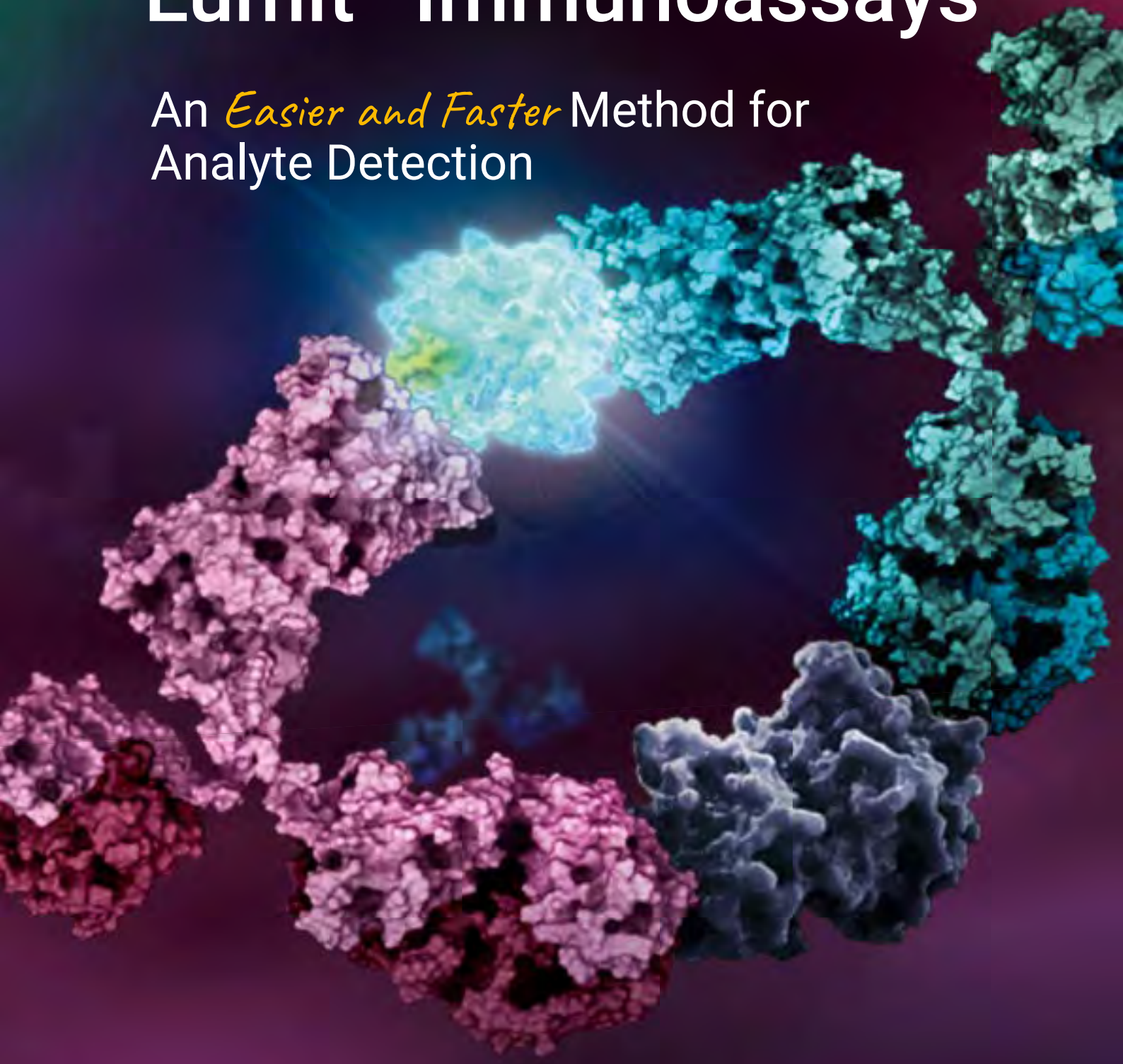
Promega

Lumit® Immunoassays

An *Easier and Faster* Method for Analyte Detection



Signaling | Metabolic Regulators | Cytokines
Protein Interaction & Degradation | Cell & Gene Therapy
Therapeutic Antibody Development

Lumit® Immunoassays

Pre-Built

Cytokines & HMGB1

- IL-1β
- IL-2
- IL-4
- IL-6
- IL-8
- IL-10
- IL-12
- IL-17A
- active IL-18
- TNF-α
- IFN-β
- IFN-γ
- VEGF-A
- HMGB1
- ...

Hormones

- Insulin
- Glucagon
- ...

Cell Proliferation

- Ki-67

Therapeutic Antibody Development

- FcRn
- FcγRI
- FcγRIIa (H131)
- FcγRIIa (R131)
- FcγRIIb
- FcγRIIIa (F158)
- FcγRIIIa (V158)
- FcγRIIIb
- ...

Cell & Gene Therapy

- dsRNA
- ...

2nd Messenger

- cAMP
- cGAMP
- ...

Signaling & Protein Degradation

- AKT
- BTK
- CHK1
- cMET
- EGFR
- ERK
- GSPT1
- H2AX
- IκBα
- JNK
- p65
- RB
- RPS6
- STAT3
- BRD4
- BTK
- SMARCA2
- SMARCA4
- ...

Browse our application notes for pre-validated targets
www.promega.com/LumitCellularSystems

Build-Your-Own

Labeling & Detection

Labeling Kit

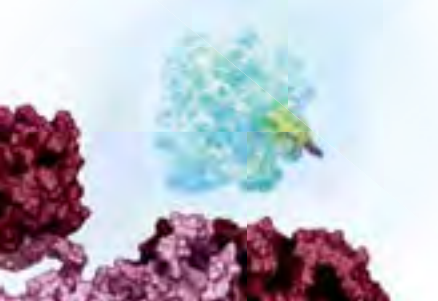
Labeled antibodies

- anti-His
- anti-GST
- anti-DYKDDDDK
- anti-human IgG
- anti-mouse IgG
- anti-rabbit IgG
- anti-goat IgG
- anti-rat IgG

Labeled Streptavidin

Detection Reagents

...



Time is precious

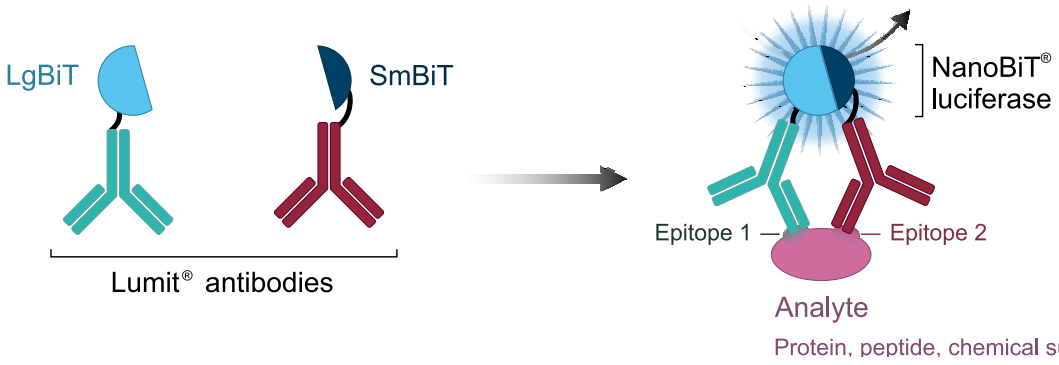
Table of Contents

1. Introduction

1.1 Lumit® Technology

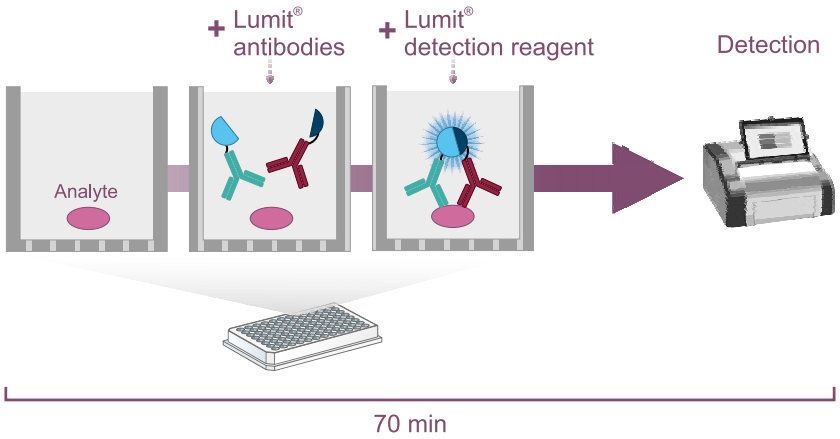
Detection and quantification of analytes are often performed using time-consuming, multi-step methods such as Western blotting and ELISA. The Lumit® technology offers a simple and fast alternative for running homogeneous immunoassays in multi-well plate formats. Its high specificity and compatibility with high-throughput screening (HTS) make it a powerful tool for scientists working in areas ranging from basic research to drug discovery.

Assay Principle



The underlying principle of Lumit® is the NanoLuc® Binary Technology (NanoBiT®). In Lumit® Immunoassays, two antibodies are chemically labeled with the small and large engineered subunits of NanoBiT luciferase, i.e., SmBiT and LgBiT. Direct or indirect binding to the analyte brings these subunits into spatial proximity, enabling them to reconstitute a functional luciferase. In the presence of the substrate furimazine, a bright luminescence is generated that is directly proportional to the amount of analyte in the sample.

Assay Workflow



Features & Benefits

- Simple homogenous workflow
  - ✓ No washing
  - ✓ No blocking
- Detection of analytes in
  - ✓ Buffer
  - ✓ Cell culture supernatants
  - ✓ Cell lysate
- Signal detection on a conventional plate-reading luminometer

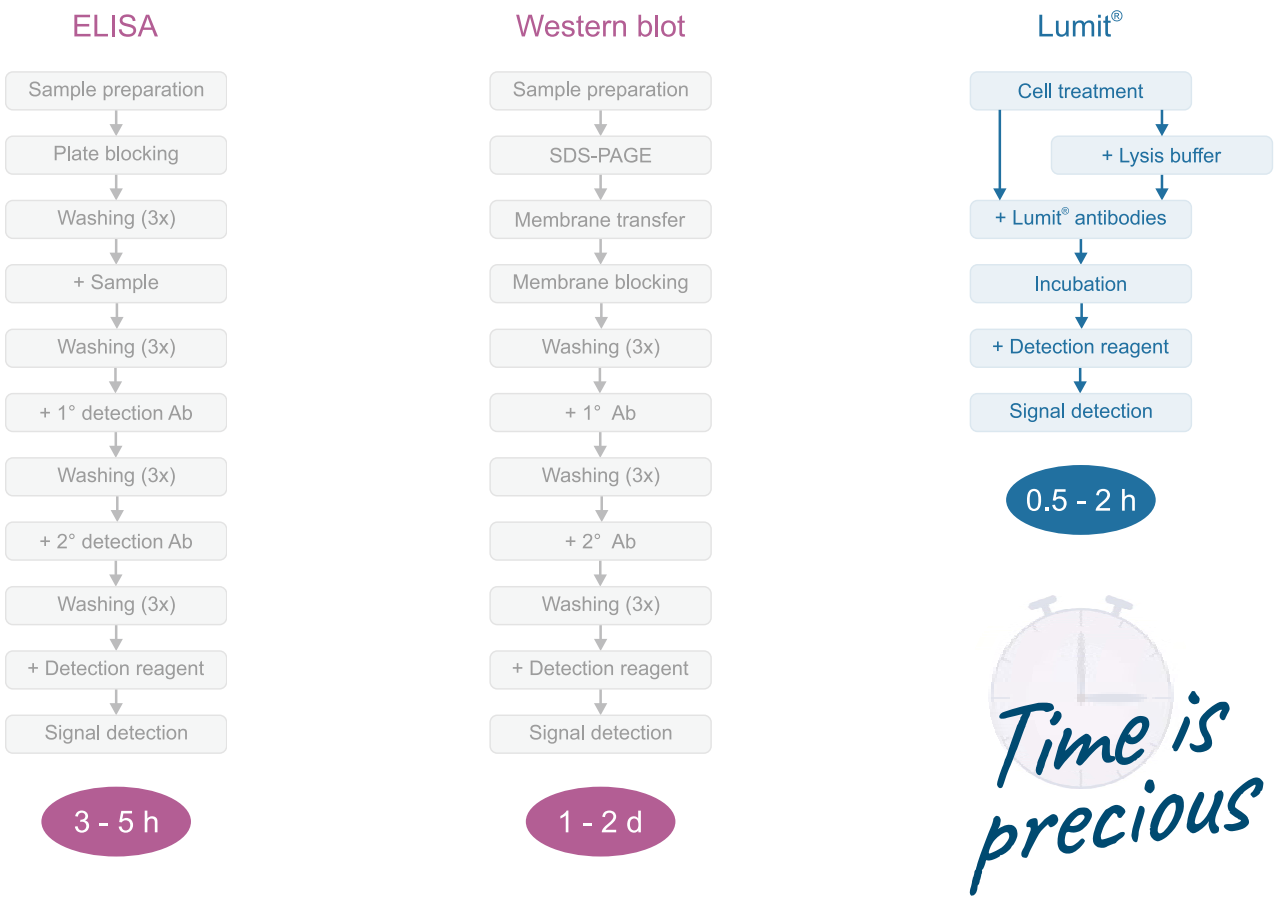
Advantages

- Simple add-and-read protocol with no washing steps
- Fast results with reduced hands-on time
- No immobilization to plates, beads, or other surfaces required
- Direct analyte measurement in cell culture plates
- Sensitive luminescence detection with a wide dynamic range
- Detection on a conventional luminometer
- High specificity and low background signal
- Easy to automate and HTS-compatible (96-well and 384-well plates)

Lumit® vs. Conventional Immunoassays

Lumit® Immunoassays are fast, add-and-read plate-based assays. No washing steps are required making Lumit® a compelling alternative to labor-intensive methods, e. g., ELISA and Western blot.

Your Short-Cut to High-Quality Data

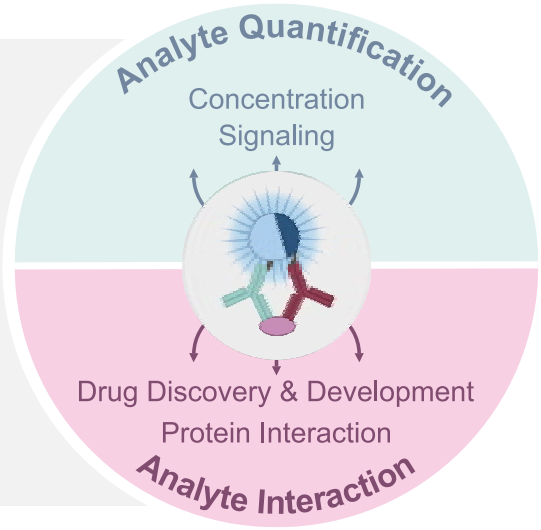


1.2 Applications & Assay Formats

A multitude of applications is supported by the different Lumit® Immunoassay formats.

Applications

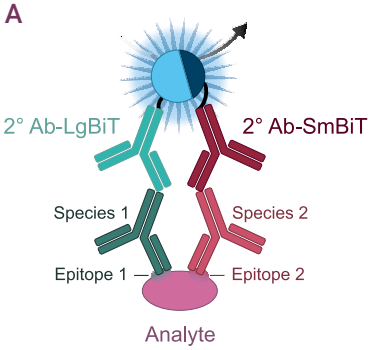
- Quantitative analyte detection in biological samples
- Competitive binding studies for proteins and small molecules
- Drug screening for proteins and small molecules
- Measurement of signaling pathway activation
- Analysis of protein interactions
- High-throughput screenings (HTS)



Analyte Quantification

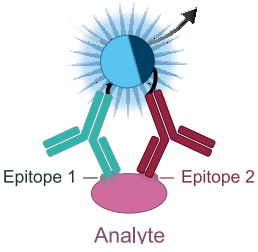
Indirect Assay Format

A



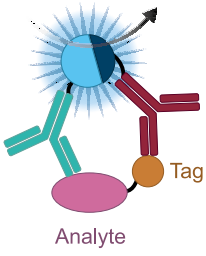
Direct Assay Format

B

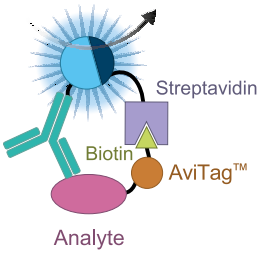


Alternative Assay Formats

C



D



The Lumit® technology supports various assay formats that enable the quantitative analysis of analytes.

(A) In the indirect assay format, two epitopes on the analyte are recognized by two different primary antibodies from different species. These antibodies are detected using BiT-labeled secondary antibodies. This format has been extensively validated for the analysis of post-translational modification (PTM) in cell lysates and is also referred to as Lumit® Immunoassay Cellular Systems.

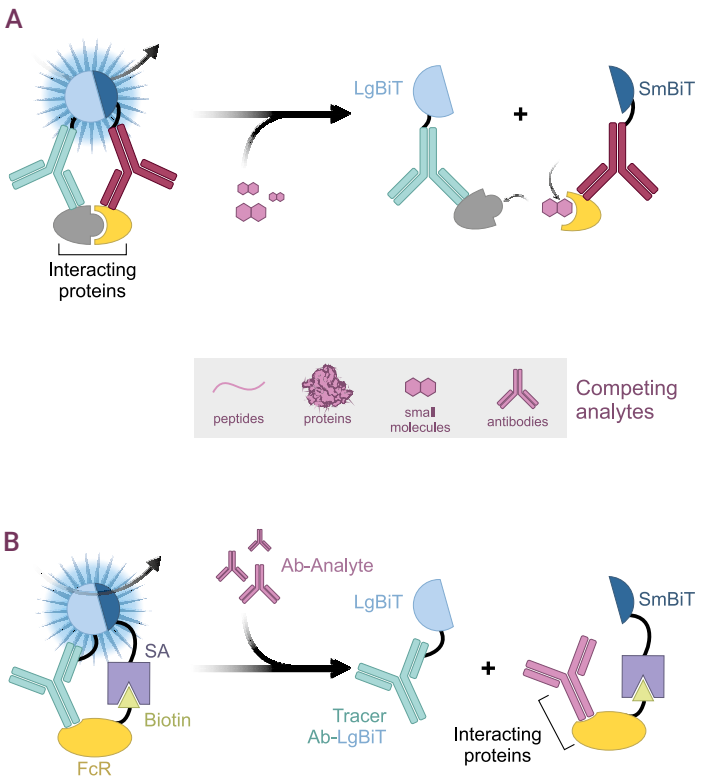
(B) The direct assay format employs two BiT-labeled primary antibodies. Alternatively, antibodies can be replaced with labeled analyte-binding proteins, as demonstrated in the Lumit® Assays for IL-18 and dsRNA.

(C) BiT-labeled anti-tag antibodies or
(D) BiT-labeled streptavidin are alternative formats available for use with tagged analytes.

Analyte Interaction

Binary interactions between analytes can be easily studied using the Lumit® technology. Different formats allow the determination and characterization of binding events, such as protein:protein and protein:ligand interactions, through competitive loss- or gain-of-signal assays.

Loss-of-Signal Binding Immunoassays

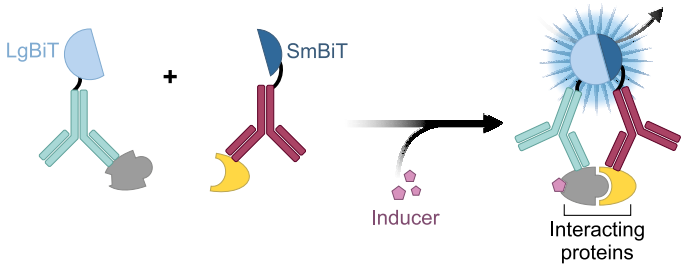


Lumit® loss-of-signal binding assays are used for determining the potency of competing analytes *in vitro*.

(A) This biochemical assay format uses two BiT-labeled primary antibodies to target the interacting proteins of interest, enabling the determination of competing analytes' (inhibitors) potency. The addition of competing analytes reduces the luminescent signal. This assay setup is exemplified by the Lumit® SARS-CoV-2 RBD:hACE2 Assay.

(B) The Lumit® FcR Binding Immunoassays are examples of competitive loss-of-signal assays. In this setup, a biotinylated Fc receptor is combined with SmBiT-labeled streptavidin (SA-SmBiT) and LgBiT-labeled antibody tracers (Tracer-Ab-LgBiT). This approach measures the affinity of analyte antibodies (Ab-Analyte) for the Fc receptor through the competitive displacement of Tracer-Ab-LgBiT.

Gain-of-Signal Binding Immunoassays



Lumit® gain-of-signal assays are used to analyze the potency of protein:protein interaction inducers. In this format, two BiT-labeled primary antibodies bind the protein pair of interest. The presence of a PPI inducer facilitates the interaction between the two proteins, resulting in a relative increase in luminescent signal.

2. Pre-Built Lumit® Immunoassays

2.1 Cytokines / HMGB1

Description & Application

Pre-built Lumit® Cytokine/HMGB1 Immunoassays are fast, plate-based, add-and-read quantification assays. Their sensitivity is emphasized by a low limit of detection (LOD) while their wide linear range mitigates the need for sample dilutions. Lumit® assays can be applied for low- or high-throughput experiments.

Principle & Workflow

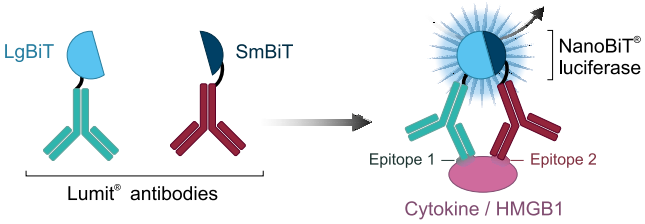
The assay kits contain analyte-specific primary antibodies, labeled with the NanoBiT® subunits LgBiT and SmBiT, an analyte standard, and detection reagent. Analyte detection within the cell culture supernatant is either performed in the presence of cells (Option #1) or upon transfer to a separate plate (Option #2). Binding of both antibodies to the analyte facilitates reconstitution of the NanoBiT® luciferase. Upon addition of detection reagent, a bright luminescent signal that is proportional to the analyte level can be recorded on a conventional plate-reading luminometer.

Assay Features

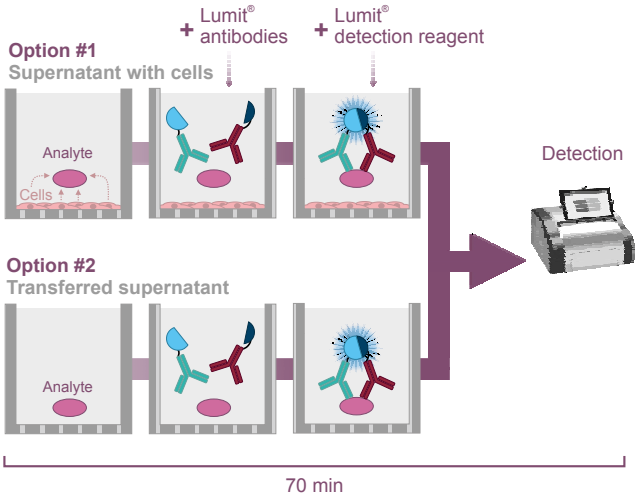
Sample material	Supernatant with cells Transferred supernatant
Sample volume	12.5 – 80 µl
Assay format	Direct assay format 96- / 384-well plates
Assay protocol	Homogeneous Add-and-read
Time required	70 minutes or less
Multiplexing option	Caspase-Glo® 1 Inflammasome Assay

> *"Lumit is much quicker, more straightforward and time efficient."*
>
> *Scientist at ImmuONE*

Assay Principle



Assay Workflow



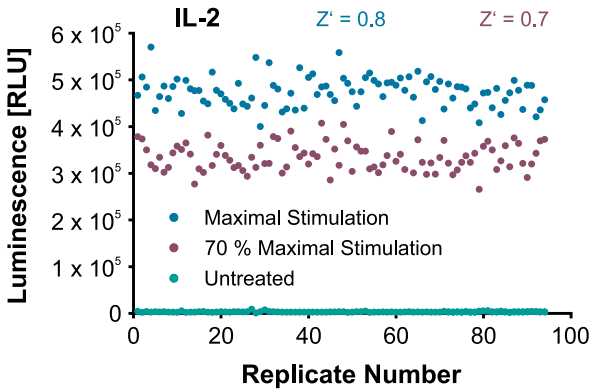
Assay	Dynamic range	LOD
Lumit® IL-2 (Human)	28.2 – 25000 pg/ml	11.2 pg/ml
Lumit® IL-4 (Human)	18.2 – 25000 pg/ml	6.7 pg/ml
Lumit® IL-6 (Human)	18.2 – 25000 pg/ml	7.5 pg/ml
Lumit® IL-10 (Human)	18.2 – 25000 pg/ml	7.4 pg/ml
Lumit® IFN-γ (Human)	7.2 – 10000 pg/ml	1.7 pg/ml
Lumit® TNF-α (Human)	18.2 – 25000 pg/ml	2.9 pg/ml
Lumit® IL-12 p70 (Human)	18.2 – 25000 pg/ml	10.4 pg/ml
Lumit® IL-1β (Human)	22 – 40000 pg/ml	10 pg/ml
Lumit® IL-1β (Mouse)	11 – 20000 pg/ml	8 pg/ml
Lumit® HMGB1 Human/Mouse Immunoassay	4 – 1000 ng/ml (hu) 3 – 2187 ng/ml (ms)	1 ng/ml 3 ng/ml
Lumit® Active IL-18 (Human)*	7.8 – 5714 pg/ml	1.9 pg/ml
Lumit® IL-8 (Human)	7.29 – 10000 pg/ml	1.7 pg/ml
Lumit® IL-17A (Human)	18.2 – 25000 pg/ml	3 pg/ml
Lumit® IFN-β (Human)	18.2 – 25000 pg/ml	5.3 pg/ml
Lumit® VEGF-A (Human)	17.3 – 7500 pg/ml	4.8 pg/ml

LOD: limit of detection (3 SD above background); hu: human; ms: mouse
* This assay uses Lumit® Flex technology, a three-component NanoLuc®-based complementation system.

Representative Data

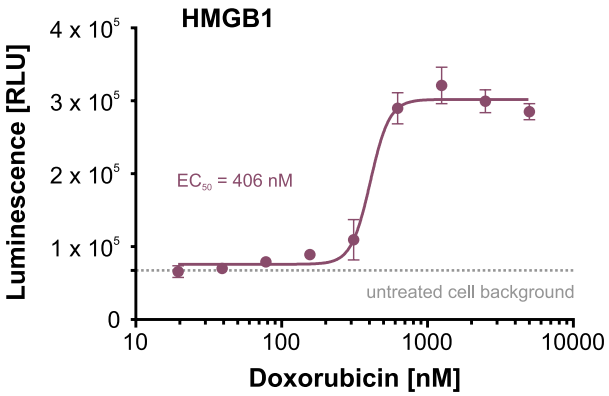
Validation of HTS-compatibility

Human PBMCs, plated at 10000 cells/well into a 384-well plate were treated with Cell Stimulation Cocktail for 24 hours at maximal or 70 % maximal levels of stimulation. For each condition, luminescence of 94 replicates was determined upon reagent addition. Z' factors determined for IL-2 release were substantially greater than 0.5, indicating amenability to screening applications.



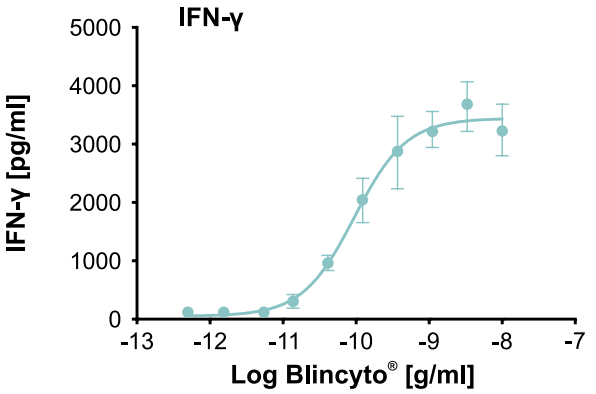
Drug-induced immunogenic cell death

Mouse EL4 cells were treated with doxorubicin for 24 hours. HMGB1 within the supernatant was quantified in the presence of cells (w/o transfer) by using Lumit® HMGB1 Immunoassay.



Detection of IFN-γ as a marker of T cell activation

Purified CD8+ T cells (effector cells) were combined with Raji B cells (target cells) and a serial dilution of Blincyto® (a CD3 and CD19 bispecific T cell engager). IFN-γ release from effector cells into the cell culture supernatant was analyzed in the presence of cells (w/o transfer) by using Lumit® IFN-γ Immunoassay.



Product Box

Lumit® Cytokine Immunoassays	Cat.# see page 36
Lumit® HMGB1 Immunoassay	Cat.# W6110, W6112

2.2 Hormones

Description & Application

Pre-built Lumit® Insulin and Glucagon Immunoassays are fast, plate-based, add-and-read quantification assays that deliver results in approximately one hour. The wide linear range of these sensitive assays mitigates the need for sample dilution. Lumit® Immunoassays are amenable for low- and high-throughput applications.

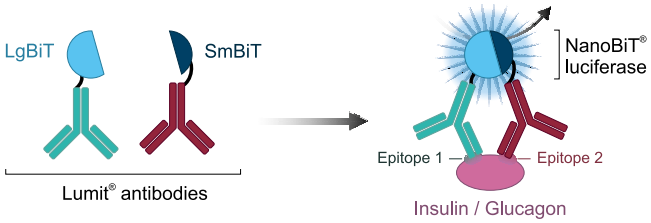
Principle & Workflow

The assay kits contain analyte-specific primary antibodies, labeled with the NanoBiT® subunits LgBiT and SmBiT, an analyte standard, and detection reagent. Analyte detection is performed in cell culture supernatant, transferred to a separate plate. Binding of both antibodies to the analyte facilitates reconstitution of the NanoBiT® luciferase. Upon addition of detection reagent, a bright luminescent signal that is proportional to analyte levels can be recorded on a conventional platereading luminometer.

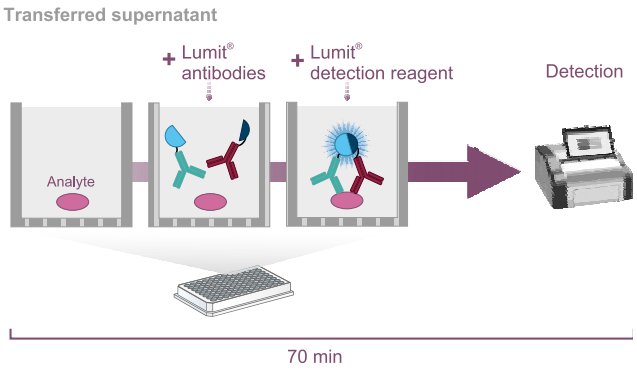
Assay Features

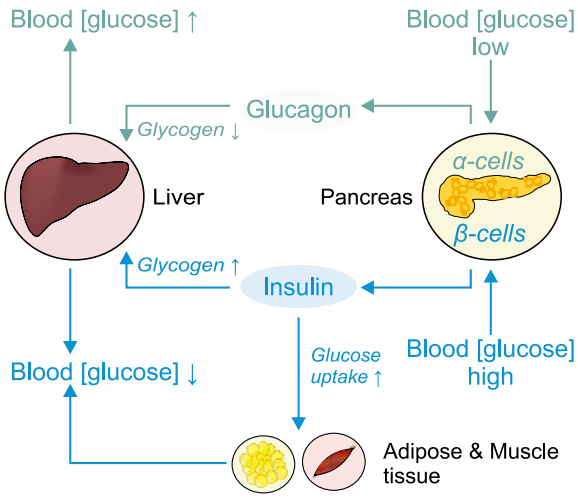
Sample material	Transferred supernatant
Sample volume	5 – 50 µl
Species compatibility	Human, mouse, rat
Assay format	Direct assay format 96- / 384-well plates
Assay protocol	Homogeneous Add-and-read
Time required	70 minutes or less
Multiplexing option	Use both Lumit® assays and analyze insulin and glucagon side-by-side to gain more information on islet function

Assay Principle



Assay Workflow





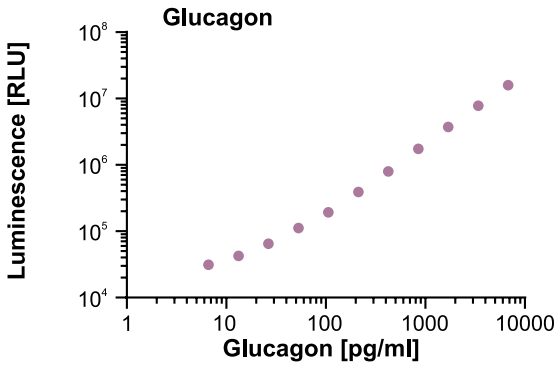
Assay	Dynamic range	LOD
Insulin	58 – 46000 pg / ml	58 pg / ml
Glucagon	3 – 7000 pg / ml	3 pg / ml

LOD: limit of detection

Representative Data

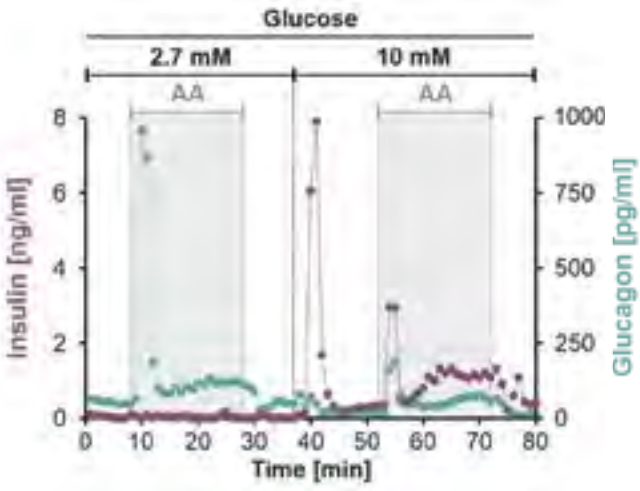
Broad dynamic range and picomolar sensitivity

The broad dynamic range enables sensitive detection without sample dilution. A dilution series of glucagon was detected in a 96-well plate by addition of Lumit® antibodies. After 1 hour of incubation, Lumit® detection reagent was added, and luminescence was measured. Analysis was performed in quadruplicates.



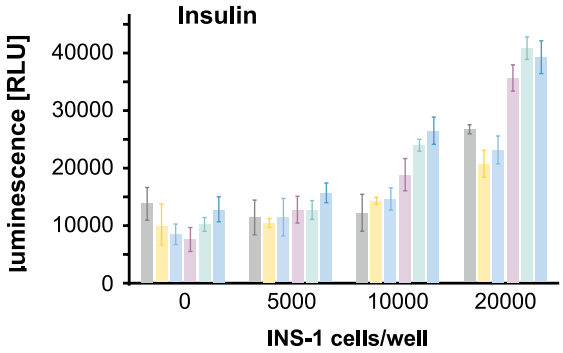
Measuring hormone secretion in perfusion experiments

In a perfusion chamber, 80 mouse islets were treated with glucose in combination with an amino acid (AA) mixture. Low and high blood glucose levels were simulated with 2.7 mM glucose and 10 mM glucose respectively. Perfusate aliquots were collected every minute. Insulin and glucagon levels were detected in 10 µl perfusate in a 384-well plate. This data was kindly provided by Drs. H. Foster and M. Merrins (University of Wisconsin VA Hospital, Madison, WI).



Monitoring insulin secretion in response to glucose

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



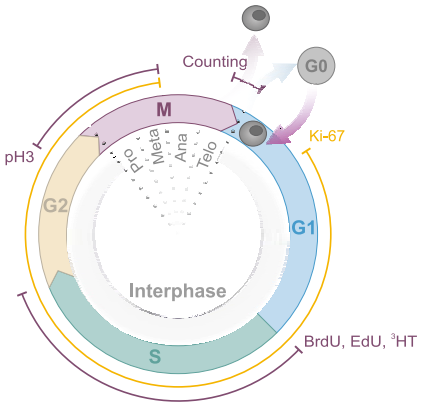
Product Box

Lumit® Glucagon Immunoassay	Cat.# W8020, W8022
Lumit® Insulin Immunoassay	Cat.# CS3037A01, CS3037A05

2.3 Cell Proliferation

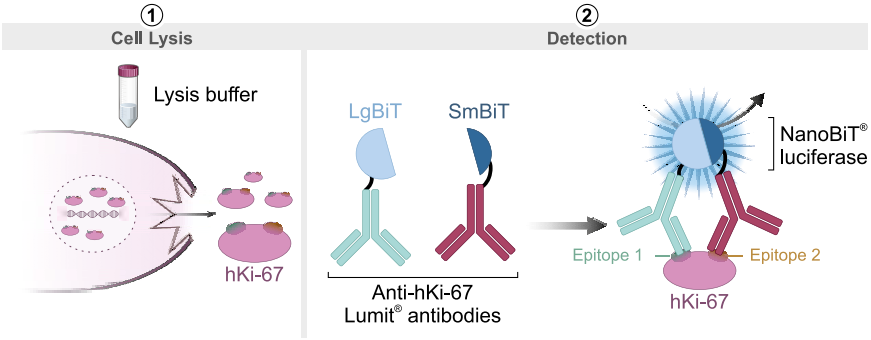
Lumit® hKi-67 Immunoassay for Cell Proliferation

Description & Application



The Lumit® hKi-67 Immunoassay for Cell Proliferation is a no-wash, plate-based assay that detects hKi-67, a well-established nuclear marker of cell proliferation expressed in all active phases of the cell cycle and is absent in resting, non-dividing cells. This assay enables researchers to confidently track proliferation changes at early time points and can be completed in under two hours. By adopting this "add-mix-measure" bioluminescent assay, researchers can streamline their workflow, reduce prep work, and accelerate time-to-results, offering a more efficient and high-throughput-compatible alternative to traditional proliferation assays (e.g., BrdU, EdU, 3H-thymidine, metabolic activity assays, and cell counting).

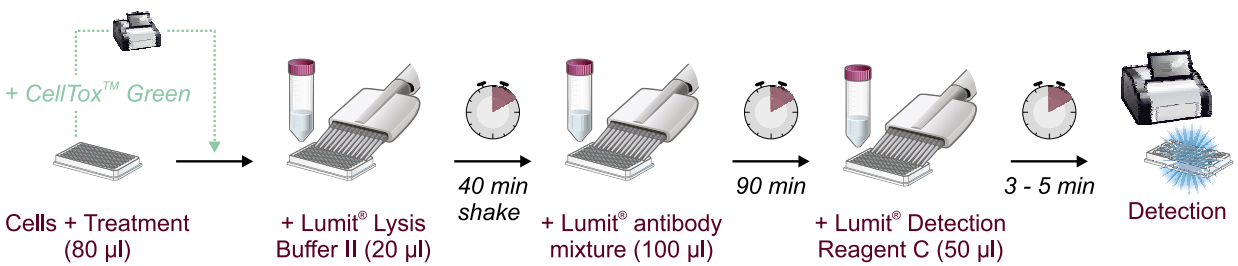
Assay Principle



Principle & Workflow

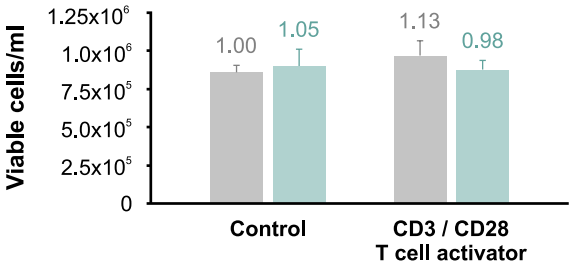
The Lumit® hKi-67 Immunoassay utilizes primary antibodies against hKi-67, labeled with NanoBiT® luciferase subunits LgBiT and SmBiT. In the presence of hKi-67, the subunits reconstitute the functional NanoBiT® luciferase, producing a bright luminescent signal upon addition of the Lumit® Detection Substrate C. This signal is directly proportional to the hKi-67 levels, providing a sensitive and specific readout of cell proliferation. Prior to antibody addition, the nuclear hKi-67 protein is released by addition of a lysis buffer to the assay plate. Optionally. the assay can be multiplexed with the included CellTox™ Green Cytotoxicity Assay to identify antiproliferative effects without cell death.

Assay Workflow

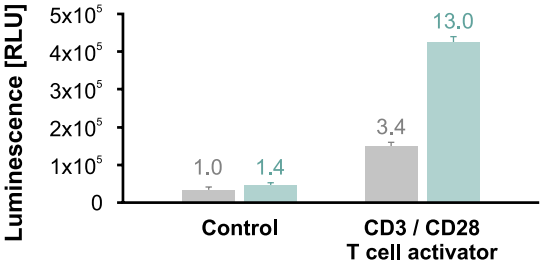


Representative Data

A



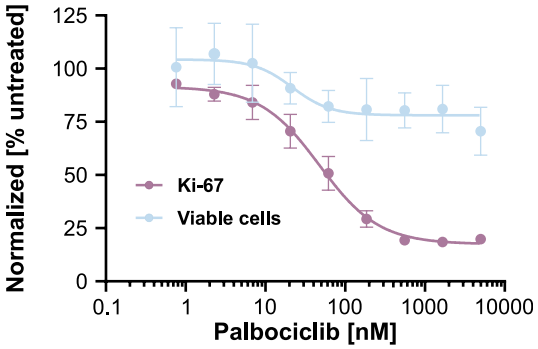
B



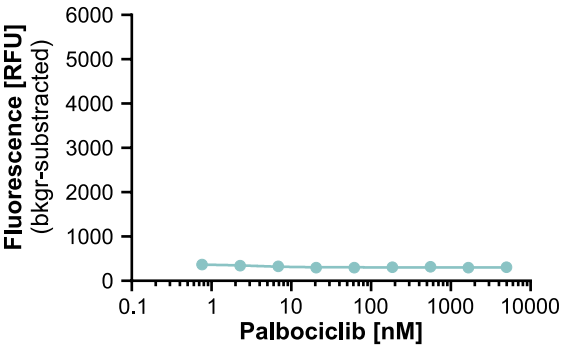
Determination of mitogenic effects

Human CD8+ T cells (STEMCELL Technologies™) were seeded at 80000 cells/well and treated with a CD3/CD28 T cell activator in the absence or presence of IL-2 (10 ng/ml) for 48 hours. The number of viable cells was determined by trypan blue staining (A) or analyzed using the Lumit® hKi-67 Immunoassay (B). hKi-67, an early marker of cell proliferation, showed a significant increase in response to T cell activation, while no changes in absolute cell number were observed.

A



B



Determination of antiproliferative effects

Jurkat cells (20000/well) were treated with a dilution series of the CDK4/6 inhibitor palbociclib for 24 hours. The hKi-67 level was assessed using the Lumit® hKi-67 Immunoassay (A, purple), while changes in the viable cell number were determined with the CyQUANT™ Direct Assay (Thermo Fisher Scientific) (A, blue) in a parallel plate. Cell death was monitored by multiplexing the Lumit® assay with the CellTox™ Green Cytotoxicity Assay (B). Without inducing cell death, palbociclib elicited an antiproliferative response, evident from a dose-dependent decrease in both the hKi-67 level and viable cell number. Compared to the DNA-stain-based measurement of viable cells, the hKi-67 assay provided a much more pronounced assay window, enabling an earlier readout with reduced incubation times.

Product Box

Lumit® hKi-67 Immunoassay for Cell Proliferation Cat.# CS3076A01

2.4 Second Messengers

Lumit® cAMP Immunoassay

Description & Application

The Lumit® cAMP Immunoassay is a homogeneous, no-wash competition assay for measuring 3',5'-cyclic adenosine monophosphate (cAMP). This key second messenger regulates metabolism, gene expression, and signal transduction. cAMP is synthesized by adenylyl cyclase upon GPCR activation and hydrolyzed by phosphodiesterases (PDEs). The assay uses competitive binding to detect cAMP level changes with high specificity and sensitivity. It is ideal for studying GPCR signaling, PDE activity, and cAMP modulators in biochemical and cellular formats.

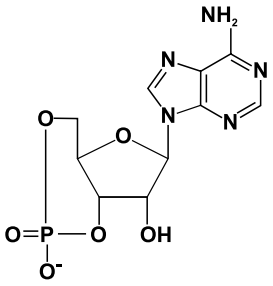
Principle & Workflow

The assay includes cAMP linked to SmBiT (Tracer cAMP-SmBiT), a cAMP-specific primary antibody, and an anti-mouse antibody conjugated to LgBiT. These components form a luminescent complex through NanoBiT® luciferase reconstitution. In the presence of free cAMP, binding of the tracer is competitively inhibited in a dose-dependent manner, resulting in reduced luminescence. After sample treatment, an antibody-tracer-Digitonin mixture is added to lyse the cells and detect cAMP. Following a 60-minute incubation at room temperature (RT), the Lumit® Detection Reagent is added to generate the luminescence signal, which is measured on a plate reader. Optionally, a 2 % TCA solution can be used to lyse cells or quench the enzymatic reaction.

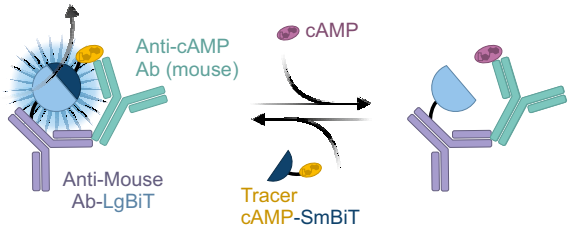
Assay Features

Sample material	Purifed enzyme, cells
Sample volume	12 – 120 µl
Assay format	Competitive assay format 96- / 384-well plates
Assay protocol	Homogeneous Add-and-read
Time required	90 minutes
Dynamic range	< 1 nM – 120 nM
LOD	0.8 nM

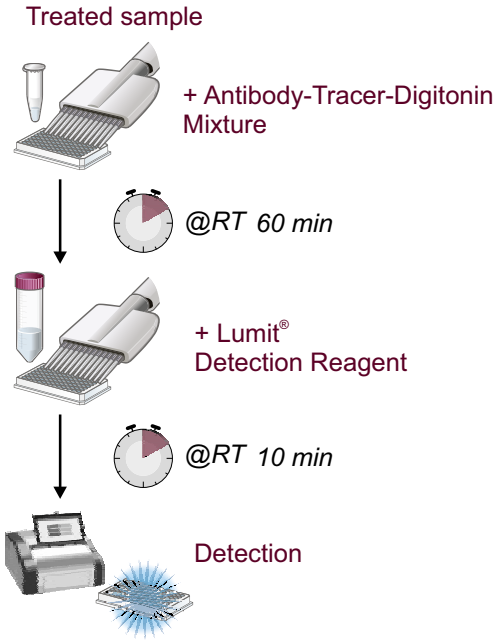
3',5'-cyclic adenosine monophosphate (cAMP)



Principle



Assay Workflow



Representative Data

Assay Specificity

Serial dilutions of cAMP and other nucleotides (cGMP, 3'3'cGAMP, and 2'3'cGAMP) were analyzed for their ability to compete with the cAMP tracer of the Lumit® cAMP Immunoassay. Except for cAMP, none of the tested nucleotides yielded a signification decrease in luminescence emphasizing assay specificity.

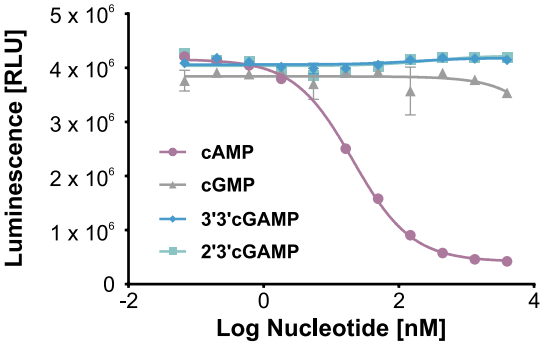
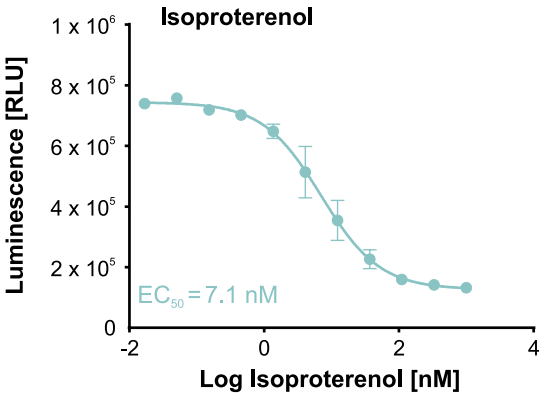
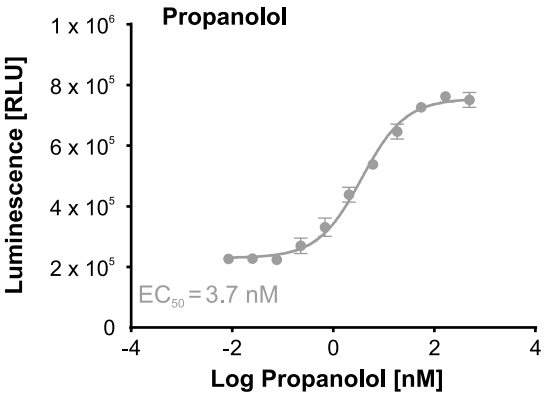


Figure adapted from Mikheil *et al.* Scientific Reports. 2024;14(1):4440

A



B



Antagonist Competition of Agonist-Induced cAMP Response

A375 cells, expressing endogenous β2-adrenergic receptors, were seeded at 1000 cells/well in 384-well plates. The following day, cells were treated with a concentration series of (A) the β-adrenergic receptor agonist isoproterenol alone, or (B) the antagonist propranolol in combination with 50 nM isoproterenol. After a 5-minute incubation at room temperature, the reactions were stopped with 2 % trichloroacetic acid (TCA), and cAMP levels were measured using the Lumit® cAMP Immunoassay.

Product Box

Lumit® cAMP Immunoassay Cat.# CS3592A08

2.4 Second Messengers

Lumit® cGAMP Immunoassay

Description & Application

The Lumit® cGAMP Immunoassay is a homogeneous, no-wash competition assay for measuring 2’,3’-cGAMP. This cyclic dinucleotide, composed of guanine and adenine monophosphate, activates the STING pathway, driving pro-inflammatory cytokine production in innate immunity. cGAMP is synthesized by cGAS upon recognition of cytosolic double-stranded DNA (dsDNA). The assay enables the identification and characterization of cGAMP modulators in biochemical and cellular formats.

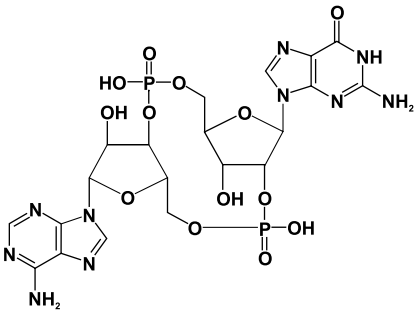
Principle & Workflow

The assay includes 2',3'-cGAMP linked to SmBiT (Tracer cGAMP-SmBiT), a cGAMP-specific primary antibody, and an anti-mouse antibody conjugated to LgBiT. These components form a luminescent complex through NanoBiT® luciferase reconstitution. In the presence of free cGAMP, binding of the tracer is competitively inhibited in a dose-dependent manner, resulting in reduced luminescence. After sample treatment, an antibody-tracer-digitonin mixture is added to lyse the cells and detect cGAMP. Following a 90-minute incubation at room temperature (RT), the Lumit® Detection Reagent is added to generate the luminescence signal, which is measured on a plate reader. Optionally, a 0.5 N HCl solution can be used to lyse cells or quench the enzymatic reaction.

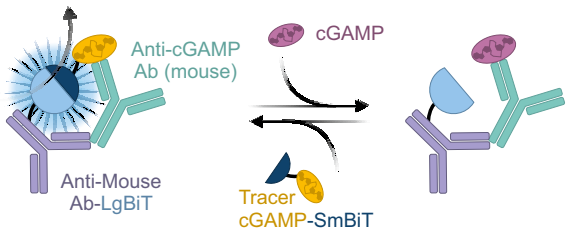
Assay Features

Sample material	Purified enzyme, cells, human serum
Sample volume	8 – 40 µl
Assay format	Competitive assay format 96- / 384-well plates
Assay protocol	Homogeneous Add-and-read
Time required	2 hours
Dynamic range	< 1 nM – 10000 nM
LOD	0.6 nM

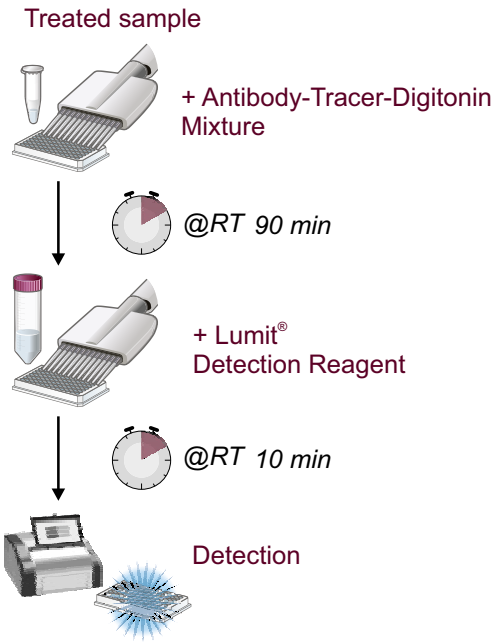
2',3'- cyclic guanosine monophosphate-adenosine monophosphate



Principle



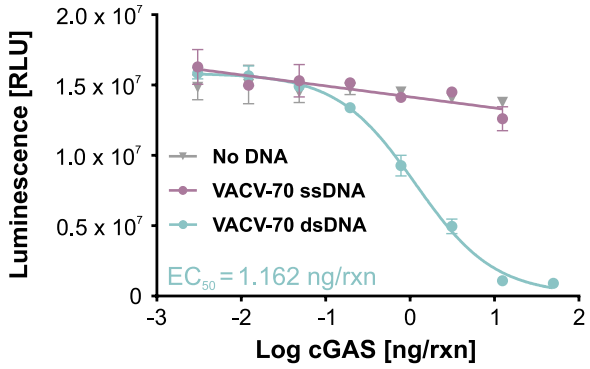
Assay Workflow



Representative Data

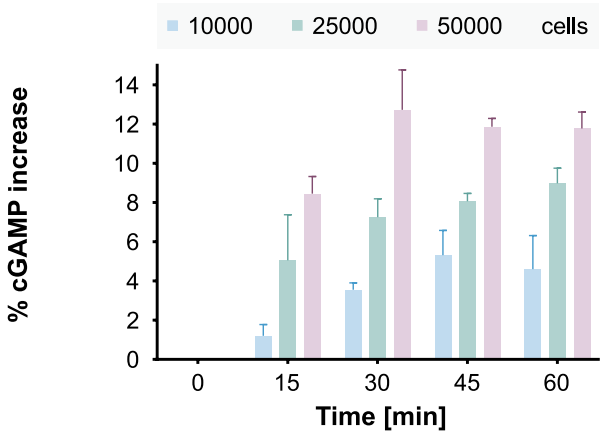
Biochemical assay of cGAS activity

cGAS reactions (40 µl) were carried out in presence of ATP (100 µM), rGTP (100 µM), and VACV-70 DNA (1 ng). After incubation for 30 minutes at 37°C the reaction was terminated with 0.5 N HCl. The cGAMP level was determined by using the Lumit® cGAMP Immunoassay. Activation of of cGAS requires dsDNA while as no activation was detected with ssDNA and the no DNA control.



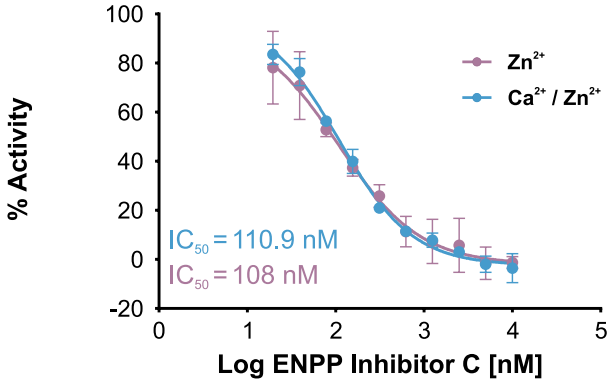
Monitoring cGAMP levels in RAW 264.7 cells

Different numbers of RAW 264.7 cells were treated with ISD dsDNA (4 µg/ml) to stimulate endogenous cGAS activity in the presence of ENPP1 Inhibitor C (60 µM). Reactions were terminated at indicated time points using 0.5 N HCl and cGAMP levels analyzed with the Lumit® cGAMP Immunoassay



Biochemical assay of ENPP1 activity/inhibition

Enzymatic ENPP1 (10 ng) reactions (40 µl) were carried out in presence of Zn^{2+} (5 µM) and cGAMP (10 nM) with and without Ca^{2+} (0.5 mM) at 37 °C. After 30 minutes, 0.5 N HCl was added to stop the reaction and cGAMP levels were quantified using the Lumit® cGAMP Immunoassay.



Figures adapted from Hsiao *et al.* Scientific Reports. 2024;14:31165

Product Box

Lumit® cGAMP Immunoassay Cat.# CS3592A05

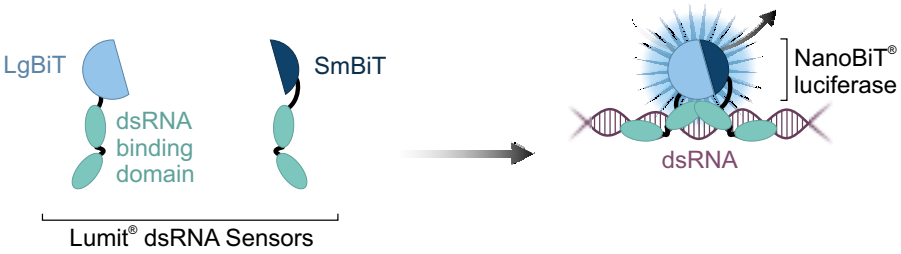
2.5 Cell & Gene Therapy

Lumit® dsRNA Detection Assay

Description & Application

The Lumit® dsRNA Detection Assay detects and quantifies double-stranded RNA (dsRNA) contamination in *in vitro* transcription (IVT) mRNA samples, which can impact RNA therapeutic development. This assay is specific, showing no cross-reactivity with single-stranded RNA (ssRNA) or DNA, and works with dsRNA as short as 30 base pairs. The assay generates a luminescent signal proportional to the dsRNA concentration. It offers a simple, wash-free workflow, suitable for high-throughput applications. Compared to traditional ELISA kits, the Lumit® dsRNA Detection Assay provides better sensitivity, accurately quantifying dsRNA in a range of 0.04 – 2.5 ng/ml.

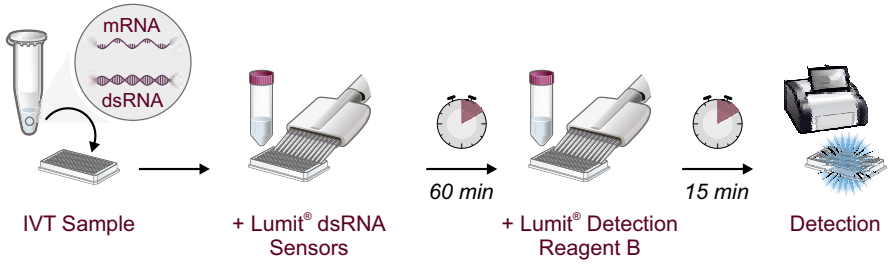
Assay Principle



Principle & Workflow

The Lumit® dsRNA Detection Assay utilizes recombinant dsRNA-binding proteins fused to the SmBiT and LgBiT subunits of NanoBiT® luciferase, respectively. In the presence of dsRNA, the two subunits reconstitute an active luciferase whose luminescent signal correlates with the dsRNA concentration. To perform the assay, dsRNA-containing samples are mixed with the Lumit® reagents in a homogeneous, solution-based format. No wash steps or immobilization are required. The resulting luminescent signal is proportional to the dsRNA content and can be easily measured using a conventional plate luminometer, making the workflow simple and high-throughput compatible.

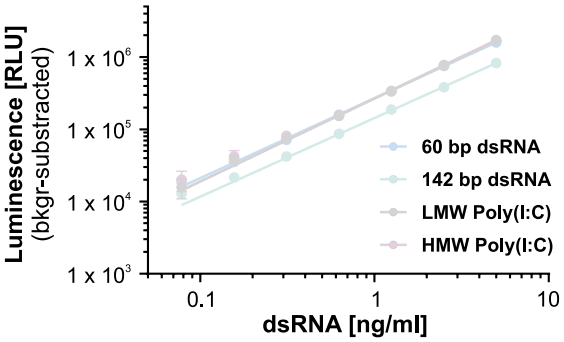
Assay Workflow

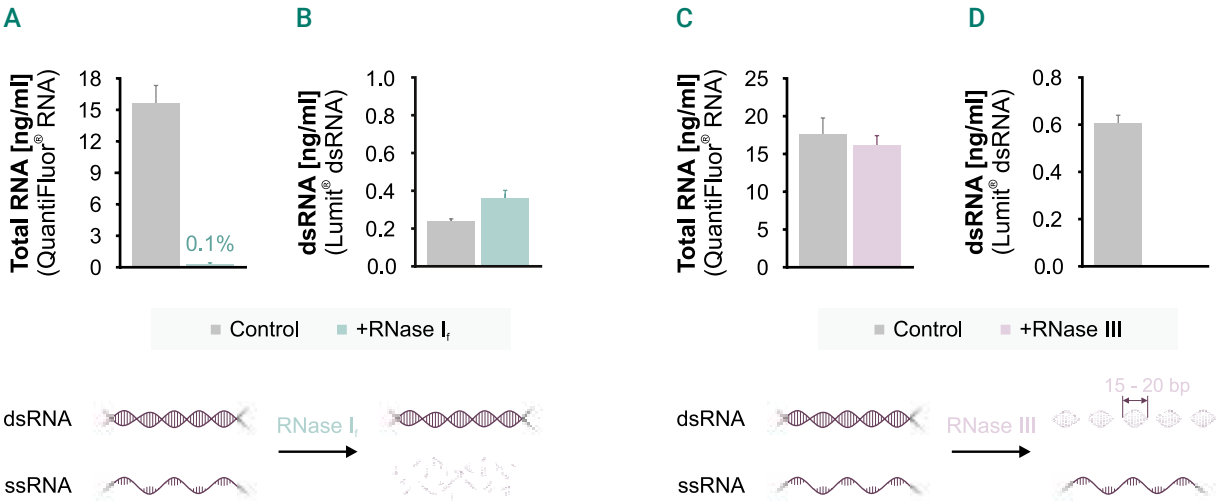


Representative Data

Independence of sequence and size

Titrations of different dsRNAs that vary in size and sequence were analyzed with the Lumit® dsRNA Detection Assay. The data shows that the assay can sensitively quantify dsRNA independent of sequence and size. The following dsRNAs were used: (1) 60 bp dsRNA (synthesized dsRNA comprised of the first 60 bp from the SARS-CoV-2 spike ORF), (2)142 bp dsRNA (commercially available "standard"), (3) LMW Poly(I:C) (synthetic dsRNA analog; 0.2 – 1 kb), (4) HMW Poly(I:C) (synthetic dsRNA analog; 1.5 – 8 kb).





Quantification of dsRNA in IVT samples with mixed RNA species

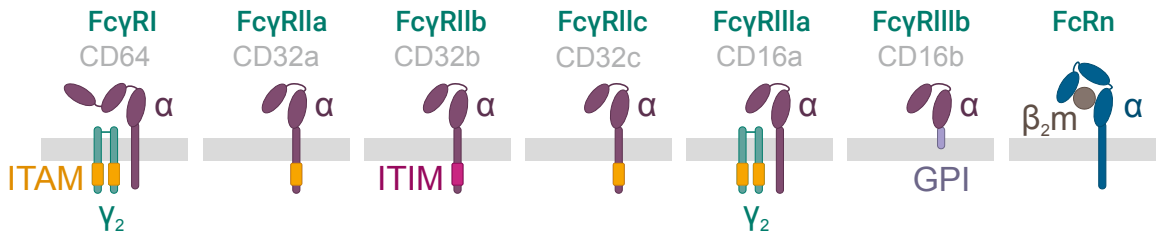
In vitro transcription (IVT) samples were treated with either RNase If (ssRNA-specific) or RNase III (dsRNA-specific). Total RNA and dsRNA were quantified using the QuantiFluor® RNA System and the Lumit® dsRNA Detection Assay. Digestion of ssRNA with RNase If significantly reduced the total RNA but did not affect the amount of dsRNA detected (A and B), while digestion of dsRNA with RNase III into fragments ≤20 bp resulted in a complete loss of signal in the Lumit® dsRNA Detection Assay without significantly affecting total RNA levels (C and D).

Product Box

Lumit® dsRNA Detection Assay	Cat.# W2041, W2042
Lumit® dsRNA Standard, 5-methylcytidine	Cat.# CS355716
Lumit® dsRNA Standard, N1-methylpseudouridine	Cat.# CS355712
Lumit® dsRNA Standard, Pseudouridine	Cat.# CS355710
Lumit® dsRNA Standard, 5-methoxyuridine	Cat.# CS355714

2.6 Therapeutic Antibody Development

The efficacy of therapeutic antibodies depends not only on the Fab fragment's binding to the target antigen but also on interactions between the Fc fragment and Fc receptors. For example, the Fc fragment's affinity for the neonatal Fc receptor (FcRn) influences antibody half-life, while its interaction with Fc gamma receptors (FcγR) determines its ability to trigger effector functions such as antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP). To ensure optimal function, candidate therapeutic antibodies should be evaluated for their Fc receptor interactions during drug development.



	FcγRI CD64	FcγRIIa CD32a	FcγRIIb CD32b	FcγRIIc CD32c	FcγRIIIa CD16a	FcγRIIIb CD16b	FcRn
Function	Activation	Activation	Inhibition	Activation	Activation	Activation	Recycling Transport
IgG binding affinities (high → low)	IgG_3	Variants H131: IgG_3; R131: IgG_3	IgG_3	IgG_3	Variants V158: IgG_3; F158: IgG_3	IgG_3	Similar for IgG_1 - IgG_4 (pH 6.0)
	IgG_1	IgG_1; IgG_1	IgG_1	IgG_1	IgG_1; IgG_1	IgG_1	
	IgG_4	IgG_2; IgG_2	IgG_4	IgG_4	IgG_2; no IgG_2	no IgG_2	
	IgG_2	IgG_4; IgG_4	no IgG_2	no IgG_2	IgG_4; no IgG_4	no IgG_4	

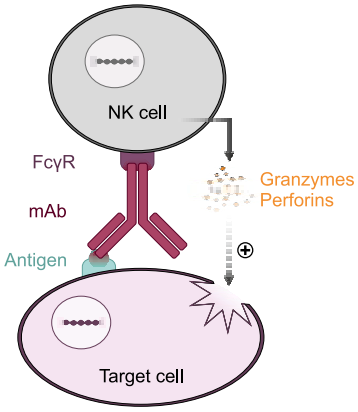
Representation of IgG-binding Fc receptors (FcγR, FcRn). Binding affinity varies among IgG subclasses. ITAM = immunoreceptor tyrosine-based activation motif; ITIM = immunoreceptor tyrosine-based inhibition motif; γ2 = FcR gamma subunit dimer; β_2m = beta-2 macroglobulin. Adapted from Bruhns, P. (2012) Blood 14;119(24):5640–9; Smith, KGC. (2010) Nat Rev Immunol May; 10(5):328–43; Hogarth, PM. (2012) Nat Rev Drug Discov Mar 30;11(4):311–31.

The neonatal Fc receptor (FcRn) is an atypical Fc receptor expressed in diverse tissues, including epithelial, endothelial, and hematopoietic cells. It binds the Fc region of IgG at acidic pH within endosomes, facilitating IgG transport across polarized barriers (e.g., epithelia) and protecting IgG from degradation, thereby extending its serum half-life. Optimizing FcRn interaction with therapeutic IgG is a critical factor in drug development, as extended half-life enhances efficacy and allows for longer dosing intervals.

Fc gamma receptors (FcγR) play a key role in immune responses by binding to the Fc region of IgG. These interactions mediate processes such as ADCC, endocytosis, phagocytosis, inflammatory mediator release, and enhanced antigen presentation. In humans, three classes of **FcγRs–FcγRI** (CD64), **FcγRIIa/b/c** (CD32a/b/c), and **FcγRIIIa/b** (CD16a/b) – have been identified on immune cells. FcγRI is a high-affinity receptor (nM range), while FcγRII and FcγRIII exhibit low to intermediate affinity (µM range). Genetic variations in Fc receptors significantly influence Fc-mediated immune responses. For instance, the FcγRIIIa polymorphism V158 exhibits higher IgG binding affinity compared to the F158 variant, impacting ADCC activity in NK cells.

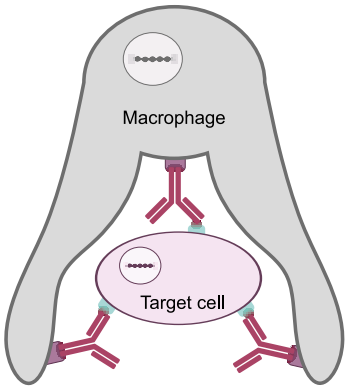
FcγR- / FcRn-mediated Cellular Functions

FcγR-mediated ADCC



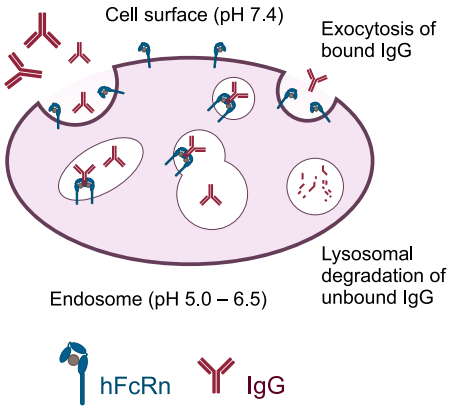
FcγR-mediated ADCC: NK cells trigger antibody-dependent cellular cytotoxicity (ADCC) upon binding the Fc region of IgG via FcγR. This activation leads to the release of cytotoxic molecules, resulting in target cell death.

FcγR-mediated ADCP



FcγR-mediated ADCP: Macrophages initiate antibody-dependent cellular phagocytosis (ADCP) upon engaging the Fc region of IgG, enhancing their ability to engulf and degrade target cells.

FcRn-mediated IgG recycling



FcRn-mediated IgG recycling: FcRn binding occurs in acidic endosomal conditions, promoting IgG uptake via pinocytosis. Unbound IgG is degraded in lysosomes, whereas FcRn-bound IgG is recycled and released through exocytosis.

2.6 Therapeutic Antibody Development

Lumit® FcγR Binding Immunoassays

Description & Application

The Lumit® FcγR Binding Immunoassays are novel homogeneous, no-wash competition assays to measure the interaction between human Fc receptors and antibodies or Fc fusion proteins. Importantly, the in-solution format circumvents experimental artifacts caused by immobilization. These assays are used in therapeutic antibody development for antibody optimization and for testing antibody potency.

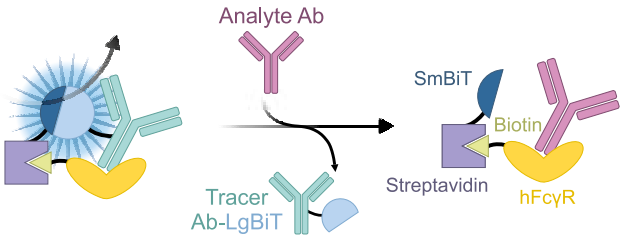
Principle & Workflow

FcγR assays consist of a LgBiT-labeled human IgG1 (Tracer Ab-LgBiT) and a biotinylated human FcγR (extracellular domain) bound to SmBiT-labeled streptavidin (hFcγR-Biotin-Streptavidin-SmBiT). In the absence of an analyte antibody (Analyte Ab), tracer binding to labeled hFcγR results in maximum luminescent signal. Binding of analyte antibodies is evident from a concentration-dependent decrease in luminescence due to competition with the tracer. These easy-to-use biochemical assays can complement and provide orthogonal data to support results from cell-based functional bioassays.

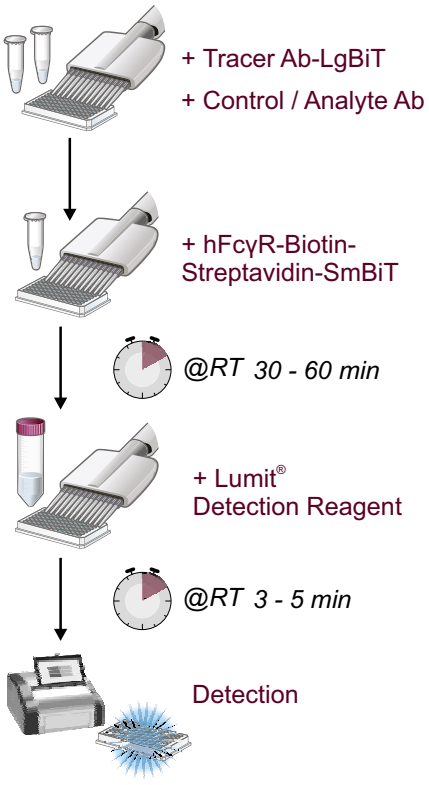
Assay Features

Sample material	Antibodies Fc proteins
Sample volume	25 µl antibody
Concentration range	4 – 4000 ng/ml
Assay format	Loss-of-signal assay 96- / 384-well plates
Assay protocol	Homogeneous Add-and-read
Time required	70 minutes or less

Assay Principle



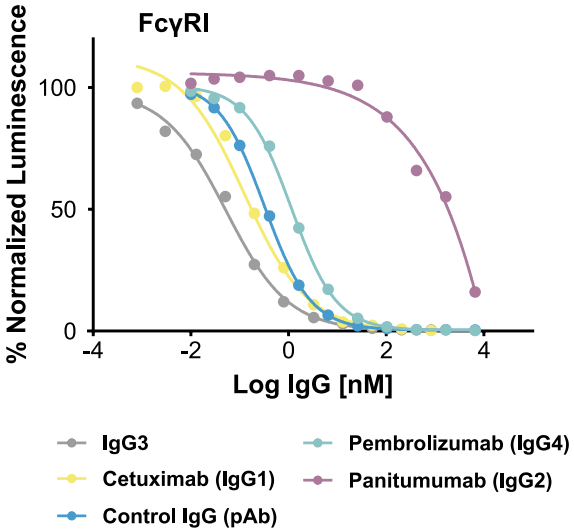
Assay Workflow



Representative Data

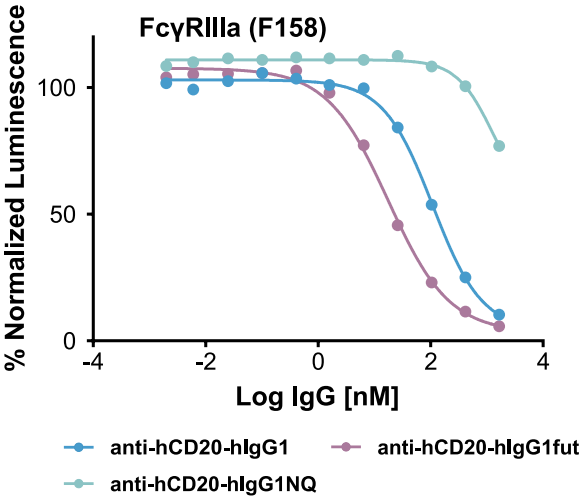
Lumit® FcγRI Binding Immunoassay

Lumit® FcγRI Binding Immunoassays are used as potency assays to complement cell-based functional Fc effector activity assays. FcγRI binds to IgG in a subclass-specific manner with IC_{50} values reflective of relative IgG affinity (IgG_3 > IgG_1 > IgG_4 >>> IgG_2).



Lumit® FcγRIIIa (F158) Binding Immunoassay

Lumit® FcγR Binding Immunoassays are used to assess antibody glycan status. IC_{50} shifts in non-fucosylated (anti-hCD20-hIgG1fut) or non-glycosylated (anti-hCD20-hIgG1NQ) antibodies were detected.



Product Box

Lumit® FcγRI Binding Immunoassay
W7080, W7081

Lumit® FcγRIIa (H131) Binding Immunoassay
W7070, W7071

Lumit® FcγRIIa (R131) Binding Immunoassay
W7060, W7061

Lumit® FcγRIIb Binding Immunoassay
W7030, W7031

Lumit® FcγRIIIa (F158) Binding Immunoassay
W7040, W7041

Lumit® FcγRIIIa (V158) Binding Immunoassay
W7050, W7051

Lumit® FcγRIIIb Binding Immunoassay
W7020, W7021

2.6 Therapeutic Antibody Development

Lumit® FcRn Binding Immunoassay

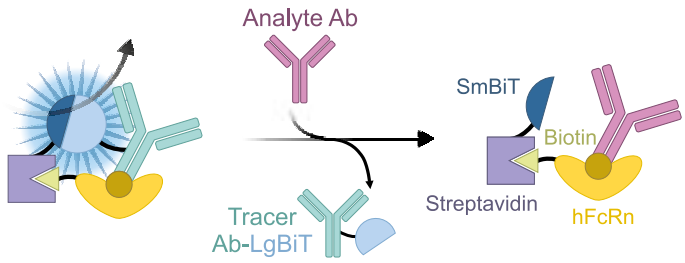
Description & Application

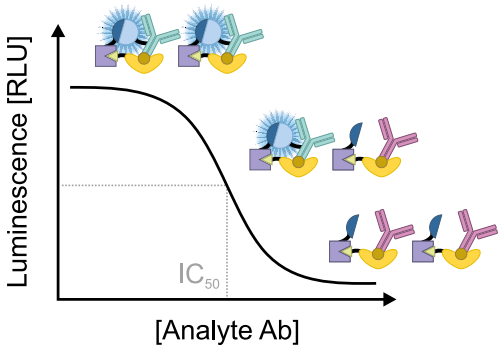
The Lumit® FcRn Binding Immunoassay is a homogeneous, no-wash competition assay to measure the interaction between human neonatal FcRn and Fc proteins, including antibodies. Importantly, the in-solution format circumvents experimental artifacts caused by immobilization. This assay is used in therapeutic antibody development to assess and tune the half-life of antibodies due to optimized binding to FcRn. In addition, it is used for determining antibody oxidation status and for detecting anti-FcRn blocking antibodies.

Principle & Workflow

The assay consists of a LgBiT-labeled human IgG1 (Tracer Ab-LgBiT) and a biotinylated human FcRn (extracellular domain) bound to SmBiT-labeled streptavidin (hFcRn-Biotin-Streptavidin-SmBiT). In the absence of an analyte antibody (Analyte Ab), tracer binding to labeled hFcRn results in maximum luminescent signal. Binding of analyte antibodies is evident from a concentration-dependent decrease in luminescence due to competition with the tracer.

Assay Principle





Assay Features

Sample material	Antibodies Fc proteins
Sample volume	25 µl antibody
Concentration range	4 – 4000 ng/ml
Assay format	Loss-of-signal assay 96- / 384-well plates
Assay protocol	Homogeneous Add-and-read
Time required	70 minutes or less

References

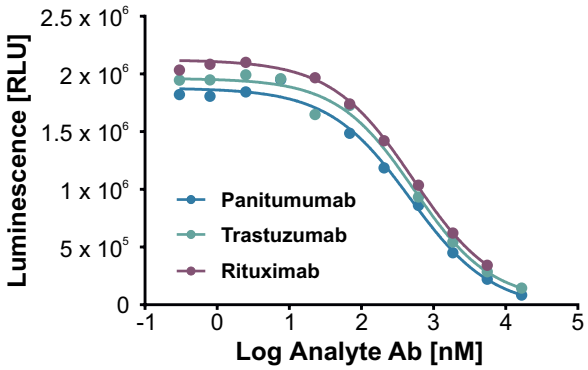
Nath, N. *et al.* (2021) *Deciphering the interaction between neonatal Fc receptor and antibodies using a homogeneous bioluminescent immunoassay.* **J Immunol. 207(4), 1211–1221.**

Tian, Z. *et al.* (2021) *Harnessing the power of antibodies to fight bone metastasis.* **Sci Adv. 7(26), eabf2051**

Representative Data

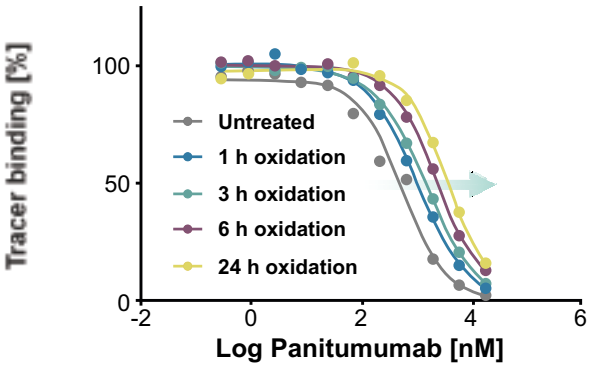
FcRn binding to a panel of therapeutic antibodies

A panel of therapeutic antibodies were tested for their affinity to FcRn by using the Lumit® FcRn Binding Immunoassay. An excellent assay window is observed for IgG/FcRn binding.



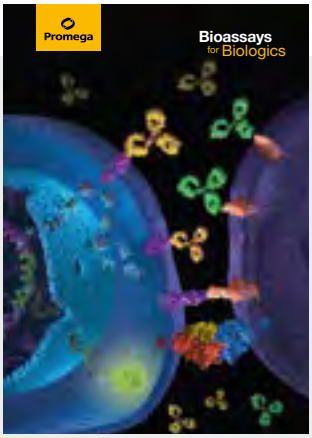
Oxidation-based loss in antibody-FcRn affinity

A therapeutic antibody was incubated with 0.3 % H_2O_2 for 1–24 hours inducing methionine oxidation. Dose-dependent, oxidation-based loss in antibody-FcRn affinity was readily detected by using the Lumit® FcRn Binding Immunoassay.



Product Box

Lumit® FcRn Binding Immunoassay	Cat.# W1151, W1152
Lumit® Canine FcRn Binding Immunoassay	Cat.# CS3019A02
Lumit® Feline FcRn Binding Immunoassay	Cat.# CS3631B01, CS3631B05



Interested in Cell-Based Reporter Bioassays for Biologics Characterization?

Find more information about mechanism of action (MOA) assays such as ADCC and ADCP in Promega's Bioassay Guide for biologics characterization and lot-release.

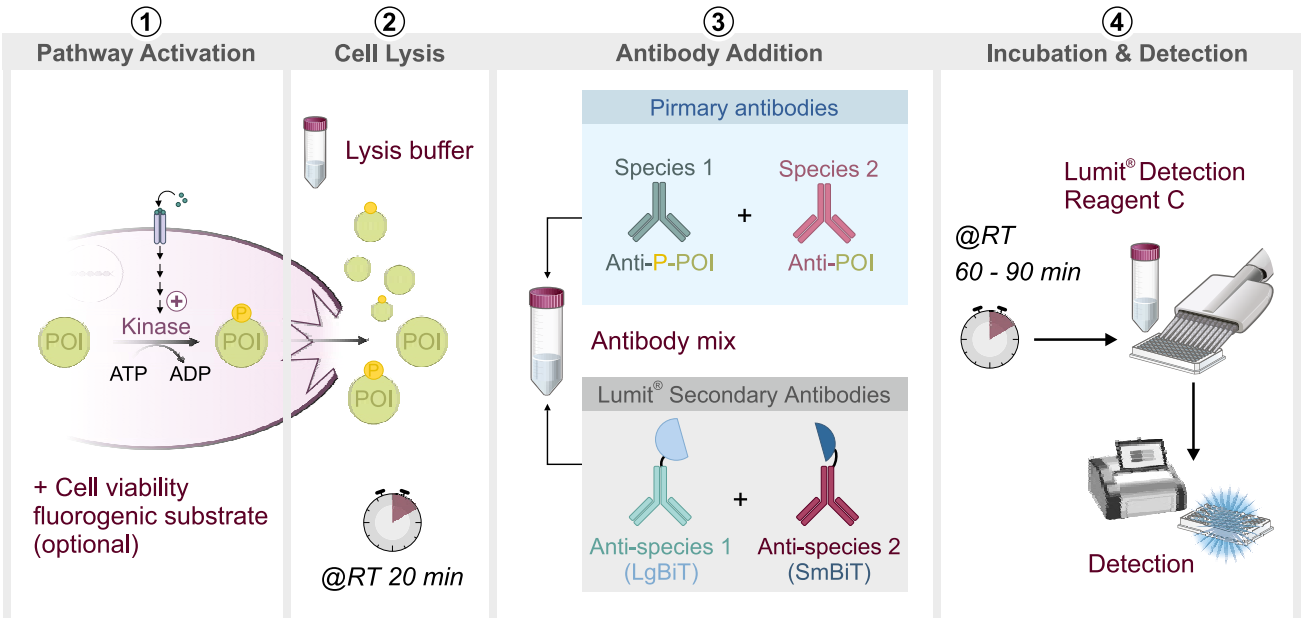
www.promega.com/BioassaysForBiologics

2.7 Signaling & Protein Degradation

Lumit® Immunoassay Cellular Systems

Description & Application

The Lumit® Immunoassay Cellular Systems have been extensively validated for analyzing kinase signaling pathways and targeted protein degradation in cell lysates. The complete kits include two different lysis buffers, analyte-specific primary antibodies, matched pre-labeled Lumit® Secondary Antibodies, and detection reagent. Kits containing only secondary antibodies and detection reagents are also available, allowing detection of analytes with your chosen primary antibodies. A growing list of pre-evaluated target proteins is available (see page 29), along with downloadable application notes detailing the primary antibodies used.

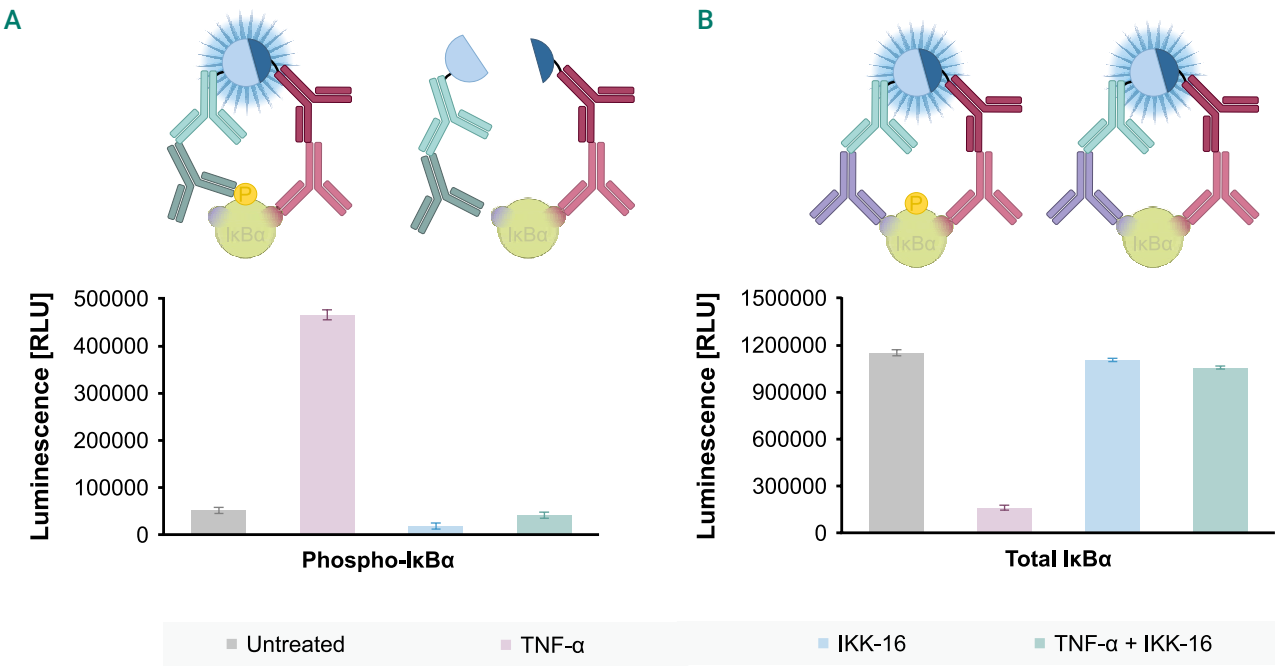


Principle & Workflow

The Lumit® Immunoassay Cellular Systems are based on the indirect Lumit® detection format in which two primary antibodies are mixed with two matching secondary antibodies that were pre-labeled with either the LgBiT or SmBiT subunit. To date, this format has been extensively validated for cell signaling analysis in cell lysates with a focus on phosphorylation as well as for measuring targeted protein degradation. The experimental workflow starts with (1) the treatment of cells to activate the signaling pathway of interest. At this point, the included cell viability fluorogenic substrate can be applied if normalization to viable cell number is desired. (2) Cells are lysed in-well by addition of a NanoBiT® compatible lysis buffer (Digitonin or Lumit® Lysis Buffer II). (3) Following addition of the antibody mix (primary and Lumit® Secondary Antibodies) and (4) incubation at room temperature for 60 to 90 minutes, the luminescent signal of the assay is determined by addition of the Lumit® detection reagent.

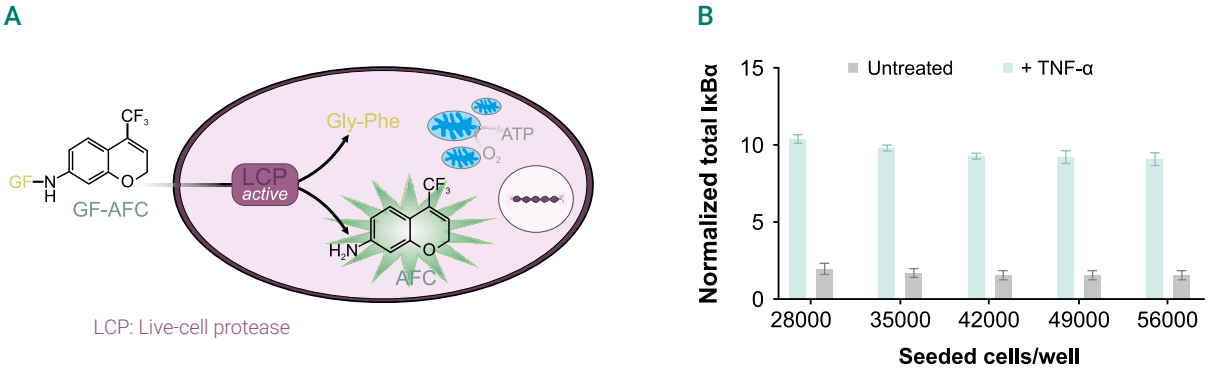
> *"Unmatched simplicity and reliability, with unbelievably fast results."*
>
> *Scientist at Arctoris*

Representative Data Signaling



Detection of phosphorylated and total proteins within the NFκB signaling pathway

MCF-7 cells were seeded at 50000 cells/well and treated with TNF-α (20 ng/ml) for 30 minutes to induce the NFκB pathway. Cells in specificity control wells were pretreated with an IKK complex specific inhibitor, IKK16 (10 µM, 1 hour). Subsequently, (A) phosphorylated IκBα (S32) or (B) total IκBα levels were determined in separate wells using two sets of primary antibodies.



LCP: Live-cell protease

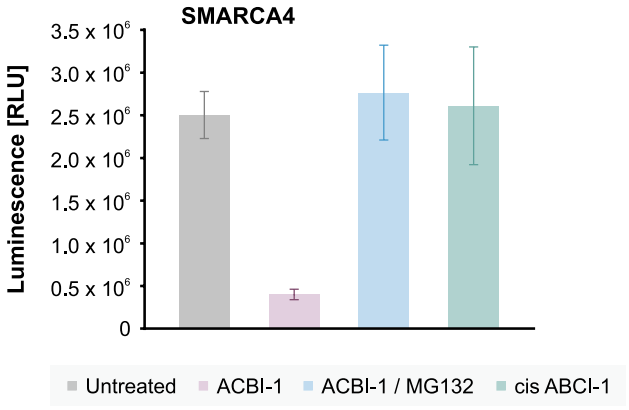
Normalization of luminescence data to viable cell number per well

Different cell numbers were treated with 50 ng/ml TNF-α for 30 minutes. (A) Cell viability fluorogenic substrate GF-AFC was added to all cells 30 minutes before lysis. This assay is based on the proteolytic processing of GF-AFC by Live-cell protease within viable cells to form a fluorescent product. Following cell lysis, an antibody mixture to detect total IκBα was added. (B) Luminescence and fluorescence were read at the end of the experiment. Normalization of Lumit® Immunoassay data to cell viability allows to easily account for well-to-well differences in cell number.

Representative Data Targeted Protein Degradation

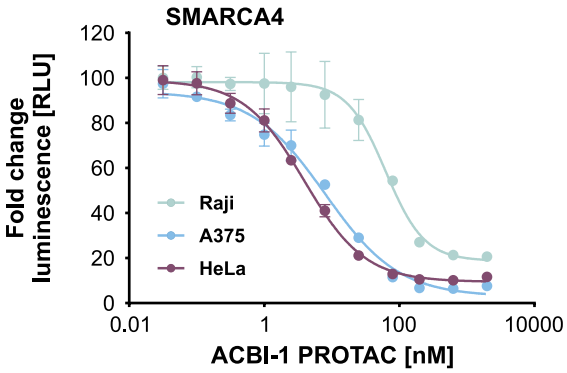
Degradation of SMARCA4 upon PROTAC® treatment

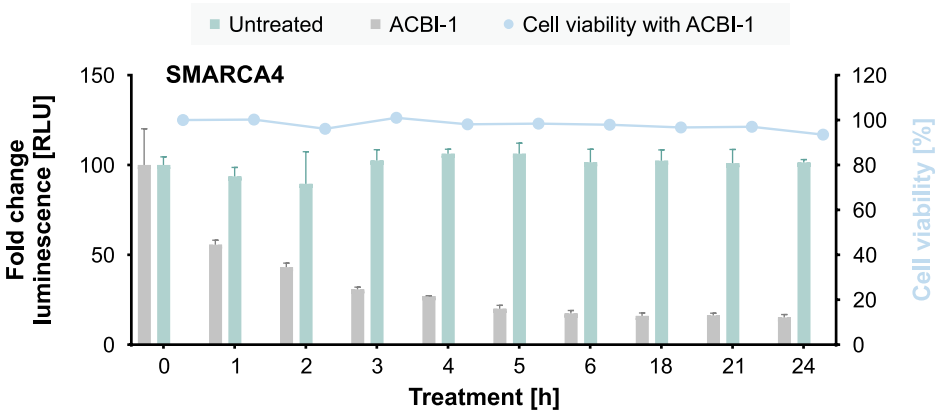
A375 cells (50000 cells/well) were treated with a single dose of ACBI-1 (250 nM) for 5 hours and targeted degradation of native SMARCA4 was assessed using the Lumit® Total SMARCA4 Immunoassay Cellular System. No degradation was observed when the stereoisomer cis ACBI-1 (250 nM) was used or when cells were co-treated with the proteasomal inhibitor MG132 (20 µM).



Cell line-dependent SMARCA4 degradation profiles

Degradation potency of the PROTAC ACBI-1 was determined in three different cell lines, i.e., Raji, A375, and HeLa. Cells were seeded (50000 cells/well) and treated with a dilution series of the PROTAC ACBI-1 for 24 hours. Different DC_{50} values were observed for Raji (62.9 nM), A375 (8.0 nM), and HeLa (3.9 nM) cells.





Time course analysis of PROTAC-induced protein degradation of SMARCA4

Targeted degradation of native SMARCA4 in A375 cells (50000 cells/well) in the presence of ACBI-1 (100 nM) was monitored every 60 minutes over a period of 24 hours. Cell viability was determined by multiplexing with the fluorogenic substrate GF-AFC.

Pre-validated Targets

A list of application notes for pre-validated targets is available to save you time and labor. These notes contain target-specific protocols, representative data, and information on the commercially available antibodies used. The following table lists pre-validated targets for which no Complete Assays are available.

Cellular Target	Total protein	Phospho-Protein	Set ID	Lysis Buffer
Phospho-4E-BP1		Yes (Ser 65)	Set 2	Digitonin lysis solution
AKT	Yes		Set 1	Lumit® Lysis Buffer II
B-cell lymphoma 6 protein (BCL-6)	Yes		Set 1	Digitonin lysis solution
β-catenin (human)	Yes	Yes (Thr 41/Ser 45)	Set 2	Digitonin lysis solution
c-Jun		Yes (Ser 63)	Set 1	Digitonin lysis solution
c-MET		Yes (Tyr 1349)	Set 1	Lumit® Lysis Buffer II
CREB	Yes	Yes (Ser 133)	Set 1	Digitonin lysis solution
EGFR		Yes (Tyr 1173)	Set 1	Lumit® Lysis Buffer II
ER (Estrogen Receptor)	Yes		Set 1	Digitonin lysis solution
GSK1-3B		Yes (Ser 9)	Set 1	Lumit® Lysis Buffer II
HER2		Yes (Tyr 1196) and (Tyr 1221/1222)	Set 1	Lumit® Lysis Buffer II
p65	Yes		Set 2	Lumit® Lysis Buffer II
Rb (Retinoblastoma Tumor Suppressor)	Yes	Yes (Ser 780)	Set 2	Digitonin lysis solution
Ribosomal Protein S6	Yes		Set 1	Lumit® Lysis Buffer II
Smad1	Yes	Yes (Ser 463/465)	Set 1	Digitonin lysis solution
Smad2	Yes	Yes (Ser 465/467)	Set 2	Digitonin lysis solution
STAT1	Yes	Yes (Ser 727) and (Tyr 701)	Set 1	Digitonin lysis solution
Phospho-STAT2		Yes (Tyr 690)	Set 1	Digitonin lysis solution

Visit our website to browse the most recent application notes!

www.promega.com/LumitCellularSystems

Product Box

Lumit® Immunoassay Cellular System – Starter Kit
Cat.# W1220

Lumit® Immunoassay Cellular System – Set 1
Cat.# W1201, W1202, W1203

Lumit® Immunoassay Cellular System – Set 2
Cat.# W1331, W1332, W1333

Lumit® Immunoassay Cellular System Complete Assays
Cat.# see page 40

Includes:

- *Lumit® Secondary Antibodies of Set 1 and Set 2*
- *Lumit® Anti-Mouse Ab-LgBiT*
  Lumit® Anti-Rabbit Ab-SmBiT
- *Lumit® Anti-Mouse Ab-SmBiT*
  Lumit® Anti-Rabbit Ab-LgBiT
- *Primary antibodies with matching Lumit® Secondary Antibodies*

3. Build-Your-Own Lumit® Immunoassays

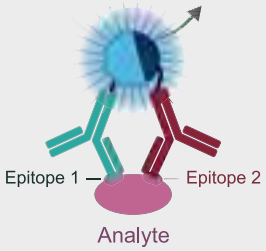
3.1 Lumit® Immunoassay Toolbox

Explore in chapter 3 how the Lumit® toolbox supports you in building an assay tailored to your needs!

Antibody / Protein Labeling Kit

Set up your own Lumit® assay by rapidly and efficiently labeling antibodies/proteins using the Lumit® Immunoassay Labeling Kit.

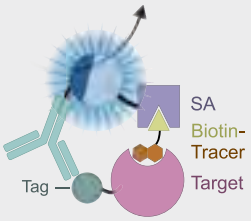
see page 31



Pre-Labeled Anti-Tag Antibodies / Streptavidin

Build your own protein:protein or protein:small molecule interaction assay using Lumit® anti-tag antibodies (for His-, GST-, FLAG®-, and human Fc-tagged proteins) and Lumit® Streptavidin (for AviTag™-tagged and biotinylated proteins).

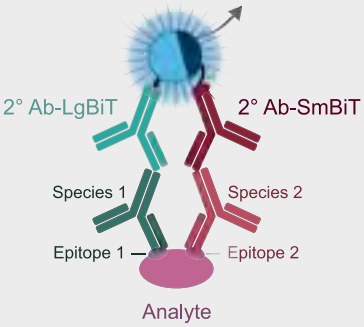
see page 32



Pre-Labeled Secondary Antibodies

Create an indirect Lumit® Immunoassay by using pre-labeled secondary antibodies against IgGs from mouse, rabbit, and goat.

see page 34



Detection Reagents

Choose between different detection reagents optimized for distinct applications, e. g., biochemical or cell-based assays.

see page 35

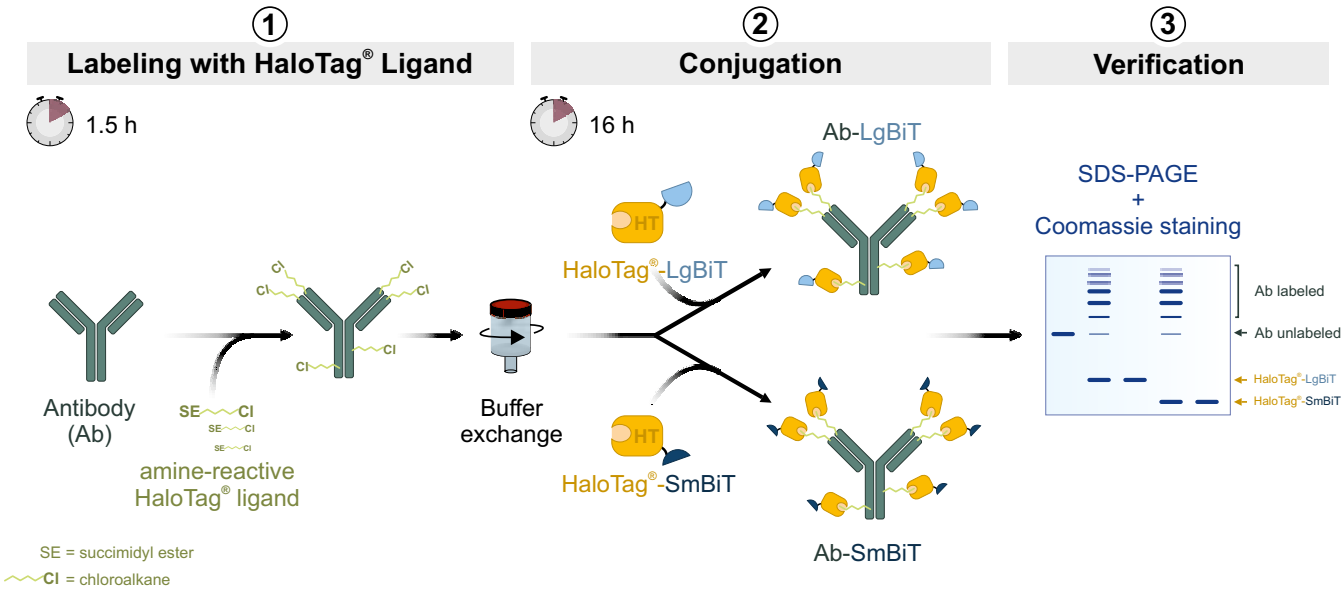
3.2 Lumit® Immunoassay Labeling Kit

Description & Application

The labeling kit is designed to conjugate antibodies / proteins to SmBiT and LgBiT supporting the development of your own Lumit® Immunoassay.

Principle & Workflow

The chemical labeling reaction is based on the HaloTag® technology. HaloTag® is a protein that covalently binds chloroalkane ligands (HaloTag® ligand) under physiological conditions and is used in a variety of applications, including antibody labeling. Labeling is a two-step process in which amine-reactive HaloTag® Succinimidyl Ester (O4) ligand reacts with primary amines of lysine amino acids on the antibodies/proteins **(1)**. For this reaction, antibodies should be in an amine-free buffer without any protein preservative. Antibodies labeled with the HaloTag® ligand are then incubated with HaloTag®-LgBiT or HaloTag®-SmBiT fusion protein to make a covalent conjugate of antibody-HaloTag®-LgBiT or antibody-HaloTag®-SmBiT **(2)**. The success of the labeling reaction is confirmed by SDS-PAGE and Coomassie staining **(3)**. Guidance for the labeling procedure is provided in the technical manual #TM602.



Product Box

Lumit® Immunoassay Labeling Kit
Cat.# VB2500

References

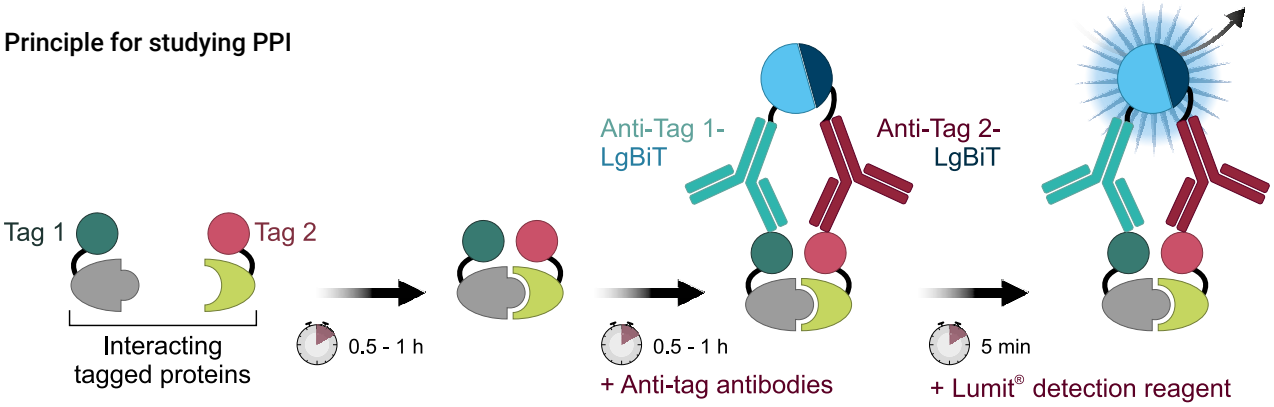
Alsulami, T. *et al.* (2021) *Development of a novel homogeneous immunoassay using the engineered luminescent enzyme NanoLuc for the quantification of the mycotoxin fumonisin B1.* **Biosens Bioelectron. 177, 112939**

3.3 Lumit® Anti-Tag Protein Interaction Reagents

Description & Application

Lumit® Anti-Tag Protein Interaction Reagents include a selection of BiT-labeled antibodies targeting common protein tags (e.g., His-, Flag®-, GST-tag, and human Fc) as well as BiT-labeled streptavidin. These reagents simplify the setup of biochemical assays to study protein:protein interactions (PPI) and screen for modulators of these interactions. Additionally, they enable the investigation of protein:small molecule interactions in a straightforward, competition-based, and HTS-compatible format.

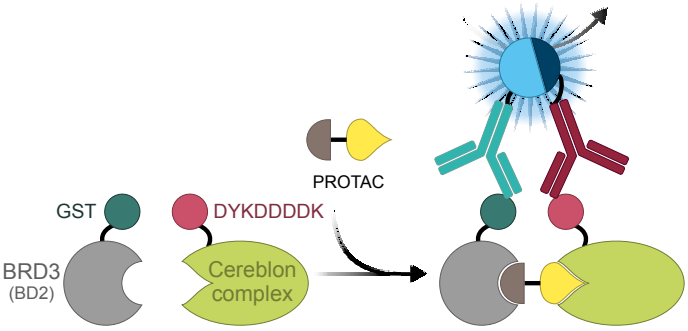
Principle for studying PPI

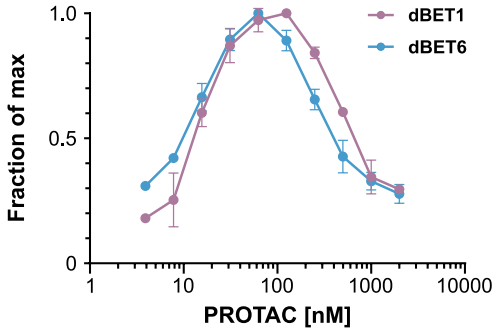


Principle & Workflow

To study protein:protein interactions, two differentially tagged proteins are incubated, with the option to include a compound that modulates the PPI of interest. After incubation, BiT-labeled Lumit® anti-tag antibodies are added. Following the addition of the Lumit® detection reagent, the luminescent signal is recorded using a plate-reading luminometer. For AviTag™-tagged or biotinylated proteins, one of the BiT-labeled Lumit® anti-tag antibodies can be replaced with Lumit® Streptavidin-LgBiT or -SmBiT.For protein:small molecule interaction studies, a biotinylated small molecule (tracer) and a tagged protein are required. The interaction between the tracer and target protein is detected using a BiT-labeled anti-tag antibody and Lumit® Streptavidin-LgBiT or -SmBiT. Target engagement by unlabeled test compounds is reflected by a decrease in the luminescent signal, caused by competitive displacement of the tracer.

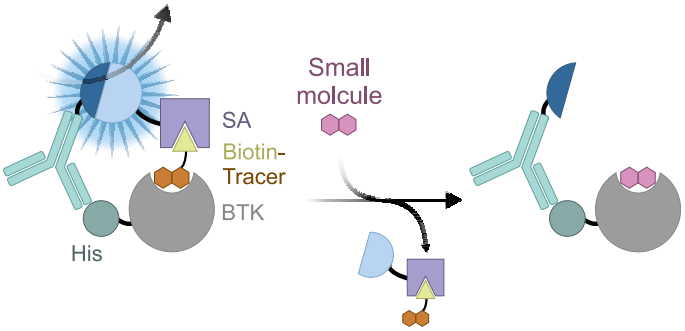
Representative Data

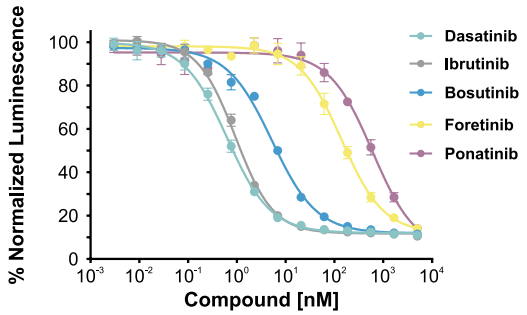




Monitor PROTAC®-induced protein:protein interactions

The ability of the PROTACs dBET1 and dBET6 to induce ternary complex formation between the Cereblon E3 ligase and BRD3 (BD2) was evaluated in a Lumit® Immunoassay. Recombinant GST-tagged BRD3 (BD2) (6.25 nM) and FLAG®-tagged Cereblon (6.25 nM) were incubated with different concentrations of PROTACs for 60 minutes. Detection was accomplished using Lumit® Anti-GST-LgBiT, Lumit® Anti-DYKDDDDK-SmBiT and Lumit® Immunoassay Detection Reagent A.





Detection and characterization of protein:small molecule interactions

The relative binding affinities of different kinase inhibitors for Bruton's tyrosine kinase (BTK) were determined in a competitive Lumit® Immunoassay. Recombinant His-tagged BTK (5 nM) was incubated with a biotinylated Ibrutinib (tracer; 37.5 nM) and kinase inhibitors at different concentrations (0.003 – 5000 µM) for 60 minutes with mild agitation. Samples were incubated for 30 minutes with a mix of Streptavidin-LgBiT and Lumit® Anti-6His-SmBiT. The fraction of tracer-bound BTK at equilibrium was detected upon addition of Lumit® Immunoassay Detection Reagent A.

Product Box

Lumit® Anti-6His-LgBiT and -SmBiT
Cat.# W1600

Lumit® Anti-GST-LgBiT and -SmBiT
Cat.# W1620

Lumit® Anti-DYKDDDDK-LgBiT and SmBiT
Cat.# W1640

Lumit® Anti-Human IgG-LgBiT and -SmBiT
Cat.# CS332214

Lumit® Streptavidin-LgBiT and -SmBiT
Cat.# W1660

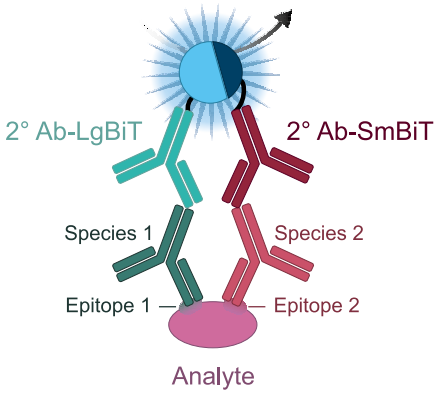
Combine with Lumit® Detection Reagent A

3.4 Lumit® Secondary Antibodies

Description & Application

Lumit® Secondary Antibodies are polyclonal BiT-labeled antibodies targeting IgG, enabling the development of Lumit® assays using the indirect immunoassay format. These antibodies are raised in donkey, immunoaffinity-purified using immobilized antigens, and conjugated to either SmBiT or LgBiT. Specificity against heavy and light chains of IgG (mouse, rabbit, and goat) has been confirmed.

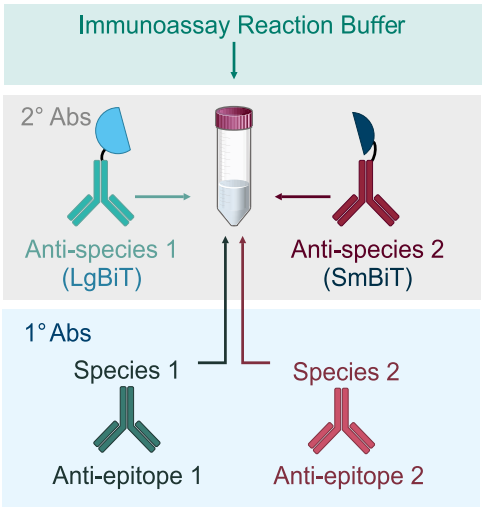
Indirect Assay Format



Principle & Workflow

To establish an indirect Lumit® Immunoassay for a new target, refer to the technical manual #TM613. In brief, users need to provide two analyte-specific primary antibodies raised in different species (e.g., mouse anti-analyte and rabbit anti-analyte). These are combined with matching Lumit® Secondary Antibodies (e.g., Lumit® Anti-Mouse Ab-LgBiT and Lumit® Anti-Rabbit Ab-SmBiT) to yield an antibody mix. Initially, it is recommended to test two or three primary antibody pairs. The best combination and concentration to use in an assay are determined through checkerboard experiments.

Preparation of Antibody Mix



Product Box

Lumit® Anti-Mouse Ab-LgBiT
Cat.# W1021, W1022

Lumit® Anti-Rabbit Ab-LgBiT
Cat.# W1041, W1042

Lumit® Anti-Rat Ab-LgBiT
Cat.# CS366187, CS366188

Lumit® Anti-Mouse Ab-SmBiT
Cat.# W1051, W1052

Lumit® Anti-Rabbit Ab-SmBiT
Cat.# W1031, W1032

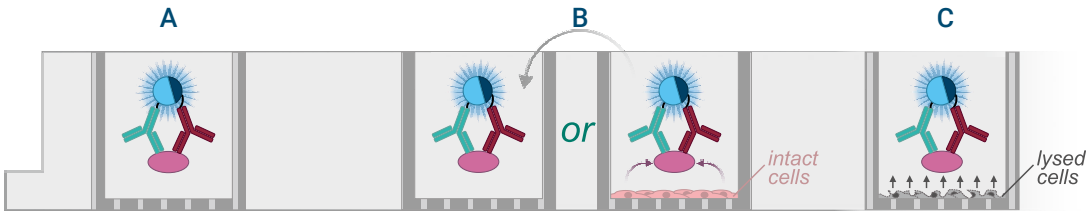
Lumit® Anti-Rat Ab-SmBiT
Cat.# CS366185, CS366186

3.5 Lumit® Immunoassay Detection Reagents

Description & Application

Lumit® Immunoassay Detection Reagents support the development of customized Lumit® Immunoassays. Three reagent types (A, B, C) are available, enabling analyte detection under varying conditions. Detection Reagent A is intended for use in the absence of cells and serum, while Detection Reagent B is optimized for cell culture supernatant containing up to 10 % FBS and/or in the presence of intact cells. Detection Reagent C is included in the Lumit® Immunoassay Lysis and Detection Kit and is recommended for cell lysates.

Lumit® Immunoassay Detection Reagents



Use for ...	• *In vitro* samples	• Cell culture supernatant	• Cell lysates
	• Free of cells	• With serum	
	• Free of serum	• With/without cells	

Principle & Workflow

Detailed guidelines for setting up Lumit® Immunoassays are available in technical manuals #TM613, #TM602, and #TM614. These manuals provide descriptions on how to optimize Lumit® Immunoassays, including tips for overcoming matrix effects and performing checkerboard experiments.

Product Box

Lumit® Immunoassay Detection Reagent A
Cat.# VB2010, VB2020, VB2030

Lumit® Immunoassay Detection Reagent B
Cat.# VB4050, VB4060

Lumit® Immunoassay Lysis and Detection Kit
Cat.# W1231, W1232, W1233

→ *Contains Lumit® Detection Reagent C*

4. Ordering Information

Pre-Built Lumit® Immunoassays

Cytokines / HMGB1

Product	Cat. #	Size
Lumit® IL-1β (Human) Immunoassay	W6010 W6012 W6011	100 assays 500 assays 1000 assays
Lumit® IL-1β (Human) Standard	W116A-C	25 µl (10 µg/ml)
Lumit® IL-1β (Mouse) Immunoassay	W7010 W7012 W7011	100 assays 500 assays 1000 assays
Lumit® IL-1β (Mouse) Standard	W119A-C	25 µl (10 µg/ml)
Lumit® IL-2 (Human) Immunoassay	W6020 W6022 W6021	100 assays 500 assays 1000 assays
Lumit® IL-2 (Human) Standard	W122A-C	25 µl (10 µg/ml)
Lumit® IL-4 (Human) Immunoassay	W6060 W6062 W6061	100 assays 500 assays 1000 assays
Lumit® IL-4 (Human) Standard	W125A-C	25 µl (10 µg/ml)
Lumit® IL-6 (Human) Immunoassay	W6030 W6032 W6031	100 assays 500 assays 1000 assays
Lumit® IL-6 (Human) Standard	W128A-C	25 µl (10 µg/ml)
Lumit® IL-8 (Human) Immunoassay	W1460 W1462 W1461	100 assays 500 assays 1000 assays
Lumit® IL-8 (Human) Standard	W146A-C	25 µl (10 µg/ml)
Lumit® IL-10 (Human) Immunoassay	W6070 W6072 W6071	100 assays 500 assays 1000 assays
Lumit® IL-10 (Human) Standard	W131A-C	25 µl (10 µg/ml)
Lumit® IL-12 p70 (Human) Immunoassay	W1850 W1852 W1851	100 assays 500 assays 1000 assays
Lumit® IL-12 (Human) Standard	W185A-C	25 µl (10 µg/ml)

Cytokines / HMGB1

Product	Cat. #	Size
Lumit® Active IL-18 (Human) Immunoassay	W1910 W1912 W1911	100 assays 500 assays 1000 assays
Lumit® Active IL-18 (Human) Immunoassay	W191A-C	25 µl (10 µg/ml)
Lumit® IL-17A (Human) Immunoassay	W1430 W1432 W1431	100 assays 500 assays 1000 assays
Lumit® IL-17A (Human) Standard	W143A-C	25 µl (10 µg/ml)
Lumit® TNF-α (Human) Immunoassay	W6050 W6052 W6051	100 assays 500 assays 1000 assays
Lumit® TNF-α (Human) Standard	W137A-C	25 µl (10 µg/ml)
Lumit® IFN-β (Human) Immunoassay	W1810 W1812 W1811	100 assays 500 assays 1000 assays
Lumit® IFN-β (Human) Standard	W149A-C	25 µl (10 µg/ml)
Lumit® IFN-γ (Human) Immunoassay	W6040 W6042 W6041	100 assays 500 assays 1000 assays
Lumit® IFN-γ (Human) Standard	W134A-C	25 µl (10 µg/ml)
Lumit® VEGF-A (Human) Immunoassay	W1810 W1812 W1811	100 assays 500 assays 1000 assays
Lumit® VEGF-A (Human) Standard	W188A-C	25 µl (10 µg/ml)
Lumit® HMGB1 (Human/Mouse) Immunoassay	W1880 W1882 W1881	100 assays 500 assays 1000 assays
Lumit® HMGB1 (Human) Positive Control	W140A-C	20 µl (500 µg/ml)

4. Ordering Information

Pre-Built Lumit® Immunoassays

Hormones

Product	Cat. #	Size
Lumit® Insulin Immunoassay Kit	CS3037A05* CS3037A07*	100[1] – 400[2] assays 500[1] – 2000[2] assays
Lumit® Glucagon Immunoassay Kit	W8020 W8022	100[1] – 400[2] assays 500[1] – 2000[2] assays

[1] 96-well; [2] 384-well

Cell Proliferation

Product	Cat. #	Size
Lumit® hKi-67 Immunoassay for Cell Proliferation	CS3076A01*	100 assays

Second Messenger

Product	Cat. #	Size
Lumit® cAMP Immunoassay	CS3592A08*	100 assays
Lumit® cGAMP Immunoassay	CS3592A05*	100 assays

Cell & Gene Therapy

Product	Cat. #	Size
Lumit® dsRNA Detection Assay	W2041 W2042	100 assays 500 assays
Lumit® dsRNA Assay Buffer (5x)	W2010	8 ml
Lumit® dsRNA Standard	W2040	15 µl (100 µg/ml)
Lumit® dsRNA Standard, 5-methylcytidine	CS355716*	15 µl (100 µg/ml)
Lumit® dsRNA Standard, N1-methylpseudouridine	CS355712*	15 µl (100 µg/ml)
Lumit® dsRNA Standard, Pseudouridine	CS355710*	15 µl (100 µg/ml)
Lumit® dsRNA Standard, 5-methoxyuridine	CS355714*	15 µl (100 µg/ml)

* This is an Early Access Material. Please inquire for more information.

Therapeutic Antibody Development

Product	Cat. #	Size
Lumit® FcγRI Binding Immunoassay	W7080 W7081	100 assays 1000 assays
Lumit® FcγRIIa (H131) Binding Immunoassay	W7070 W7071	100 assays 1000 assays
Lumit® FcγRIIa (R131) Binding Immunoassay	W7060 W7061	100 assays 1000 assays
Lumit® FcγRIIb Binding Immunoassay	W7030 W7031	100 assays 1000 assays
Lumit® FcγRIIIa (F158) Binding Immunoassay	W7040 W7041	100 assays 1000 assays
Lumit® FcγRIIIa (V158) Binding Immunoassay	W7050 W7051	100 assays 1000 assays
Lumit® FcγRIIIb Binding Immunoassay	W7020 W7021	100 assays 1000 assays
Lumit® FcRn Binding Immunoassay	W1151 W1152	100 assays 1000 assays
Lumit® Canine FcRn Binding Immunoassay	CS3019A02*	100 assays
Lumit® Feline FcRn Binding Immunoassay	CS3631B01* CS3631B05*	100 assays 1000 assays

Signaling & Protein Degradation

Product	Cat. #	Size
Lumit® Immunoassay Cellular System – Starter Kit	W1220	200 assays
Lumit® Immunoassay Cellular System – Set 1	W1201 W1202 W1203	100 assays 1000 assays 10000 assays
Lumit® Immunoassay Cellular System – Set 2	W1331 W1332 W1333	100 assays 1000 assays 10000 assays

* This is an Early Access Material. Please inquire for more information.

4. Ordering Information

Pre-Built Lumit® Immunoassays

Signaling & Protein Degradation –
Lumit® Immunoassay Cellular Systems (ICS) Complete Assays

Product	Cat. #	Size
Lumit® Phospho-AKT (Ser 473) ICS	CS3397A07* CS3397A08*	100 assays 1000 assays
Lumit® Total BTK ICS	CS366152* CS366156*	100 assays 1000 assays
Lumit® Phospho-BTK (Tyr 223) ICS	CS3397A19* CS3397A20*	100 assays 1000 assays
Lumit® Total BRD4 ICS	CS366168* CS366172*	100 assays 1000 assays
Lumit® Phospho-CHK1 (Ser 317) ICS	CS366144* CS366148*	100 assays 1000 assays
Lumit® Total CK1α ICS	please enquire	100 assays 1000 assays
Lumit® p-cMET (Tyr 1234/1235) ICS	CS366136* CS366140*	100 assays 1000 assays
Lumit® Total EGFR ICS	CS366120* CS366124*	100 assays 1000 assays
Lumit® Phospho-EGFR (Tyr 1068) ICS	CS366104* CS366108*	100 assays 1000 assays
Lumit® Phospho-ERK (Thr 202) ICS	CS3397A03* CS3397A04*	100 assays 1000 assays
Lumit® Total GSPT1 ICS	CS366904* CS366908*	100 assays 1000 assays
Lumit® Phospho-H2AX (Ser 139) ICS	CS366112* CS366116*	100 assays 1000 assays
Lumit® Total IκBα ICS	CS3397A58* CS3397A62*	100 assays 1000 assays
Lumit® Phospho-IκBα (Ser 32) ICS	CS3397A15* CS3397A16*	100 assays 1000 assays
Lumit® Total IKFZ1 ICS	please enquire	100 assays 1000 assays
Lumit® Total IKFZ2 ICS	please enquire	100 assays 1000 assays

* This is an Early Access Material. Please inquire for more information.

Signaling & Protein Degradation –
Lumit® Immunoassay Cellular Systems (ICS) Complete Assays

Product	Cat. #	Size
Lumit® Total IKFZ3 ICS	please enquire please enquire	100 assays 1000 assays
Lumit® Phospho-JNK (Thr 183/Tyr 185) ICS	CS3397A34* CS3397A38*	100 assays 1000 assays
Lumit® Phospho-p65 (Ser 536) ICS	CS3397A42* CS3397A46*	100 assays 1000 assays
Lumit® Phospho-RB (Ser 807/811)	CS3397A26* CS3397A30*	100 assays 1000 assays
Lumit® Phospho-RPS6 (Ser 240/244) ICS	CS366176* CS366180*	100 assays 1000 assays
Lumit® Total SALL4 ICS	please enquire please enquire	100 assays 1000 assays
Lumit® Total SMARCA2 ICS	CS366128* CS366132*	100 assays 1000 assays
Lumit® Total SMARCA4 ICS	CS366160* CS366164*	100 assays
Lumit® Total STAT3 ICS	CS3397A50* CS3397A54*	100 assays
Lumit® Phospho-STAT3 (Tyr 705) ICS	CS3397A11* CS3397A12*	100 assays

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Need Technical Assistance?

Please contact our technical service:

www.promega.com/support/tech-support

4. Ordering Information

Build-Your-Own Lumit® Immunoassays

Lumit® Immunoassay Labeling Kit

Product	Cat. #	Size
Lumit® Immunoassay Labeling Kit	VB2500	1 kit

Lumit® Anti-Tag Protein Interaction Reagents

Product	Cat. #	Size
Lumit® Anti-6His-LgBiT and -SmBiT • Lumit® Anti-6His-LgBiT • Lumit® Anti-6His-SmBiT	W1600	20 µl each
Lumit® Anti-6His-LgBiT	W1601	200 µl
Lumit® Anti-6His-SmBiT	W1611	200 µl
Lumit® Anti-GST-LgBiT and -SmBiT • Lumit® Anti-GST-LgBiT • Lumit® Anti-GST-SmBiT	W1620	20 µl each
Lumit® Anti-GST-LgBiT	W1621	200 µl
Lumit® Anti-GST-SmBiT	W1631	200 µl
Lumit® Anti-DYKDDDDK-LgBiT and -SmBiT • Lumit® Anti-DYKDDDDK-LgBiT • Lumit® Anti-DYKDDDDK-SmBiT	W1640	20 µl each
Lumit® Anti-DYKDDDDK-LgBiT	W1641	200 µl
Lumit® Anti-DYKDDDDK-SmBiT	W1651	200 µl
Lumit® Anti-Human IgG-LgBiT and -SmBiT • Lumit® Anti-Human IgG-LgBiT • Lumit® Anti-Human IgG-SmBiT	CS332214*	20 µl each
Lumit® Anti-Human IgG-LgBiT	CS332222*	200 µl
Lumit® Anti-Human IgG-SmBiT	CS332223*	200 µl
Lumit® Streptavidin-LgBiT and -SmBiT [1)] • Lumit® Streptavidin-LgBiT • Lumit® Streptavidin-SmBiT	W1660	20 µl each
Lumit® Streptavidin-LgBiT	W1661	200 µl
Lumit® Streptavidin-SmBiT	W1671	200 µl
Lumit® Anti-Mouse Ab-LgBiT and -SmBiT	refer to page 43	
Lumit® Anti-Rabbit Ab-LgBiT and -SmBiT	refer to page 43	

[1)] Can be used with AviTag™-tagged proteins or biotinylated proteins/small molecules.
* This is an Early Access Material. Please inquire for more information.

If not indicated otherwise, the number of assays is given for 96-well plates.

Lumit® Secondary Antibodies

Product	Cat. #	Size
Lumit® Immunoassay Cellular System – Set 1	W1201	100 assays
	W1202	1000 assays
	W1203	10000 assays
Lumit® Immunoassay Cellular System – Set 2	W1331	100 assays
	W1332	1000 assays
	W1333	10000 assays
Lumit® Anti-Mouse Ab-LgBiT	W1021	30 µl
	W1022	300 µl
Lumit® Anti-Mouse Ab-SmBiT	W1051	30 µl
	W1052	300 µl
Lumit® Anti-Rabbit Ab-LgBiT	W1041	30 µl
	W1042	300 µl
Lumit® Anti-Rabbit Ab-SmBiT	W1031	30 µl
	W1032	300 µl
Lumit® Anti-Rat Ab-LgBiT	CS366187*	30 µl
	CS366188*	300 µl
Lumit® Anti-Rat Ab-SmBiT	CS366185*	30 µl
	CS366186*	300 µl

Lumit® Immunoassay Detection Reagents

Product	Cat. #	Size
Lumit® Immunoassay Detection Reagent A	VB2010	500 assays
	VB2020	5000 assays
	VB2030	50000 assays
Lumit® Immunoassay Detection Reagent B	VB4050	100 Assays
	VB4060	1000 Assays
Lumit® Immunoassay Lysis and Detection Kit	W1231	100 assays
	W1232	1000 assays
	W1233	10000 assays

* This is an Early Access Material. Please inquire for more information.

For Research Use Only. Not for Use in Diagnostic Procedures.

5. References

Build-Your-Own Lumit® Immunoassay

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- Alves, J. *et al.* (2021) *A bioluminescent and homogeneous SARS-CoV-2 spike RBD and hACE2 interaction assay for antiviral screening and monitoring patient neutralizing antibody levels.* **Sci Rep. 11(1):18428**

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- Nath, N. *et al.* (2021) *Deciphering the interaction between neonatal Fc receptor and antibodies using a homogeneous bioluminescent immunoassay.* **J Immunol. 207(4):1211–1221**
- Tian, Z. *et al.* (2021) *Harnessing the power of antibodies to fight bone metastasis.* **Sci Adv. 7(26):eabf2051**

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- Yang, B. *et al.* (2022) *Discovery of a potent GIPR peptide antagonist that is effective in rodent and human systems.* **Mol Metab. 66:101638**
- Viloria, K. *et al.* (2023) *GC-globulin/vitamin D-binding protein is required for pancreatic α-cell adaptation to metabolic stress.* **Diabetes. 72(2):275-289**

Lumit® Cytokine Immunoassays

- Reed, B. and Landry GL. (2023) *Diglycolic acid inhibits succinate dehydrogenase activity, depletes mitochondrial membrane potential, and induces inflammation in an SH-SY5Y neuroblastoma model of neurotoxicity in vitro.* **Toxicol Appl Pharmacol. 15:463:116414**

Lumit® Anti-Tag Protein Interaction Reagents

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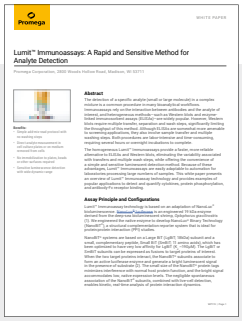
Lumit® cGAMP Immunoassay

- Hsiao, K. *et al.* (2024) *Homogeneous and bioluminescent biochemical and cellular assay for monitoring cGAMP and enzymes that generate and degrade cGAMP.* **Sci Rep. 14(1):31165**

Lumit® cAMP Immunoassay

- Mikheil, D. *et al.* (2024) *A bioluminescent and homogenous assay for monitoring GPCR-mediated cAMP modulation and PDE activity.* **Sci Rep. 14(1):4440**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WHITE PAPER

Lumit™ Immunoassays: A Rapid and Sensitive Method for Analyte Detection

Promega Corporation, 2800 Woods Hollow Road, Madison, WI 53711

Abstract

Assay Principle and Configurations

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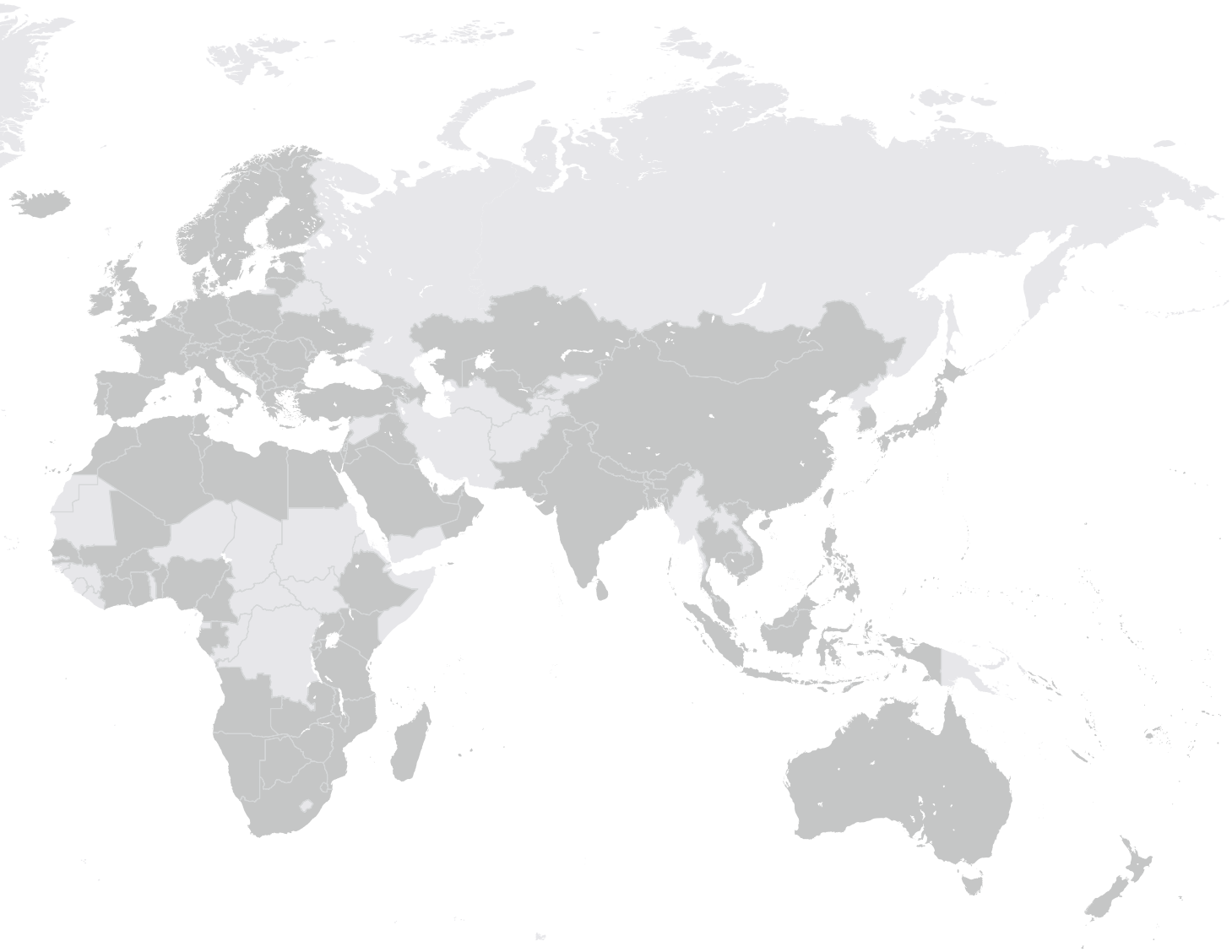

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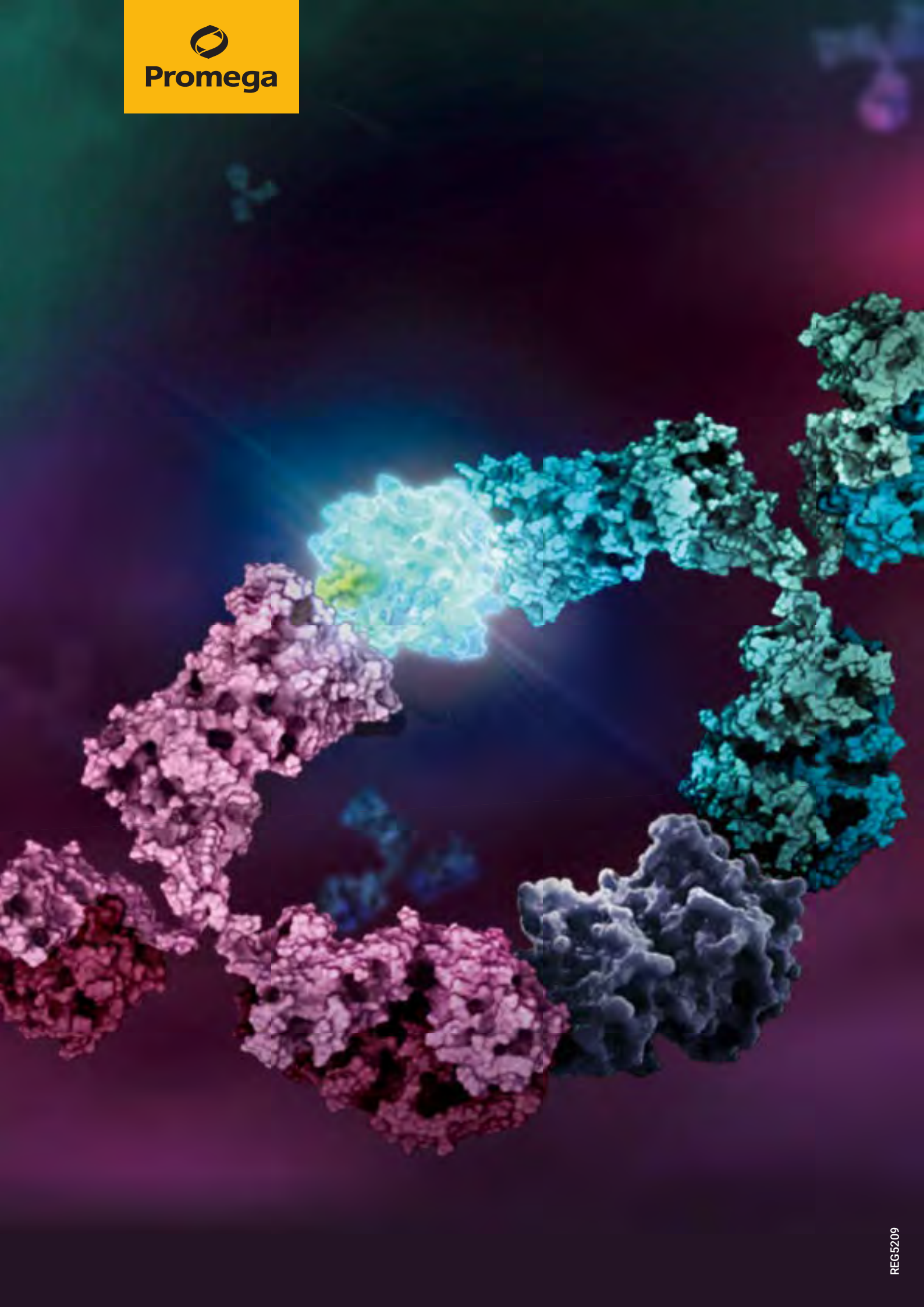

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