009;106(9):3473–3478. doi:10.1073/pnas.0900088106.

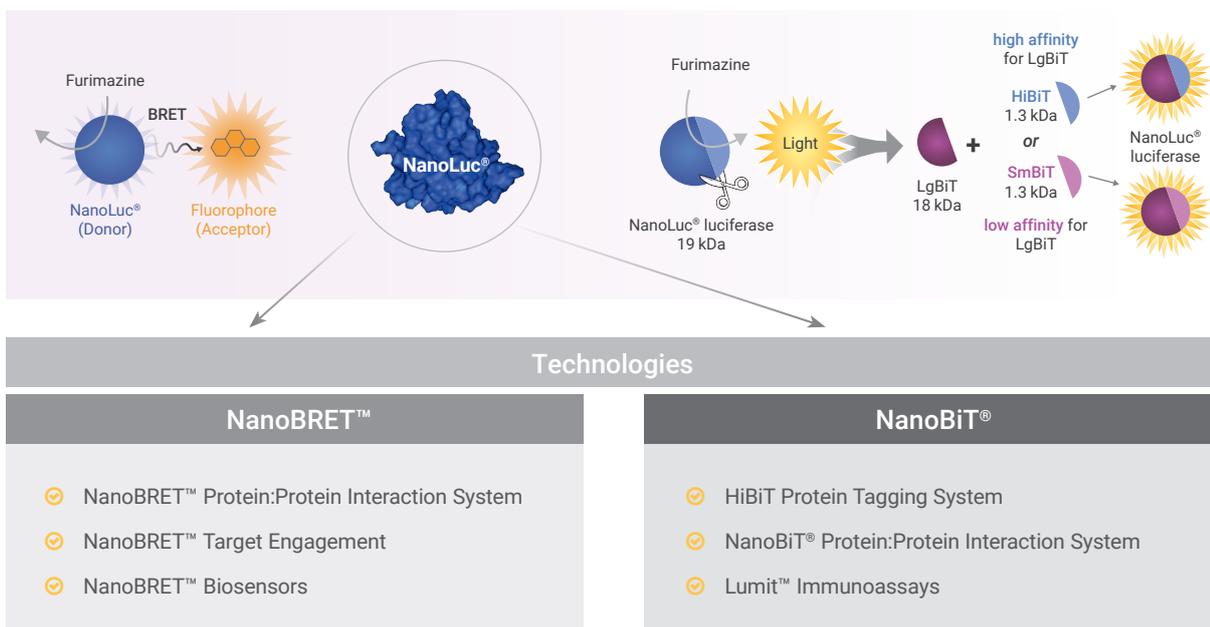
6. Protein Biology & Cellular Responses

NanoLuc® Platform Technologies

NanoLuc® (Nluc) is a genetically evolved luciferase derived from the catalytic subunit of the *Oplophorus* luciferase, isolated from the deep-sea shrimp *Oplophorus gracilirostris*. In combination with its optimized substrate furimazine, NanoLuc® generates an exceptionally **bright signal** making it the ideal reporter for applications that demand small cell numbers, low expression levels, and high sensitivity. When used as a protein reporter, its **small size** (19 kDa) minimizes artificial impact on fusion partner proteins.



Since its development in 2012, NanoLuc® became the cornerstone of the **NanoBRET™** and **NanoBiT®** technology platforms that harbor many powerful technologies to explore protein dynamics at physiologically relevant levels in **live cells**, including **protein interaction, trafficking**, or changes in **protein abundance** (e.g., targeted protein degradation). NanoLuc®-based technologies equip you with a screening-compatible toolbox to understand cellular protein biology in ways that have never been possible before.



Explore the Capabilities of NanoLuc® Technology Platforms

Protein Quantification

The **HiBiT Protein Tagging System** enables fast quantification of proteins in cells, on the cell surface or within the cell culture supernatant with high sensitivity. Proteins of interest are fused to the high-affinity peptide tag HiBiT and detected in a homogeneous add-and-read assay by providing the complementary LgBiT subunit and the luciferase substrate. The various detection reagents available allow for either endpoint or time-course analysis in live cells for up to 72 hours. See also pages 28/29.

Protein:Protein Interaction

The **NanoBRET™ and NanoBiT® Protein:Protein Interaction (PPI) Systems** enable direct analysis of protein interaction dynamics in a live-cell context. Both technologies offer great HTS amenability and thus can be deployed for the identification and development of PPI inhibitors.

The **NanoBRET™ PPI System** utilizes bioluminescence resonance energy transfer (BRET) in a proximity-based assay that detects protein interactions by measuring energy transfer from a luciferase donor to a fluorescent acceptor. The two interacting proteins of interest are fused to either NanoLuc® or the HaloTag®. The HaloTag® protein can be labeled irreversibly with a spectrally optimized chemical fluorophore. For more information visit pages 30/31.

NanoBiT® PPI Assays are intensity-based assays that rely on two optimized split subunits of NanoLuc® luciferase. The interaction of LgBiT and SmBiT fusion proteins is evident from a luminescent signal due to the reconstitution of the functional NanoBiT® luciferase. The low affinity of SmBiT for the LgBiT counterpart ensures high signal specificity and reversibility of the interaction. See also pages 32/33.

Immunodetection

Lumit™ Immunoassays provide a powerful alternative to conventional immunodetection approaches, e.g., ELISA and western blot. These in-solution assays make use of antibodies labeled with the NanoBiT® subunits LgBiT and SmBiT. Analytes can be reliably detected within hours in cell culture supernatants as well as in cell lysates without the need for any washing or transfer steps. Lumit™ Immunoassays are easily adaptable to automation for laboratories processing large numbers of samples. Learn more on pages 34/35.

HiBiT Protein Tagging System

Applications

Quantification of protein expression, stabilization, degradation, and secretion, autophagy, membrane receptor trafficking, monitoring viral entry and release.

Description

The HiBiT Protein Tagging System facilitates the setup of easy-to-quantify and highly sensitive protein assays, monitoring regulated changes in cellular protein abundance. HiBiT simplifies protein tagging in live cells, providing a streamlined, antibody-free detection protocol that requires only a luminometer for signal quantification. With a sensitivity down to the endogenous protein expression level and the convenience of a single-reagent-addition step, HiBiT technology opens a universe of possibilities for researchers studying protein biology.

Principle

HiBiT is an 11 amino acid peptide tag that can be attached to any protein-of-interest (POI) by either inserting the coding sequence of a POI into a HiBiT fusion vector or by CRISPR/Cas9-mediated gene tagging. The detection of HiBiT-tagged proteins is performed by using reagents that contain the optimized 17.6 kDa LargeBiT (LgBiT)-subunit of the NanoLuc[®] luciferase and its substrate furimazine. High-affinity binding of HiBiT to the inactive luciferase subunit LgBiT restores the activity of the highly active luciferase enzyme and enables the quantification of HiBiT-tagged proteins at even low expression levels and with an exceptionally broad linear range.

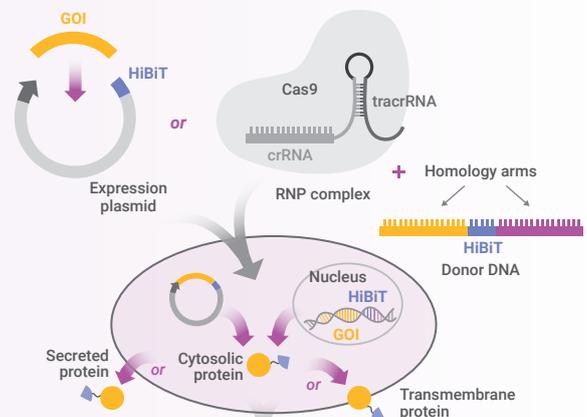
Quantification Possibilities of HiBiT-Tagged Proteins: (A) Real-time kinetic studies by co-expression of the LgBiT subunit in combination with non-lytic Nano-Glo[®] substrates. (B) Quantification of total HiBiT-tagged proteins, or extracellular detection of cell-surface or secreted proteins. (C) Western blot analysis of HiBiT-tagged proteins.

For pricing and more information about the product, visit:



Principle of HiBiT Tagging System

Tag Gene-of-Interest with HiBiT



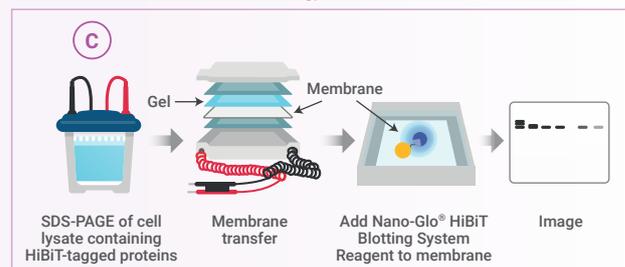
Detect HiBiT-tagged protein



or



or

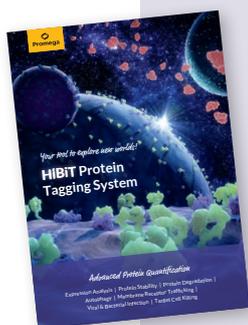


Features

- ✓ **Small Tag Size:** Minimizes interference with native protein function
- ✓ **One-Step Protocol:** No antibodies or washing steps required
- ✓ **Robust:** Large linear detection range < 7 logs
- ✓ **High-Throughput:** 96- or 384-well format

Catalog Information

Product	Quantity	Cat.#
Nano-Glo® HiBiT Lytic Detection System <i>Detection of HiBiT-tagged proteins in cell lysates</i>	10 ml	N3030
	100 ml	N3040
	10 x 100 ml	N3050
Nano-Glo® HiBiT Extracellular Detection System <i>Quantify HiBiT-tagged proteins expressed on the cell surface</i>	10 ml	N2420
	100 ml	N2421
	10 x 100 ml	N2422
Nano-Glo® HiBiT Blotting System <i>Detection of HiBiT-tagged proteins on Western blot membranes</i>	10 ml	E2510
	100 ml	E2520
	10 x 100 ml	E2530
HiBiT Control Protein	20 µg	N3010
Mouse Anti-HiBiT mAb		<i>Please contact us</i>
HiBiT Fusion Vectors		
pBiT3.1 HiBiT MCS Vectors (N-, C-terminal, N-terminal secreted)	20 µg	N2361, N2371, N2381
HiBiT CMV-neo Flexi® Vectors (N-, C-terminal, N-terminal secreted)	20 µg	N2401, N2391, N2411



For more information download our brochure:

Learn more about the possibilities of the HiBiT protein tagging and quantification system.

[www.promega.com/
ProteinTaggingSystem](http://www.promega.com/ProteinTaggingSystem)



To monitor real-time expression levels of HiBiT-tagged proteins in cells use our **Live-Cell Detection Substrates:**

- ✓ Nano-Glo® Live Cell Assay System
- ✓ Nano-Glo® Endurazine™ Substrate
- ✓ Nano-Glo® Vivazine™ Substrate

Peer-Reviewed Publications

Schwinn MK, *et al.* **CRISPR-mediated tagging of endogenous proteins with a luminescent peptide.** ACS Chem. Biol. 2017;13(2):467–474. doi: 10.1021/acschembio.7b00549.

Oh-hashii K, Furuta E, Fujimura K, Hirata Y. **Application of a novel HiBiT peptide tag for monitoring ATF4 protein expression in Neuro2a cells.** Biochem. Biophys. Rep. 2017;12:40–45. doi:10.1016/j.bbrep.2017.08.002.

Sasaki M, *et al.* **Development of a rapid and quantitative method for the analysis of viral entry and release using a NanoLuc luciferase complementation assay.** Virus Res. 2018;243:69–79. doi:10.1016/j.virusres.2017.10.015.

NanoBRET™ Protein:Protein Interaction Assays

Applications

Monitoring protein:protein interaction (PPI) in live cells at physiologically relevant expression levels; kinetic measurements of PPI induction or inhibition; small molecule/off-target screenings; peptide library screenings; validation of *in vitro* data; generation of biosensors.

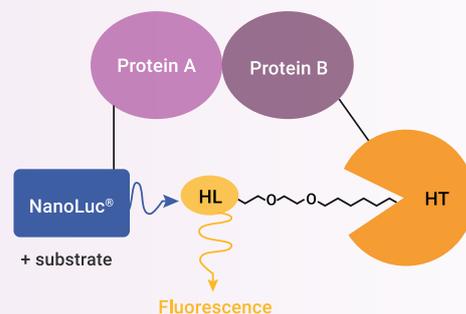
Description

The NanoBRET™ Technology represents a robust, HTS-compatible method for investigating PPIs in living mammalian cells. The method enables dynamic real-time measurements of PPIs at physiologically relevant expression levels and can be applied to monitor both induction and inhibition of a PPI of interest. Assays can be performed in 96- or 384-well formats with low variability and high reproducibility.

Principle

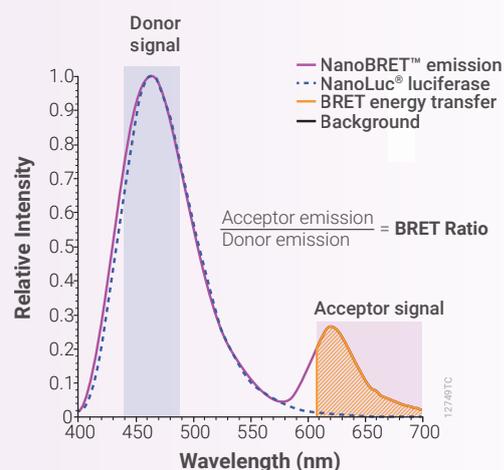
NanoBRET™ Technology is a bioluminescence resonance energy transfer (BRET)-based method using NanoLuc® luciferase as BRET energy donor and HaloTag® protein, labeled with the NanoBRET™ 618 Fluorophore, as energy acceptor to measure the interaction of specific protein pairs. The bright, blue-shifted donor signal and red-shifted acceptor create an optimal spectral overlap, increased signal, and lower background compared to conventional BRET assays. NanoBRET™ PPI Starter Systems provide the vectors required to generate NanoLuc® luciferase and HaloTag® protein fusions with the target proteins of interest.

For pricing and more information about the product, visit:



HL: HaloTag® NanoBRET™ 618 Ligand HT: HaloTag® protein

The large spectral separation of donor (NanoLuc®, Emmax: 460 nm) and acceptor signals (NanoBRET™ 618 fluorophore, Emmax: 618 nm) facilitates an improved signal-to-noise ratio. The signals are recorded in two channels: (1) Donor channel at 460 nm using a bandpass filter, and (2) acceptor channel at 610 nm using a long pass filter. The BRET ratio is determined by dividing the acceptor signal by the donor signal.



Measuring PPIs in living cells with NanoBRET™ Technology requires the expression of the two interacting proteins of interest (A, B) as fusion proteins with either NanoLuc® luciferase or the HaloTag® protein, respectively. If the interaction partners are in close proximity (< 10 nm), the NanoBRET™ 618 fluorophore will be excited by the NanoLuc® luciferase via BRET.

Features

- ✓ **Live-Cell Assay:** Measure protein:protein interactions in their native environment
- ✓ **Physiological Expression Levels:** Study induction and inhibition of protein interactions using full-length proteins expressed at physiologically relevant levels
- ✓ **Robust:** Low assay variability and high reproducibility (high Z' factor)
- ✓ **High-Throughput:** 96- or 384-well format

Catalog Information

Product	Quantity	Cat. #
NanoBRET™ PPI Assay Starter Systems <i>Includes fusion vectors, positive controls, and detection reagent</i>		N1811, N1821
NanoBRET™ Nano-Glo® Detection System <i>Single-time-point or < 2-hour analysis</i>	200 assays	N1661
	1,000 assays	N1662
	10,000 assays	N1663
NanoBRET™ Nano-Glo® Kinetic Detection System <i>Kinetic assays ≥ 2 hours</i>	200 assays	N1571
	1,000 assays	N1572
	10,000 assays	N1573
„Ready-to-use“ NanoBRET™ Assays *		
NanoBRET™ BRD4/Histone H3.3 Interaction Assay	1x	N1830
NanoBRET™ cMyc/MAX Interaction Assay	1x	N1870
NanoBRET™ KRas/BRAF Interaction Assay	1x	N1880

* All ready-to-use NanoBRET™ assays contain, in addition to the optimized and validated vector constructs, the control pair p53/MDM2 and reagents for 200 assays (96-well). A complete list of the ready-to-use NanoBRET™ assays can be found at www.promega.com/nanobret.



For more information download our brochure:

Read the complete guide to investigate the dynamics of protein:protein interactions in live cells with the NanoBRET™ technology.

www.promega.com/NanoBretTechnology-brochure



New: RAS Pathway PPI assay collection

Optimized NanoBRET™ interaction assays for wild-type and common mutants of KRAS/NRAS/HRAS with BRAF/CRAF/ARAF binding partners. Assays are also available to study RAF dimerization and downstream signaling interactions.

For more information please contact us.

Peer-Reviewed Publications

Machleidt T, *et al.* **NanoBRET – A novel BRET platform for the analysis of protein-protein interactions.** ACS Chem. Biol. 2015;10(8):1797–804. doi: 10.1021/acschembio.5b00143.

Demont EH, *et al.* **1,3-Dimethyl benzimidazolones are potent, selective inhibitors of the BRPF1 bromodomain.** ACS MED. Chem. Lett. 2014;5(11):1190–1195. doi:10.1021/ml5002932

White CW, *et al.* **Using NanoBRET and CRISPR/Cas9 to monitor proximity to a genome-edited protein in real-time.** Sci. Rep. 2017;7(1):3187. doi: 10.1038/s41598-017-03486-2.

NanoBiT[®] Protein:Protein Interaction Assays

For pricing and more information about the product, visit:



Applications

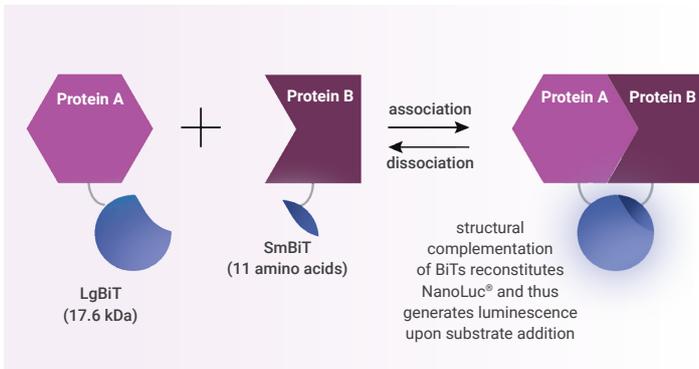
Monitoring protein:protein interaction (PPI) in live cells at physiologically relevant expression levels; kinetic measurements of PPI induction or inhibition; small molecule/off-target screenings; peptide library screenings; validation of *in vitro* data; generation of biosensors.

Description

The NanoLuc[®] Binary Technology (NanoBiT[®]) is a NanoLuc[®] complementation assay designed for the sensitive detection of PPIs in living cells. This technology enables setting up real-time or endpoint PPI assays. In contrast to other available complementation assays, NanoBiT[®] permits the investigation of PPIs at physiological relevant expression levels. Unlike related approaches based on fragmented fluorescent proteins, NanoBiT[®] complementation is fully reversible and thus allows the measurement of both, protein association and dissociation, over a broad dynamic range.

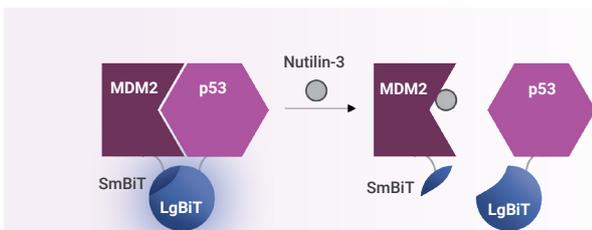
Principle

The NanoBiT[®] system is based on two engineered fragments – Large BiT (LgBiT) and Small BiT (SmBiT) – of the intensely bright NanoLuc[®] luciferase. The BiT fragments have been independently optimized for stability and minimal self-association and are expressed as fusions to target proteins of interest. Interaction of the target proteins facilitates BiT fragment complementation yielding a bright, luminescent enzyme.



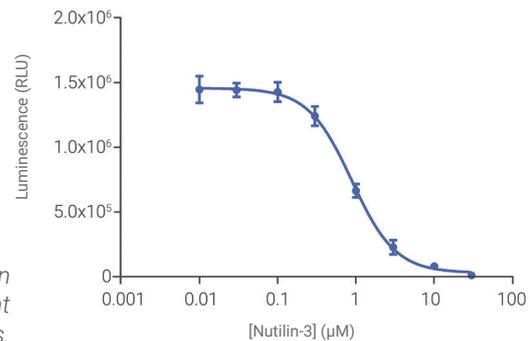
Principle of NanoBiT[®] for Studying PPI Dynamics Inside Living Cells

Structural complementation of the two optimized BiT fragments following interaction of protein A and B. For a PPI assay, protein A and protein B are expressed as fusion proteins to LgBiT and SmBiT, respectively. Interaction of fusion partners leads to structural complementation of the BiT fragments, generating a functional enzyme with a bright, luminescent signal.



Inhibition of p53/MDM2 PPI by Nutlin-3

Dose-dependent inhibition of p53:MDM2 interaction by Nutlin-3. Performed in HEK293 cells after transient transfection of LgBiT-p53 and SmBiT-MDM2 fusion constructs.



Features

- ✔ **Live-Cell Assay:** Measure real-time protein:protein interactions in their native environment
- ✔ **Small Tag Size:** Minimizes interference with native protein function
- ✔ **Highly Dynamic System:** Reversible protein complementation reporter to study protein association and disassociation
- ✔ **High-Throughput:** 96-, 384, 1536-well format

Catalog Information

Product	Quantity	Cat. #
NanoBiT® PPI Assay Starter Systems <i>Includes fusion vectors, positive control PPI pair, negative control vector, and Nano-Glo® Live Cell Assay System</i>	200 assays	N2014, N2015
	200 assays	N2011
Nano-Glo® Live Cell Assay System	1,000 assays	N2012
	10,000 assays	N2013
NanoBiT® PPI Control Pair (FKBP, FRB)	20 µg (each)	N2016



For more information download our brochure:

Download the complete guide to measure the dynamics of protein:protein interactions in live cells with the NanoBiT® technology.

www.promega.com/NanoBiTTechnology-brochure



Peer-Reviewed Publications

Dixon A *et al.* **NanoLuc complementation reporter optimized for accurate measurement of protein interactions in cells.** ACS Chem. Biol. 2015;11(2):400–408. doi:10.1021/acscchembio.5b00753.

Shintani Y, *et al.* **β-Arrestin1 and 2 differentially regulate PACAP-induced PAC1 receptor signaling and trafficking.** PLoS One. 2018;13(5). doi:10.1371/journal.pone.0196946.

Koushyar S, *et al.* **The prohibitin-repressive interaction with E2F1 is rapidly inhibited by androgen signalling in prostate cancer cells.** Oncogenesis. 2017;6(5):400–408. doi:10.1038/oncsis.2017.32.

Lumit™ Immunoassays

Applications

Analyte quantification and interaction studies; competitive binding studies and drug screening for proteins and small molecules; measurement of signaling pathway activation.

Description

Detection and quantification of analytes is often performed with time-consuming multi-step methods such as western blotting or ELISA. Lumit™ Immunoassays are a simple and faster alternative to run homogeneous immunoassays in multi-well formats. The bioluminescent assays are highly specific and more reliable by eliminating the variability associated with multiple wash steps.

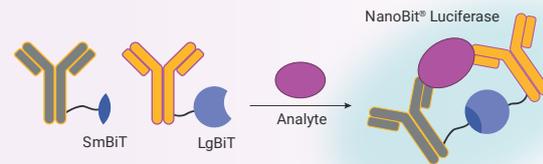
Principle

Lumit™ Immunoassays are based on NanoLuc® Binary Technology (NanoBiT®). In Lumit™ Immunoassays, antibodies are chemically labeled with the small and large subunits of NanoLuc® luciferase, known as SmBiT and LgBiT, respectively. Direct or indirect binding to the analyte yields spatial proximity of the labeled antibodies, allowing SmBiT and LgBiT to form an active NanoBiT® luciferase. In the presence of its substrate furimazine a luminescent signal can be detected directly proportional to the amount of the analyte present in the sample.

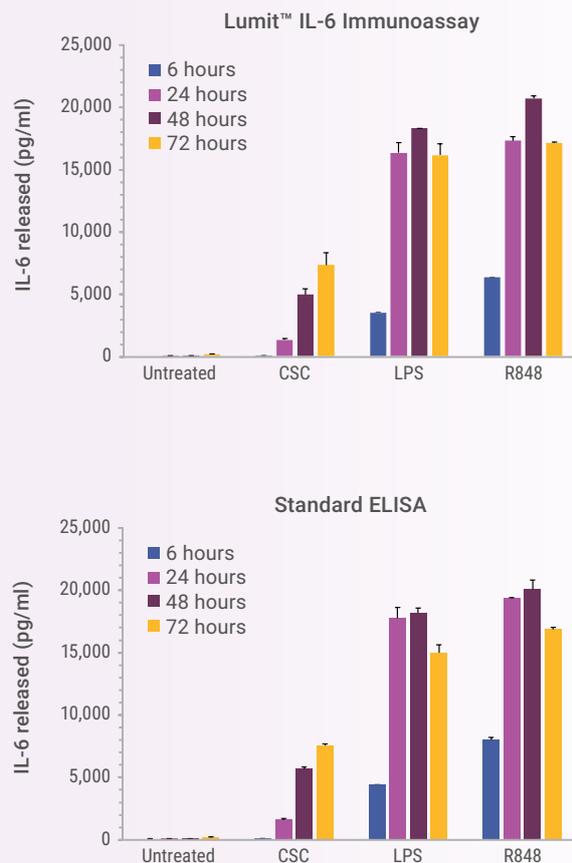
Features

- ✔ **Fast One-Step Immunoassays:** Simple add-and-read protocols; no washing, blocking, or immobilization
- ✔ **Large Dynamic Range:** Reduce sample dilutions needed with assays that are linear over 4 logs
- ✔ **Perform Directly on Cell Culture Samples:** Assay cell supernatants directly on cell plates – no transfer step required
- ✔ **Scalable and High-Throughput:** Scalable from 96- to 384-well formats.

Principle of Lumit™ Immunoassays



Compared Data of Lumit™ vs. ELISA IL-6 Quantification

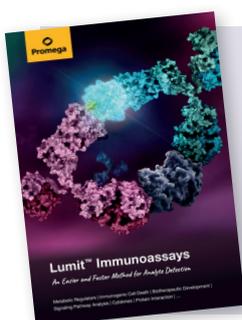


The Lumit™ IL-6 (Human) Immunoassay (upper panel) and standard ELISA (lower panel) were used to quantify IL-6 release from PBMCs. The results are similar, while the Lumit™ Assay was completed in one fifth the time of the ELISA, with no transfer, wash steps or plate washer needed.

Product Overview

Lumit™ Immunoassays		
Pre-Built	Pre-Validated	Build-Your-Own
<p>Cytokines</p> <ul style="list-style-type: none"> • IL-2 • IL-1β • IL-6 • IL-4 • IL-10 • IFN-γ • TNFα 	<p>DAMP</p> <ul style="list-style-type: none"> • HMGB1 	<p>Labeling & Detection</p> <ul style="list-style-type: none"> • Labeling Kit • Labeled Antibodies <ul style="list-style-type: none"> » anti-His » anti-GST » anti-FLAG® » anti-human IgG » anti-mouse IgG » anti-rabbit IgG » anti goat IgG • Labeled Streptavidine • Detection Reagents
<p>Fc Receptor Binding</p> <ul style="list-style-type: none"> • FcRn • FcγRI • FcγRIIA (H131) • FcγRIIA (R131) • FcγRIIIA (V158) • FcγRIIIA (F158) 	<p>Metabolic Regulators</p> <ul style="list-style-type: none"> • Insulin • Glucagon 	
	<p>SARS-CoV-2</p> <ul style="list-style-type: none"> • Spike RBD:hACE2 Interaction • Spike RBD Mutants 	
	<p>Cellular Systems</p> <ul style="list-style-type: none"> • AKT • BCL6 • BTK • c-Jun • CREB • ERK1 • Estrogen Receptor • IκBα • JNK • STAT1/2/3 • Smad1 • 4E-BP1 • β-catenin • p65 • RB • Smad2 	

- ✓ **Pre-Built Assays:** Ready-to-use immunoassays with pre-labeled antibodies are available for direct detection of cytokines, HMGB1, insulin, glucagon, Fc receptor, or SARS-CoV-2 Spike binding.
- ✓ **Pre-Validated Assays:** Monitor cellular signaling pathway activation through specific nodes of phosphorylation with customer-provided primary antibodies. A full list of pre-validated phosphorylation targets using commercially available primary antibodies can be sent by request. Applications notes containing protocols, example data, and ordering information for the primary antibodies are available on our website.
- ✓ **Build-Your-Own Assays:** Perform labeling of antibodies with the Lumit™ Immunoassay Labeling Kit and Detection Reagents to **build your own Lumit™ Immunoassay for additional targets.**



For more information download our brochure:

Discover the versatile possibilities of Lumit™ technology for protein detection and analyte binding.

www.promega.com/Lumit-brochure



7. Protein & Antibody Characterization

Proteases for Mass Spectrometry

Mass spectrometry (MS) is a powerful analytical tool used for proteome-wide analysis and characterization of proteins from a variety of organisms and cell types.

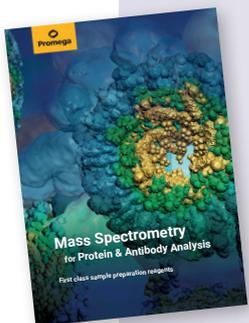
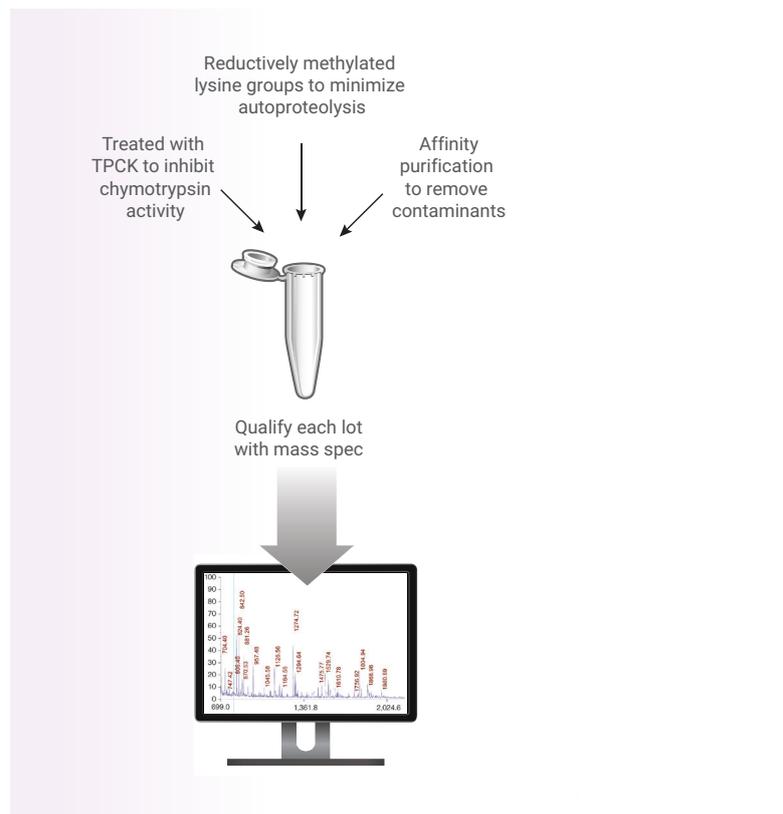
Recent advances in mass spectrometry provide tools for:

- ✔ Protein identification
- ✔ Protein characterization
- ✔ Relative and absolute quantitation
- ✔ The study of post-translational modifications
- ✔ Protein:protein interactions

Protein analysts worldwide rely on the first-class and consistent product quality of Promega proteases, including trypsin and alternative proteases, as evidenced by thousands of references in papers over the last 20 years. Our mass spectrometry products are used for many proteomics research and drug development applications, including identification and characterization of biotherapeutic antibodies and evaluation of post-translational modifications that may affect antibody efficacy or stability.

Promega has the tools, expert staff, and state-of-the-art facilities to support complete custom solutions for protein analysis. The flexible manufacturing enables us to offer customized product sizes, format, and packaging to meet your needs.

Trypsin is the first choice when it comes to protein cleavage in preparing sample for mass spec- and HPLC-based analysis. The high quality of Promega's trypsin is achieved by reductive methylation of lysine residues, chymotrypsin inactivation (treatment with TPCK) and subsequent affinity purification. The consistent quality from lot-to-lot is achieved through stringent production standards and quality controls.



For more information download our brochure:



The brochure **Mass Spectrometry for Protein & Antibody Analysis** will help you find the perfect solutions for your protein analysis.

www.promega.com/MassSpec-brochure

Trypsin Platinum, Mass Spectrometry Grade

Application

Recombinant trypsin for most accurate biotherapeutic protein characterization and specialized proteomics applications.

Description

Trypsin Platinum is designed for analytical scientists looking for the most accurate and efficient protein characterization with mass spectrometry and RP-HPLC-UV. Elimination of non-specific cleavage activity commonly present in the existing proteomics and MS grade trypsins, superior autoproteolytic resistance, and improved proteolytic efficiency makes Trypsin Platinum an ideal tool for the biotherapeutic protein characterization.

Principle

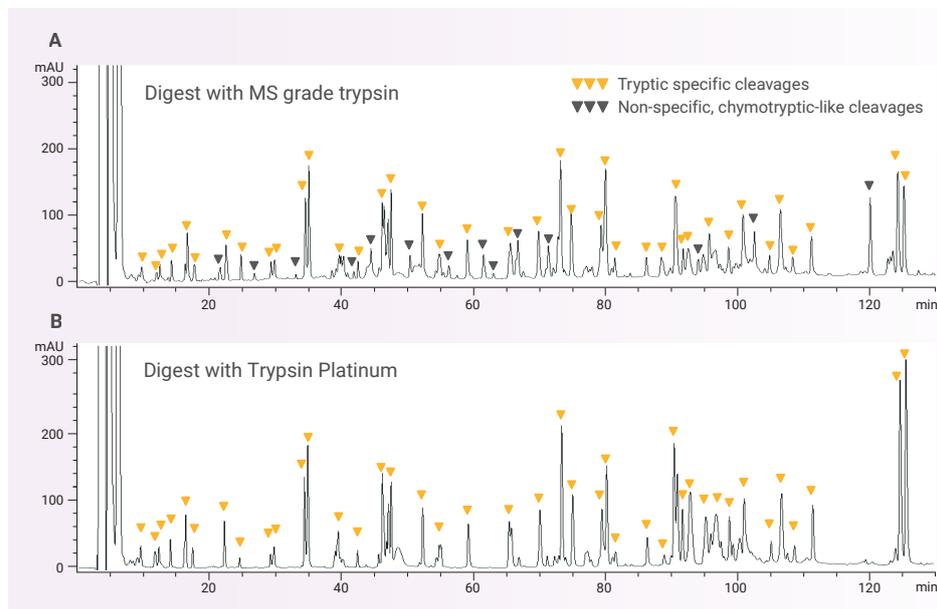
Prior to digestion a protein is denatured, reduced and alkylated. We recommend GuHCl as a protein denaturing agent in the sample preparation procedure. Trypsin Platinum is then added, and a protein is digested overnight at 37°C. To achieve the most efficient digestion, 1:10 Trypsin Platinum:protein ratio is recommended. To digest particularly proteolytically resistant domains or sites, 1:3 ratio can be used. The resulting peptides are desalted and analyzed by mass spectrometry.

For pricing and more information about the product, visit:



Features

- ▼ **Outstanding Cleavage Specificity:** Free of detectable non-specific cleavage activity even at 1:1 trypsin:protein ratio and extended digestion period
- ▼ **Superior Autoproteolytic Resistance:** Autoproteolysis is maximally suppressed to maintain high trypsin activity and avoid interference of tryptic autoproteolytic peptides with peptide analysis
- ▼ **Enhanced Proteolytic Activity:** efficient digestion of proteolytically resistant cleavage sites
- ▼ **Free of Contaminating Proteins of Animal Origin**
- ▼ **Superior Purity and Lot-to-Lot Reproducibility:** Tightly regulated production with rigorous quality control assures the highest purity and stability



Trypsin Platinum (Panel B) is free of non-specific cleavage activity compared to MS grade trypsin (Panel A). Panitumumab (Vectibix®) antibody used as a model protein substrate was digested with MS grade trypsin and Trypsin Platinum. The digestion reactions used a 1:10 trypsin:protein ratio. The digests were analyzed with RP-HPLC-UV.

ProAlanase, Mass Spectrometry Grade

Application

Site-specific endoprotease that targets proline and alanine for protein analysis/characterization, *de novo* sequencing peptide mapping, disulfide bond mapping, post-translational modifications (PTMs) analysis of proteins such as histones, paleoproteomics.

For pricing and more information about the product, visit:



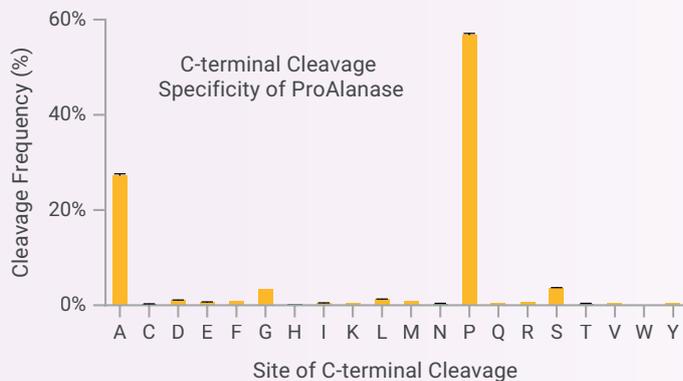
Description and Principle

ProAlanase is an endoprotease that preferentially cleaves proteins on the C-terminal side of proline and, to a lesser extent, alanine amino acids. Isolated and purified from the fungus *Aspergillus niger*, ProAlanase is active at acidic pH (~1.0–5.5), unlike most proteases used in bottom-up proteomics, which require slightly basic pH (~7.0–9.0).

Utilizing alternative proteases helps to provide complete sequence coverage and to identify all PTMs. However, like Trypsin, alternative proteases such as Lys-C, Asp-N, Glu-C & Arg-C also cleave at charged residues, introducing bias to regions within proteins that are digested. Use of ProAlanase prevents this bias because it cleaves at unique, non-charged sites in the proteome.

Features and Benefits

- ✔ **Unique Cleavage Profile:** Cleavage C-terminal to proline and alanine residues can increase protein sequence coverage as well as PTM identifications by cleaving at unique sites in the proteome not targeted with conventional proteases.
- ✔ **Digestion at Low pH:** Activity at acidic pH (~1.0–5.5) minimizes disulfide bond scrambling and other artificial non-enzymatic PTMs like deamidation. Additional denaturants are typically not needed at this low pH.
- ✔ **Fast Digestion:** Digest samples in 1 to 2 hours rather than the typical 4 hours to overnight.



Human K562 extract was digested with ProAlanase at pH 1.5 for 2 hours at 37°C using a 1:100 enzyme:substrate ratio. Peptides were analyzed by LC-MS/MS on a Q-Exactive Plus. Data were searched using Byonic™ software with no enzyme specified.

Catalog Information

Product	Quantity	Cat.#
Trypsin		
Trypsin Platinum	100 µg	VA9000
Trypsin Gold, Mass Spec Grade	100 µg	V5280
Sequencing Grade Modified Trypsin	100 µg, 100 µg (5 x 20 µg)	V5117, V5111
Immobilized Trypsin	2 ml	V9012
Trypsin/Lys-C Mix, Mass Spec Grade	20 µg, 100 µg, 100 µg (5 x 20 µg)	V5071, V5072, V5073
Rapid Digestion – Trypsin	100 µg	VA1060
Rapid Digestion – Trypsin/Lys-C	100 µg	VA1061
Antibody Characterization/Fragmentation		
AccuMAP™ Low pH Protein Digestion Kit	10 reactions, 100 reactions	VA1040, VA1050
IdeS Protease	5,000 u, 25,000 u	V7511, V7515
IdeZ Protease	5,000 u, 25,000 u	V8341, V8345
ISOQUANT® Isoaspartate Detection Kit	100 reactions	MA1010
Specific Alternative Proteases		
Arg-C, Sequencing Grade	10 µg	V1881
Asp-N, Sequencing Grade	2 µg	V1621
Glu-C, Sequencing Grade	50 µg (5 x 10 µg)	V1651
Lys-C, Mass Spec Grade	20 µg	VA1170
rAsp-N, Mass Spec Grade	10 µg	VA1160
rLys-C, Mass Spec Grade	15 µg	V1671
ProAlanase, Mass Spec Grade	5 µg, 15 µg	VA2161, VA2171
Glycosidases		
PNGase F	500 units (10 u/µl)	V4831
Endo H	10,000 units (500 u/µl), 50,000 units (500 u/µl)	V4871, V4875
Surfactants		
ProteaseMAX™ Surfactant, Trypsin Enhancer	1 mg, 5 mg (5 x 1 mg)	V2071, V2072
SoluMAX™ Surfactant	5 mg	Please contact us
Protein Extracts and Peptide Mix for LC/MS Monitoring		More on www.promega.com

Peer-Reviewed Publications

Samodova D, *et al.* **ProAlanase is an effective alternative to trypsin for proteomics applications and disulfide bond mapping.** MCP 2020; 19 (12), 2139–56. doi:10.1074/mcp.TIR120.002129.

Saveliev S, *et al.* **Trypsin/Lys-C protease mix for enhanced protein mass spectrometry analysis.** Nature Methods 2013; 10, i-ii. doi:10.1038/nmeth.f.371.

Saveliev S, *et al.* **Mass spectrometry compatible surfactant for potimized in-gel protein digestion.** Anal. Chem. 2013; 85 (2) 907–14. doi.org/10.1021/ac302423t.

8. Nucleic Acid Analysis

DNA Sequencing & Fragment Analysis Spectrum Compact CE System

The Spectrum Compact CE System is an integrated and efficient instrument that brings you the independence to perform Sanger sequencing and fragment analysis in your laboratory, under your control, and at your convenience.

It is designed for use with existing sequencing chemistries using fluorescently labeled dideoxynucleotide triphosphate and 4-, 5- and 6-dye STR kits from Promega and other commercially available kits.



Features

- ✔ Benchtop 4-capillary electrophoresis instrument compatible with up to 6-dye chemistries
- ✔ Processes up to 32 samples in a single run and is compatible with most commercially available kits
- ✔ Flexible raw data analysis, even with already available versions of analysis software
- ✔ Prefilled, plug-and-play consumables offer maximal flexibility and resource efficiency
- ✔ Controlled by the integrated touchscreen or by Remote Access Software
- ✔ On-site instrument and software installation, operational training



For more information, visit:
[www.promega.com/
SpectrumCompact](http://www.promega.com/SpectrumCompact)



Genomic Essentials – Molecular Biology Tools

With the Genomic Essentials products, you have everything you need for DNA & RNA analysis:

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- ✔ GoScript® Reverse Transcriptase for efficient cDNA synthesis
- ✔ RNasin® and RNasin® Plus – RNases inhibitors

DNA Amplification – PCR & qPCR

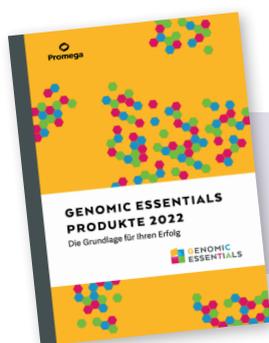
- ✔ GoTaq® G2 DNA Polymerase – for end-point PCR
- ✔ GoTaq® probe-based or dye-based products for qPCR methods

Next-Generation and Sanger Sequencing

- ✔ ProNex® Size Selective Purification System
- ✔ ProDye™ Terminator Sequencing System – for improved fluorescent Sanger sequencing using a proprietary thermostable DNA polymerase included in the master mix

And much more like...

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- ✔ Competent cells
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- ✔ Markers
- ✔ Transfection reagents



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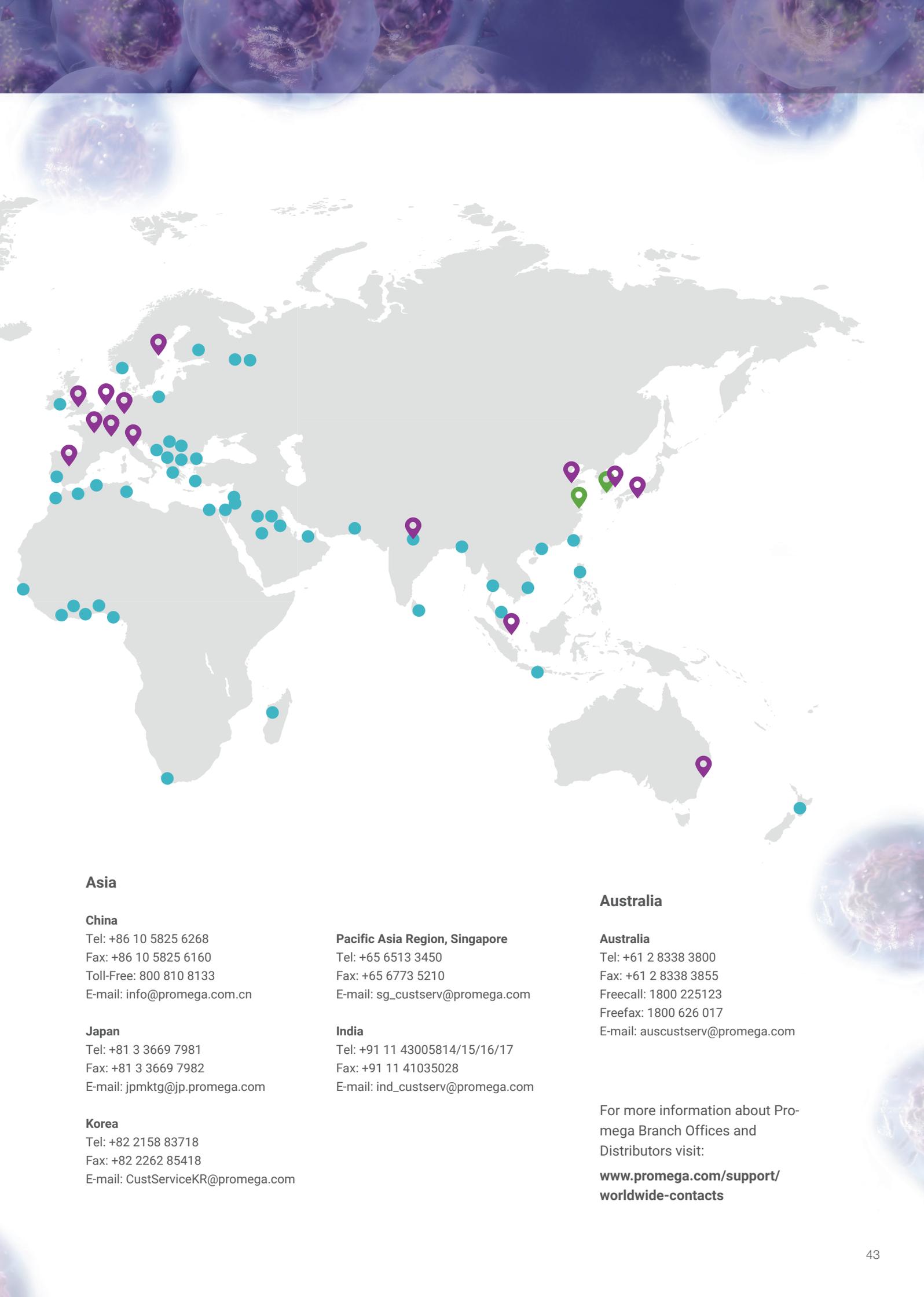
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