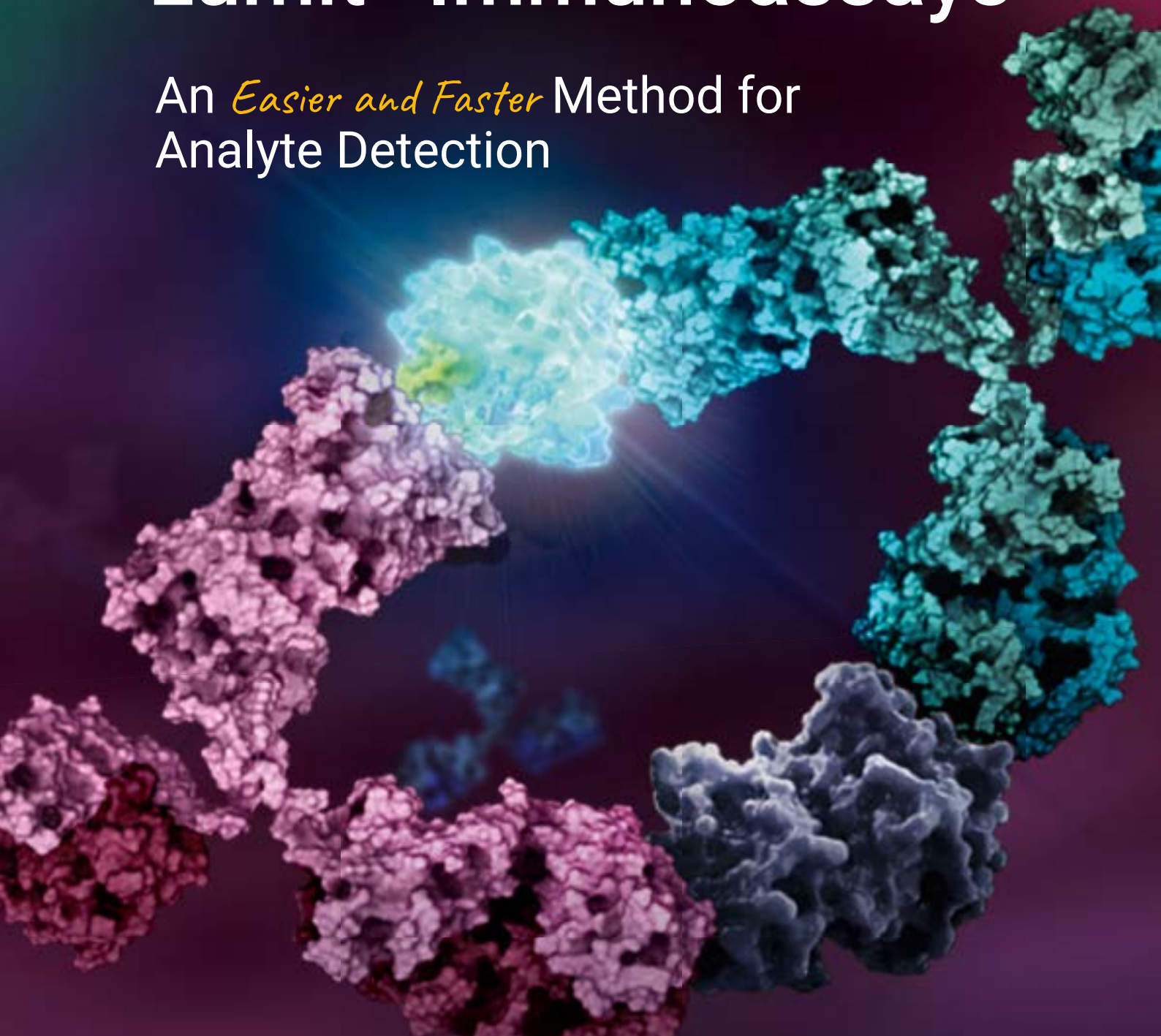


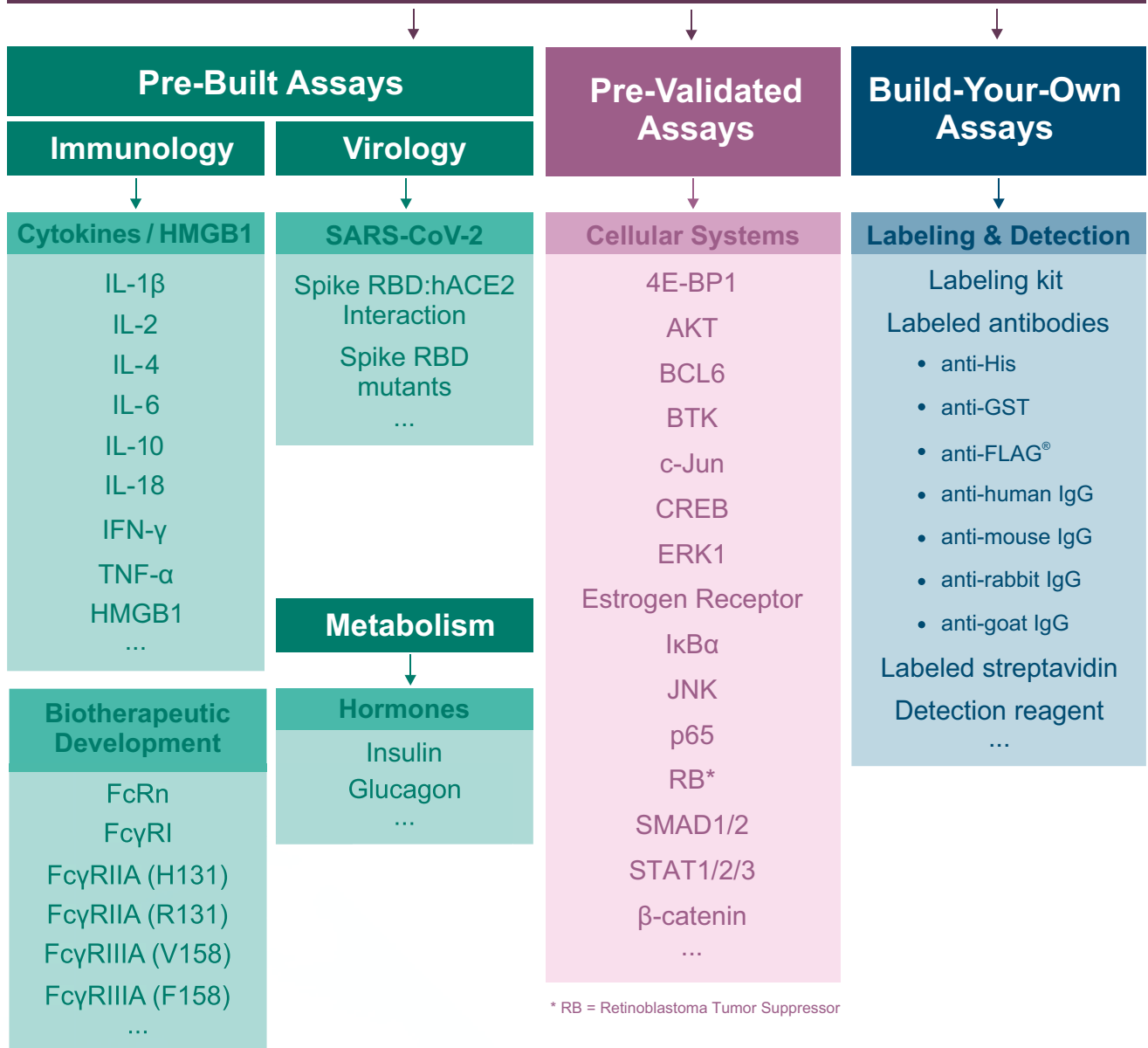
Lumit[®] Immunoassays

An *Easier and Faster* Method for
Analyte Detection



Metabolic Regulators | Immunogenic Cell Death | Cytokines
Biotherapeutic Development | Signaling Pathway Analysis
Protein Interaction

Lumit[®] Immunoassays



Time is precious

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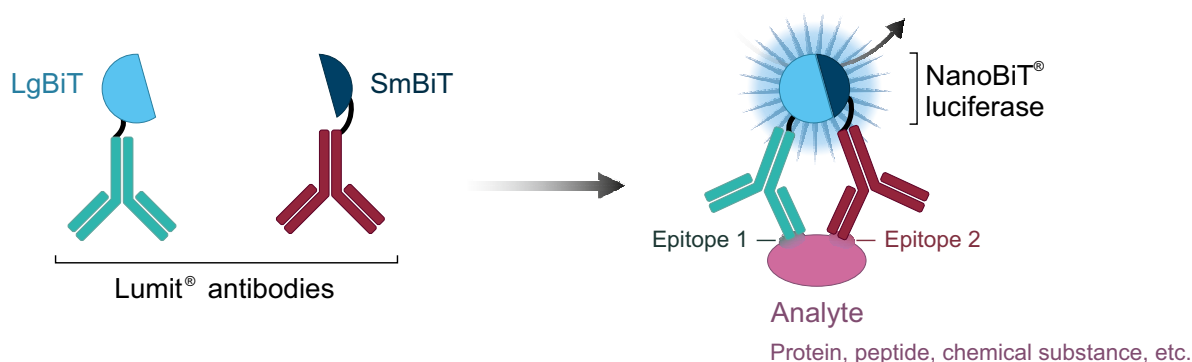
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1. Introduction

1.1 Lumit® Technology

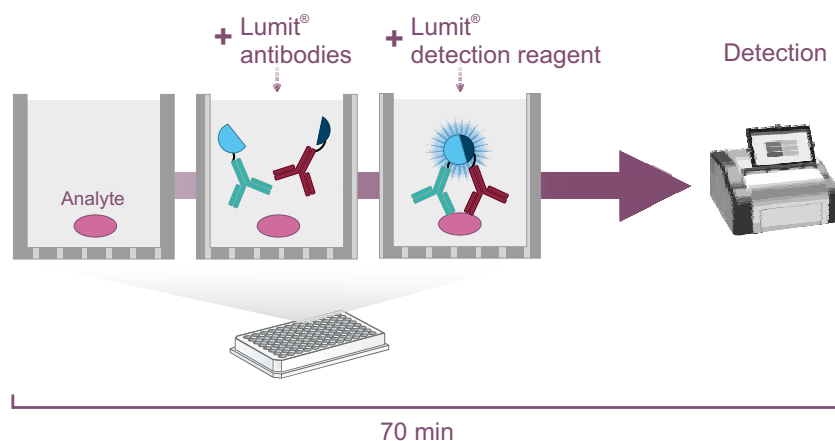
Detection and quantification of analytes is often performed using time-consuming multi-step methods such as Western blotting and ELISA. The Lumit® technology is a simple and fast alternative to run homogeneous immunoassays in multi-well plate formats. Its high specificity and amenability to high-throughput screening (HTS) make it a powerful tool for scientists doing 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 subunits of NanoLuc® luciferase, i.e. SmBiT and LgBiT, respectively. Direct or indirect binding to the analyte yields spatial proximity of the labeled antibodies and enables SmBiT and LgBiT to reconstitute the NanoBiT® luciferase. In presence of its substrate furimazine, a bright luminescence can be detected that is directly proportional to the amount of analyte present 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

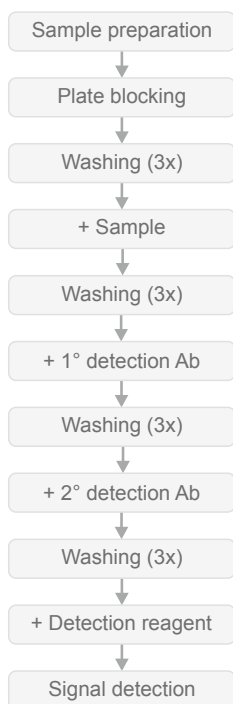
- Simple add-and-read protocol with no washing steps
- Fast results with reduced hands-on time
- No immobilization to plates, beads, or other surfaces required
- Direct analyte measurement in cell culture plates
- Sensitive luminescence detection with a wide dynamic range
- Detection on a conventional 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.

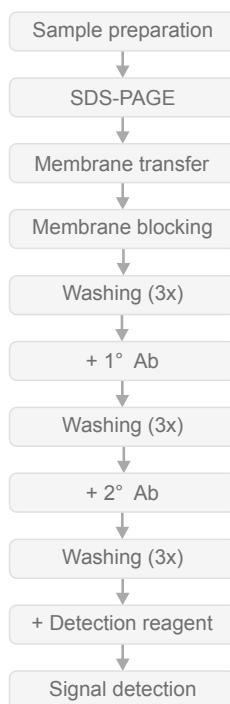
Your Short-Cut to High-Quality Data

ELISA



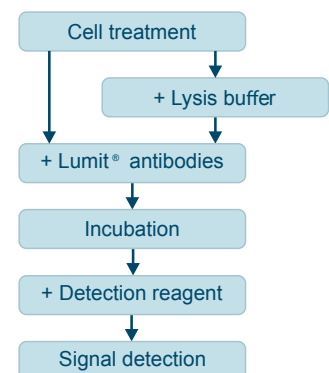
3 – 5 h

Western blot



1 – 2 d

Lumit®



0.5 – 2 h

Time is precious

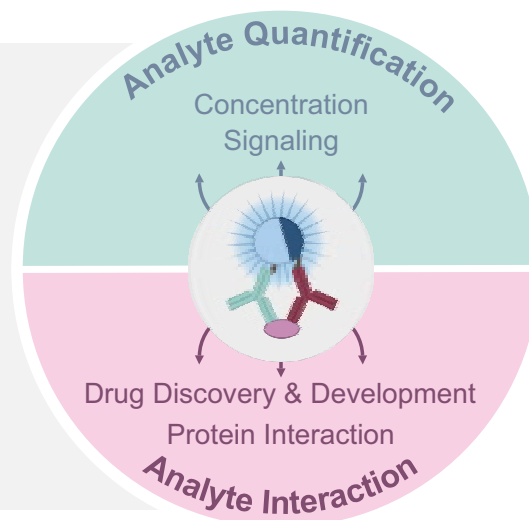
1. Introduction

1.2 Applications & Assay Formats

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

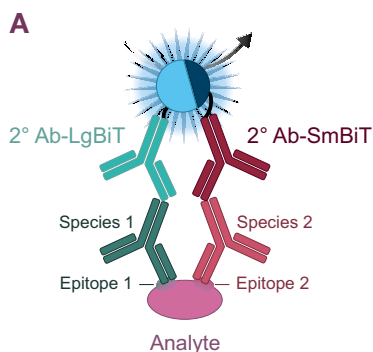
Applications

- Analyte quantification 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)

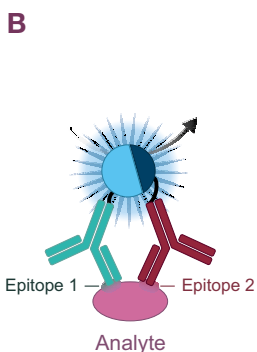


Analyte Quantification

Indirect Assay Format



Direct Assay Format

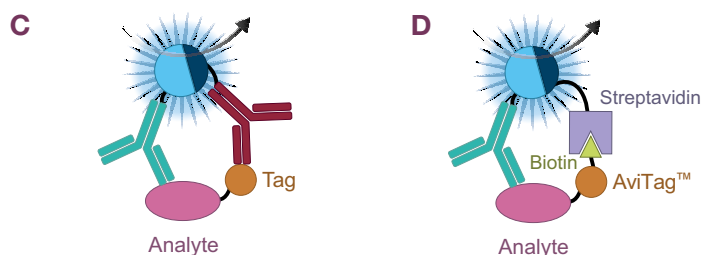


The Lumit® technology supports different assay formats that enable quantitative analysis of analytes.

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

(B) The direct assay format utilizes two BiT-labeled primary antibodies.

Alternative Assay Formats



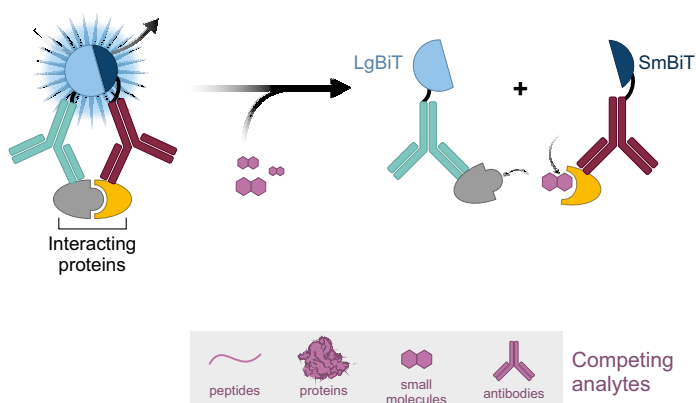
Alternatives of these formats exist for tagged analytes that involve either **(C)** BiT-labeled anti-tag antibodies or **(D)** BiT-labeled streptavidin to detect proteins that carry biotin.

Analyte Interaction

The binary interaction between two analytes can be easily explored using the Lumit[®] technology. Various formats enable determination and characterization of analyte binding events (e.g. protein:protein and protein:ligand interactions) in competitive loss-of-signal as well as gain-of-signal assays.

Loss-of-Signal Binding Immunoassays

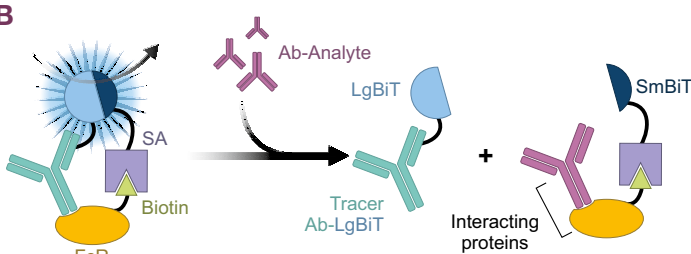
A



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

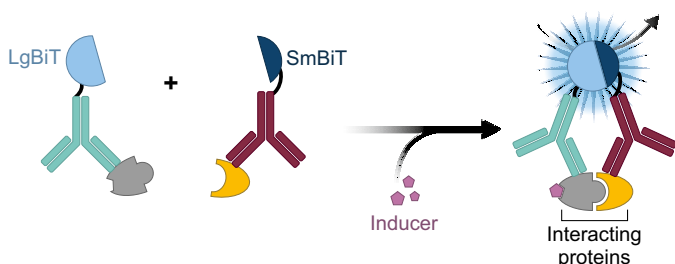
(A) In this biochemical assay format, two BiT-labeled primary antibodies against the protein interactors of interest are used for determining the potency of competing analytes (inhibitors). Upon addition of competing analytes, the luminescent signal decreases. This type of assay setup is used by the Lumit[®] SARS-CoV-2 RBD:hACE2 Assay.

B



(B) The setup of Lumit[®] FcR Binding Immunoassay is another example of a competitive loss-of-signal assay. In this assay format, biotinylated FcR is combined with SmBiT-labeled streptavidin (SA-SmBiT) and a LgBiT-labeled antibody tracer (Tracer-Ab-LgBiT). This enables determination of analyte antibody (Ab-Analyte) affinity towards FcR by competitive displacement of the Tracer-Ab-LgBiT.

Gain-of-Signal Binding Immunoassays



Lumit[®] gain-of-signal assays are used for inducer potency analyses of a protein: protein pair of interest. In this format, two BiT-labeled primary antibodies against the protein pair of interest are simultaneously present with a PPI-inducer. The interaction of the two proteins leads to a relative increase in the luminescent signal.

2. Pre-Built Lumit[®] Immunoassays

2.1 Lumit[®] Assays for the Detection of Cytokines / HMGB1

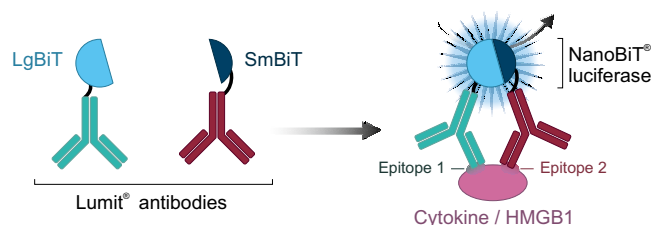
Description & Application

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.

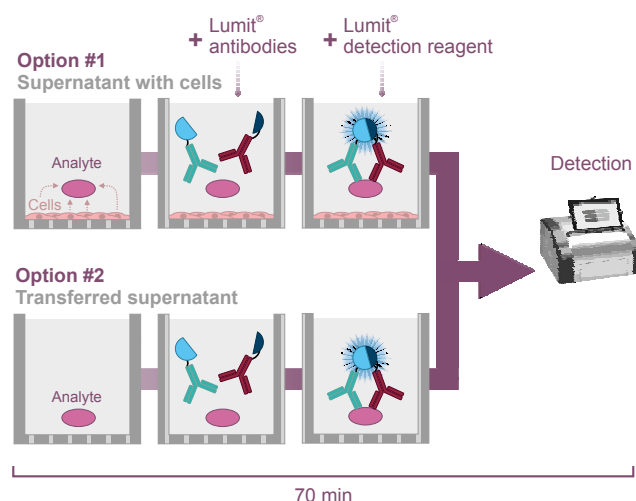
Principle & Workflow

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 Principle



Assay Workflow



Assay Features

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

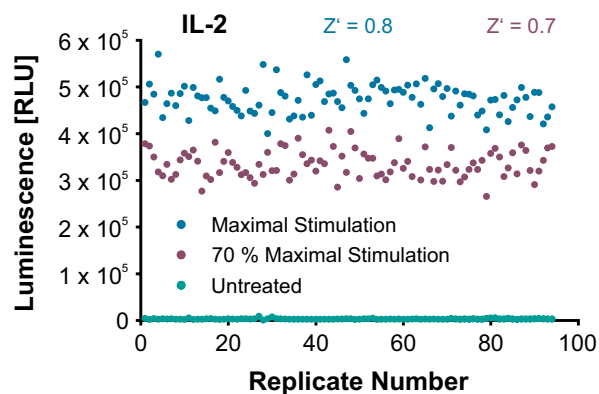
Assay	Dynamic Range	LOD
Human IL-1β	22–40000 pg/ml	10 pg/ml
Mouse IL-1β	11–40000 pg/ml	8 pg/ml
Human IL-2	14–25000 pg/ml	13 pg/ml
Human IL-4	14–25000 pg/ml	13 pg/ml
Human IL-6	6–25000 pg/ml	6 pg/ml
Human IL-10	18–25000 pg/ml	18 pg/ml
Human IFN-γ	2–10000 pg/ml	2 pg/ml
Human TNF-α	6–25000 pg/ml	6 pg/ml
Human/Mouse HMGB1	7–729 pg/ml (Hu) 3–2187 pg/ml (Ms)	1 ng/ml (Hu) 3 ng/ml (Ms)

LOD: limit of detection

Representative Data

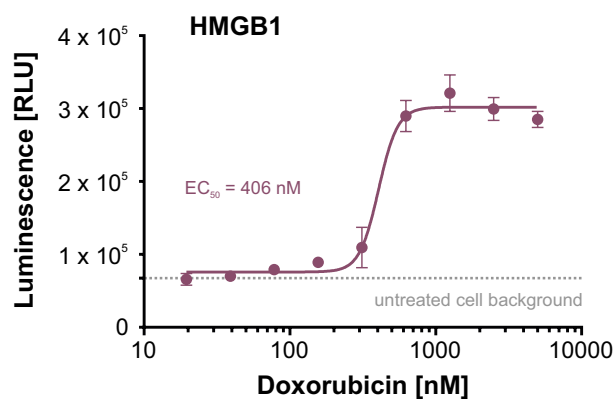
Validation of HTS-compatibility

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.



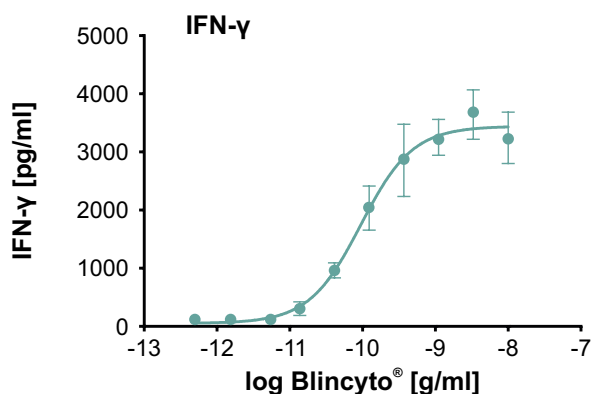
Drug-induced immunogenic cell death

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 30
 Lumit[®] HMGB1 Immunoassay Cat.# W6110, W6112



2. Pre-Built Lumit® Immunoassays

2.2 Lumit® Assays for the Detection of Metabolic Regulators

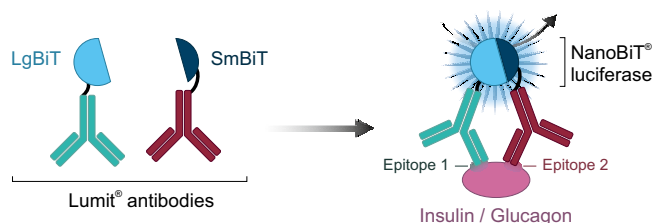
Description & Application

Pre-built Lumit® Insulin and Glucagon 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 dilution. Lumit® Immunoassays are amenable for low- and high-throughput applications.

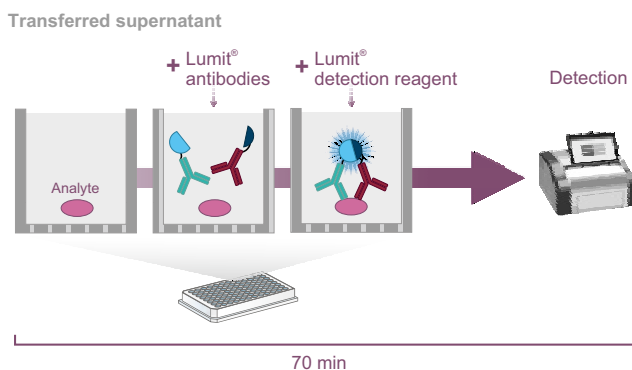
Principle & Workflow

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 plate-reading luminometer.

Assay Principle



Assay Workflow

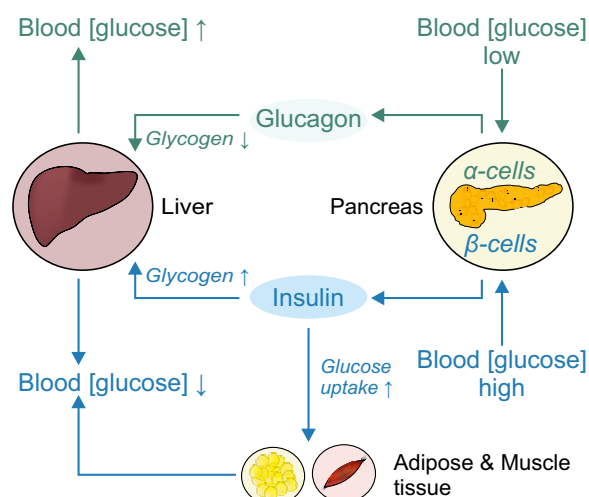


Assay	Dynamic Range	LOD
Insulin	58–46000 pg/ml	58 pg/ml
Glucagon	3–7000 pg/ml	3 pg/ml

LOD: limit of detection

Assay Features

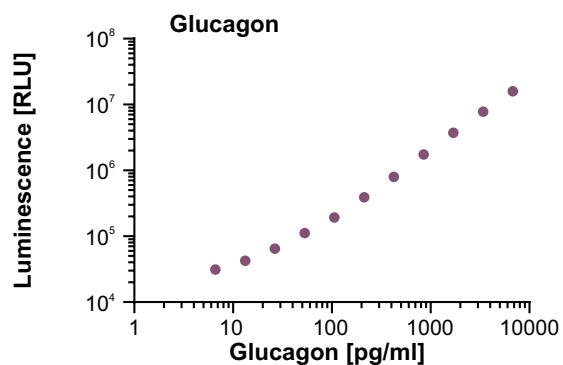
Sample material	Transferred supernatant
Sample volume	5–50 µl
Assay format	Direct assay format 96- /384-well plates
Implementation	Homogeneous Add-and-read
Time required	70 minutes or less
Multiplexing option	Use both Lumit® assays and analyze insulin and glucagon side-by-side to gain more information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



Representative Data

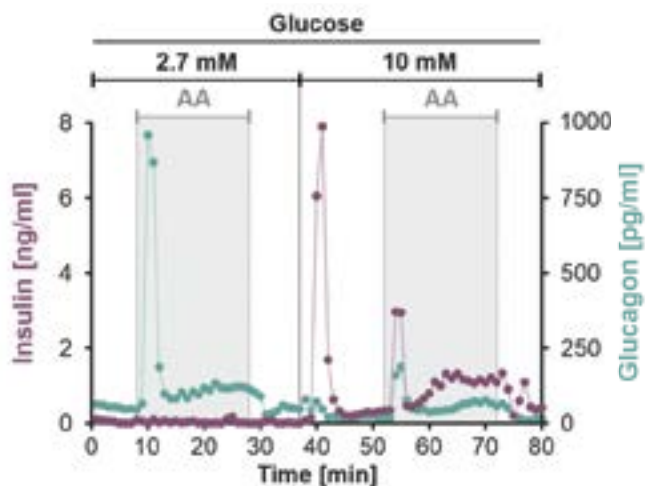
Broad dynamic range and picomolar sensitivity

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.



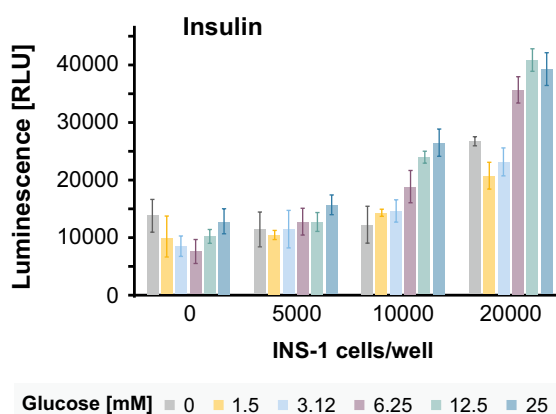
Measuring hormone secretion in perfusion experiments

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® Insulin Immunoassay Cat.# CS3037A01, CS3037A05

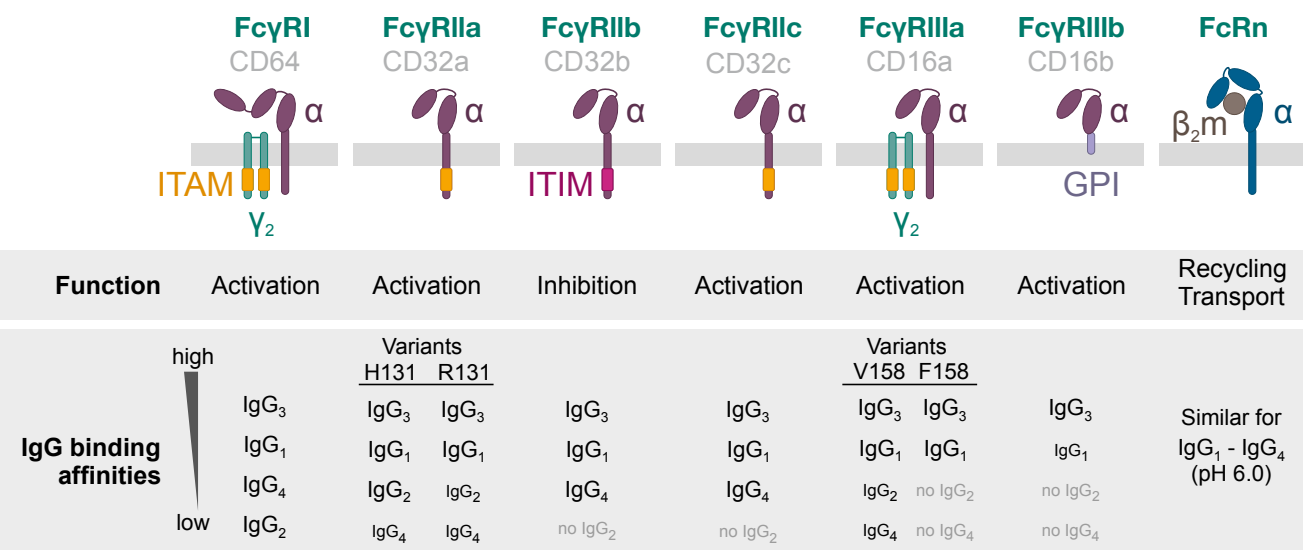
Lumit® Glucagon Immunoassay Cat.# W8020, W8022



2. Pre-Built Lumit[®] Immunoassays

2.3 Lumit[®] Fc Receptor Binding Assays for Biotherapeutic Development

The efficacy of therapeutic antibodies does not only depend on the binding activity of the Fab fragment to the target antigen, but also depends on the binding activity between Fc fragment and Fc receptors. For example, the affinity of the Fc fragment for the neonatal Fc receptor (FcRn) influences the half-life of antibodies, while its binding affinity towards Fc gamma receptors (FcγR) impacts the ability of an antibody to elicit cellular effector functions such as ADCC (antibody-dependent cellular cytotoxicity) and ADCP (antibody-dependent cellular phagocytosis). Therefore, candidate therapeutic antibodies must be tested against a panel of Fc receptors during drug development.



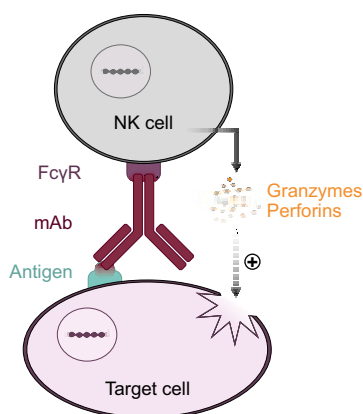
Representation of IgG-binding Fc receptors (FcγR, FcRn). Binding affinity varies among IgG subclasses. ITAM = immunoreceptor tyrosine-based activation motif; ITIM = immunoreceptor tyrosine-based inhibition motif; γ₂ = FcR gamma subunit dimer; β₂m = beta-2 macroglobulin. Adapted from Bruhns, P. (2012) 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 a multifunctional atypical Fcγ receptor expressed in diverse tissues throughout the body, including epithelia, endothelia, and cells of hematopoietic origin. FcRn binds to the Fc region of IgG antibodies at acidic pH within endosomes. FcRn functions include the transport of IgG throughout polarized cells barriers (e.g., epithelia) and the protection of IgGs from degradation, thereby regulating the half-life of antibodies in serum. Therefore, the investigation of FcRn interaction with therapeutic IgG is a key parameter that needs to be optimized during the drug development process as an extended half-life contributes to better efficacy and long dosing intervals.

Fc gamma receptors (FcγR) mediate a variety of biological responses by binding to the Fc region of IgG. These include antibody-dependent cellular cytotoxicity, endocytosis, phagocytosis, release of inflammatory mediators and augmentation of antigen presentation. In humans, three groups of FcγR have been described across a variety of cell types: **FcγRI** (CD64), **FcγRIIa/b/c** (CD32a/b/c) and **FcγRIIIa/b** (CD16a/b). These are expressed in different combinations at the surface of various immune cells. FcγRI is classified as a high-affinity receptor (nM K_D) while FcγRII and FcγRIII are classified as low- to intermediate-affinity receptors (μM K_D). Several studies showed that the different Fc receptor genotypes have a significant influence on Fc-mediated effects. For example, FcγRIIIa, responsible for ADCC activity of NK cells, shows as polymorphic variant V158 higher binding activity for IgG₁ when compared to variant F158.

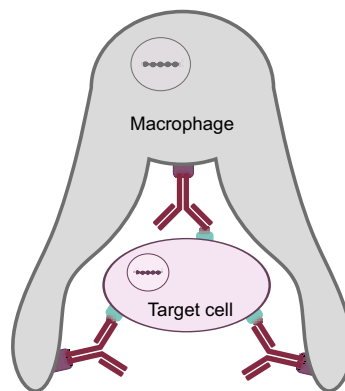
FcγR- / FcRn-mediated Cellular Functions

FcγR-mediated ADCC



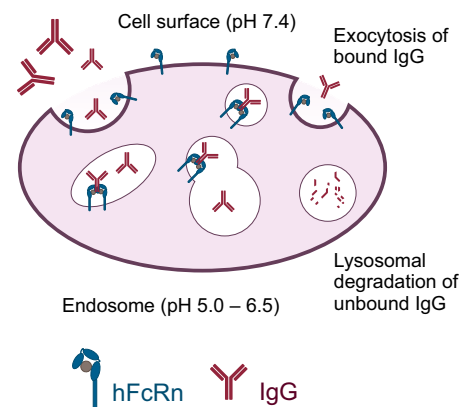
Antibody-Dependent Cellular Cytotoxicity (ADCC) is mediated by NK cells that are activated by binding the Fc part of IgG via FcγR. Stimulation of cytotoxic effector functions results in target cell death.

FcγR-mediated ADCP



Antibody-Dependent Cellular Phagocytosis (ADCP) is induced by interaction of macrophage FcγR with the Fc region of IgG which in turn augments their phagocytic activity.

FcRn-mediated IgG recycling



The interaction of FcRn IgG is promoted by acidic conditions within endosomes upon pinocytosis. The unbound IgG fraction is degraded in lysosomes along with other proteins while FcRn-bound IgGs are retained and released via exocytosis.

2. Pre-Built Lumit® Immunoassays

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.

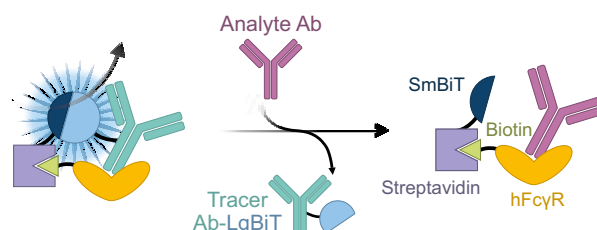
Principle & Workflow

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.

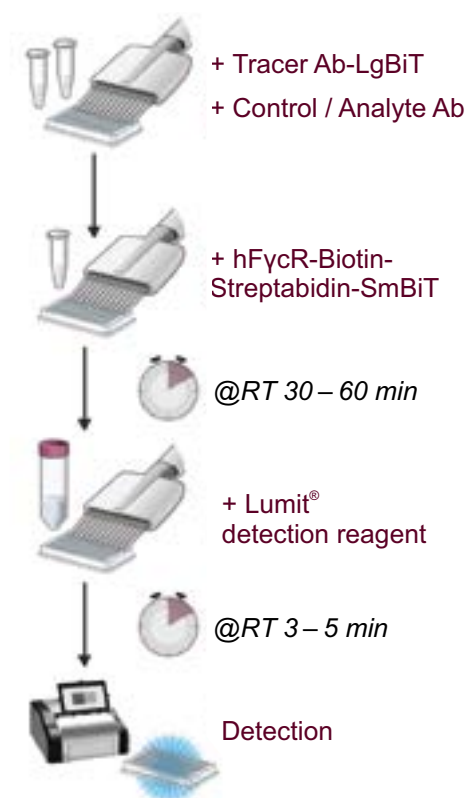
Assay Features

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

Assay Principle



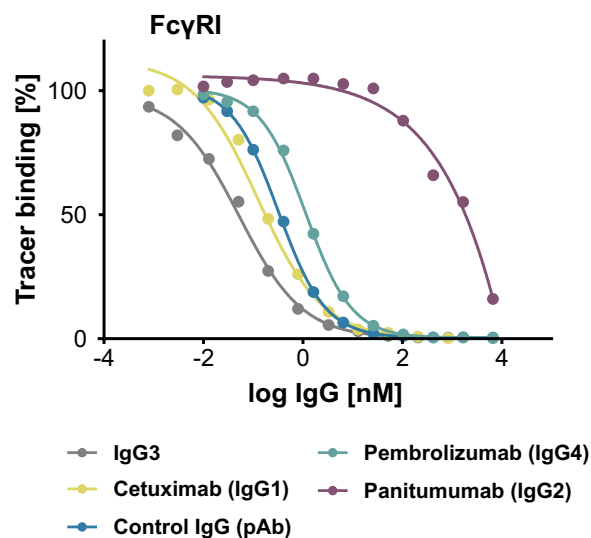
Assay Workflow



Representative Data

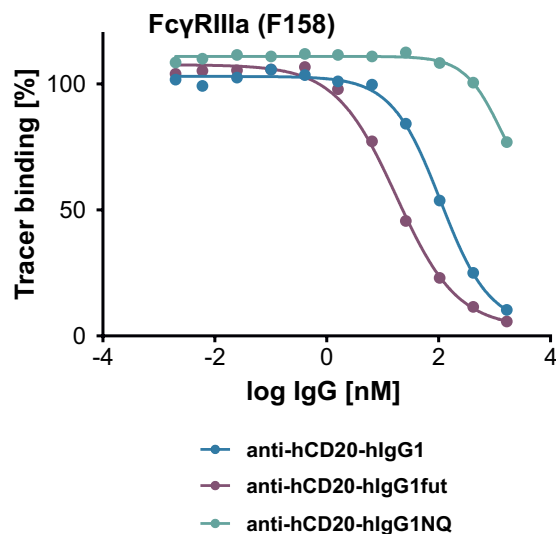
Lumit® FcγRI Binding Immunoassay

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 IC₅₀ values reflective of relative IgG affinity (IgG₃ > IgG₁ > IgG₄ >>> IgG₂).



Lumit® FcγRIIIa (F158) Binding Immunoassay

Lumit® FcγR Binding Immunoassays are used to assess antibody glycan status. IC₅₀ shifts in non-fucosylated (anti-hCD20-hIgG1fut) or non-glycosylated (anti-hCD20-hIgG1NQ) antibodies were detected.



Product Box

Lumit® FcγRI Binding Immunoassay

Cat.# CS3041A01

Lumit® FcγRIIIa (H131) Binding Immunoassay

Cat.# CS3041A02

Lumit® FcγRIIIa (R131) Binding Immunoassay

Cat.# CS3041A03

Lumit® FcγRIIIa (V158) Binding Immunoassay

Cat.# CS3041A04

Lumit® FcγRIIIa (F158) Binding Immunoassay

Cat.# CS3041A05



2. Pre-Built Lumit[®] Immunoassays

Lumit[®] FcRn Binding Immunoassay

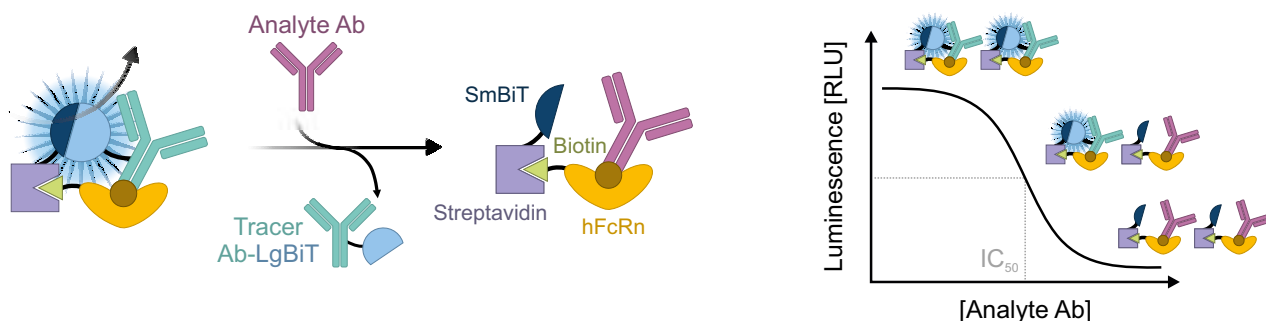
Description & Application

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 ng/ml to 4 µg/ml
Assay format	Loss-of-signal assay 96-/384-well plates
Implementation	Homogeneous Add-and-read
Time required	70 minutes or less

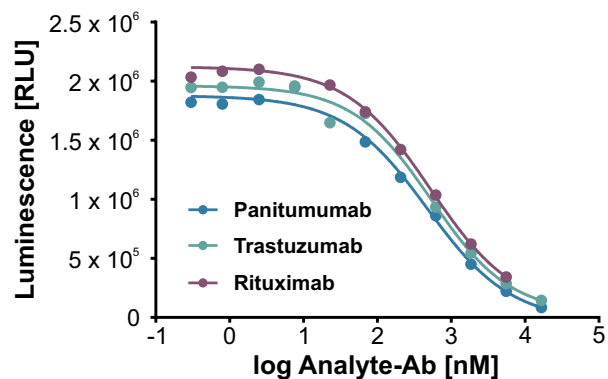
References

- 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

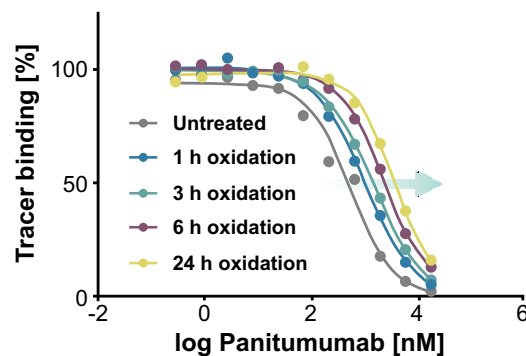
FcRn binding to a panel of therapeutic antibodies

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.



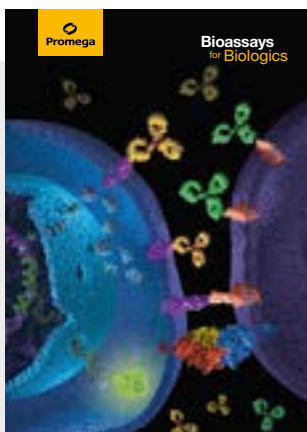
Oxidation-based loss in antibody-FcRn affinity

A therapeutic antibody was incubated with 0.3 % H₂O₂ 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



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. Pre-Built Lumit® Immunoassays

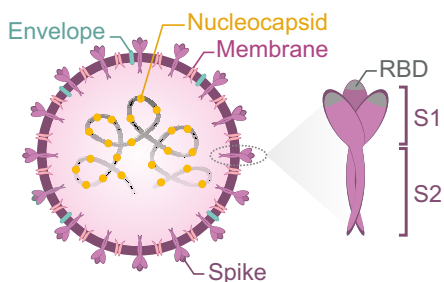
2.4 SARS-CoV-2 Protein:Protein Interaction Immunoassays

Lumit® SARS-CoV-2 RBD:hACE2 Immunoassay

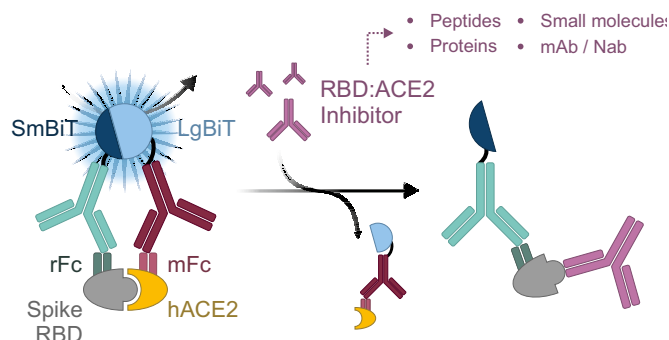
Description & Application

The Lumit® SARS-CoV-2 RBD:hACE2 Immunoassay detects the interaction between the SARS-CoV-2 spike protein's receptor binding domain (RBD) and the human angiotensin converting enzyme 2 (ACE2) protein. The fact that this interaction is fundamental for host cell infection renders it an important target for therapeutic intervention. This biochemical, HTS-compatible assay is used to screen for RBD:hACE2 interaction inhibitors and serves as a surrogate neutralization test to monitor neutralizing antibodies (NAb) in plasma or serum of convalescent patients or vaccinated individuals.

SARS-CoV-2 Spike Protein



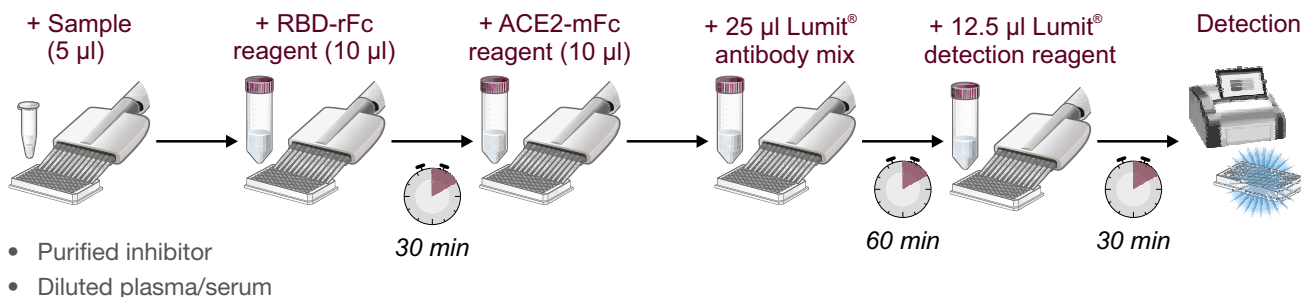
Assay Principle



Principle & Workflow

The Lumit® SARS-CoV-2 RBD:hACE2 Immunoassay utilizes recombinant rabbit Fc-RBD (rFc-RBD) and mouse Fc-hACE2 (mFc-hACE2) fusion proteins that are recognized by Lumit® anti-rabbit-SmBiT and anti-mouse-LgBiT antibodies, respectively. Molecules that negatively affect the interaction are evident from a relative decrease in the luminescent signal. Recombinant rFc-RBD and sample are mixed, followed by subsequent additions of the other assay components. The bioluminescent signal can be recorded on a conventional plate-luminometer.

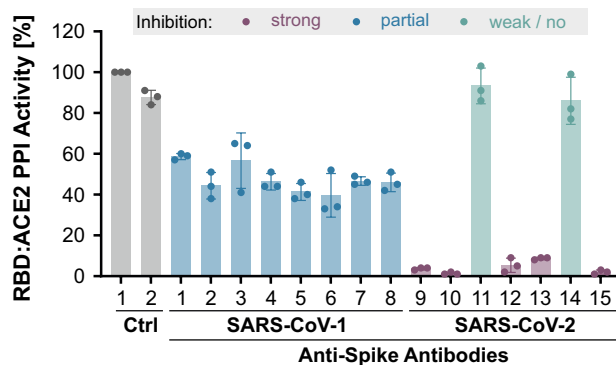
Assay Workflow



Representative Data

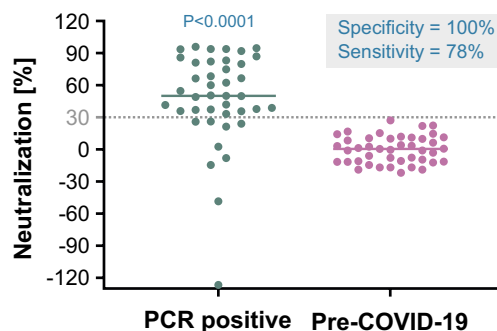
Screening for candidate therapeutic antibodies

A mini-library of 15 commercially available antibodies/antibody fragments against SARS-CoV-1 or -2 was screened at a single dose. Data were normalized to the no antibody control (Ctrl 1). Antibodies were grouped according to their potency to block the RBD:ACE2 interaction in strong, partial, and weak/no inhibition. The use of an anti-SARS-CoV-2 nucleocapsid antibody (Ctrl 2) confirmed assay specificity.



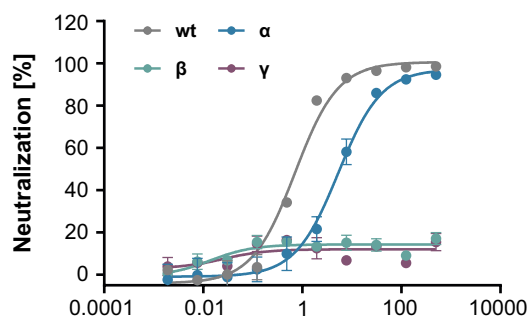
Detection of neutralizing antibodies in patient-derived samples

A cohort of pre-pandemic (n = 43) and PCR positive plasma samples (n = 41) from Madison, WI (USA) was analyzed. Samples dilutions (1:20) were pre-incubated with the RBD-rFc for 30 minutes at RT prior to addition of the other assay components. The threshold was set at 30 % neutralization resulting in 100 % assay specificity and 78 % assay sensitivity.



Monitoring antibody neutralization efficacy across SARS-CoV-2 variants

The potency of an anti-SARS-CoV-2 spike antibody to neutralize different variants-of-concern was compared. In comparison to wildtype, RBD mutations inherent to the α -variant seem not to significantly affect neutralization, while mutations within the RBD of the β - and γ -variants nearly completely abrogated the potency for neutralization of the anti-SARS-CoV-2 spike antibody.



All figures adapted from Alves *et. al.* 2021

Product Box

Lumit® SARS-CoV-2 Spike RBD:hACE2 Immunoassay wt
Cat.# CS3163B01, CS3163B02

SARS-CoV-2 RBD N501Y (rabbit Fc) α
Cat.# CS3163B01C, CS3163B02C

SARS-CoV-2 Spike RBD K417N, E484K, N501Y (rabbit Fc) β
Cat.# CS3163B01D, CS3163B02D

SARS-CoV-2 Spike RBD K417T, E484K, N501Y (rabbit Fc) γ
Cat.# CS3163B01E, CS3163B02E

Combine with

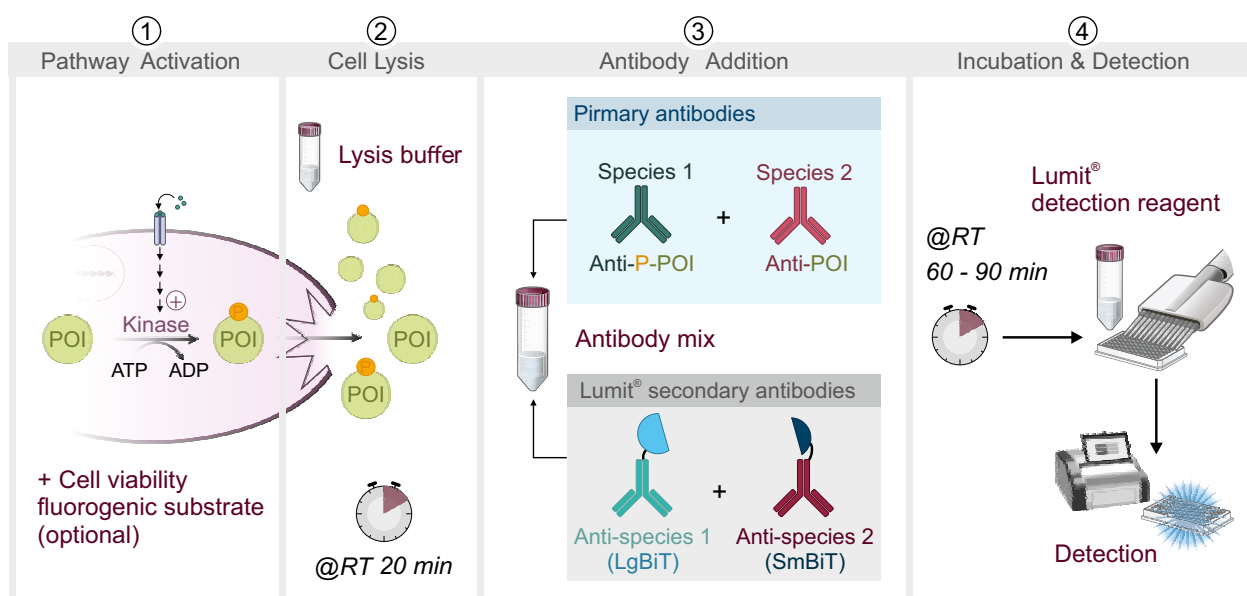


3. Pre-Validated Lumit[®] Immunoassays

3.1 Lumit[®] Immunoassay Cellular Systems

Description & Application

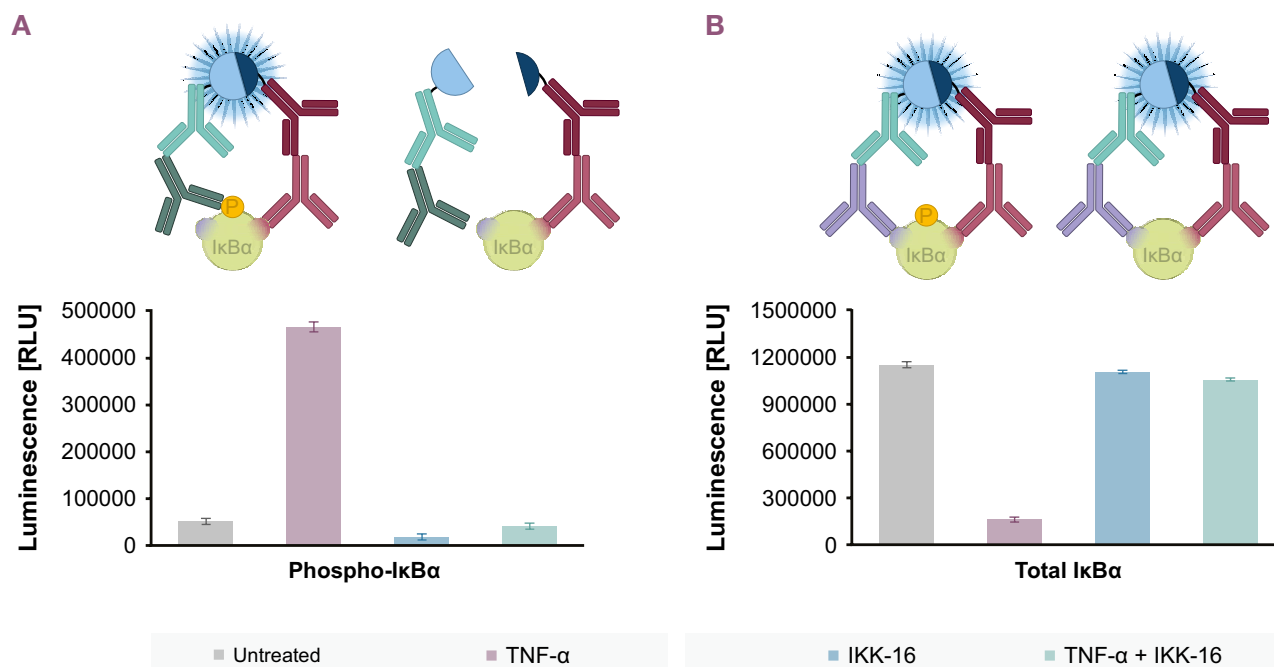
The Lumit[®] Immunoassay Cellular Systems have been extensively validated for kinase signaling pathway analysis in cells. The assay kits contain a lysis buffer, a set of pre-labeled Lumit[®] secondary antibodies, and detection reagents. Together with a matching pair of primary antibodies (not included) this immunoassay allows for easy signaling pathway analysis. As summarized on page 22, there is a growing number of application notes for different targets and pathways available. Each application note provides a protocol as well as information on the primary antibodies used.



Principle & Workflow

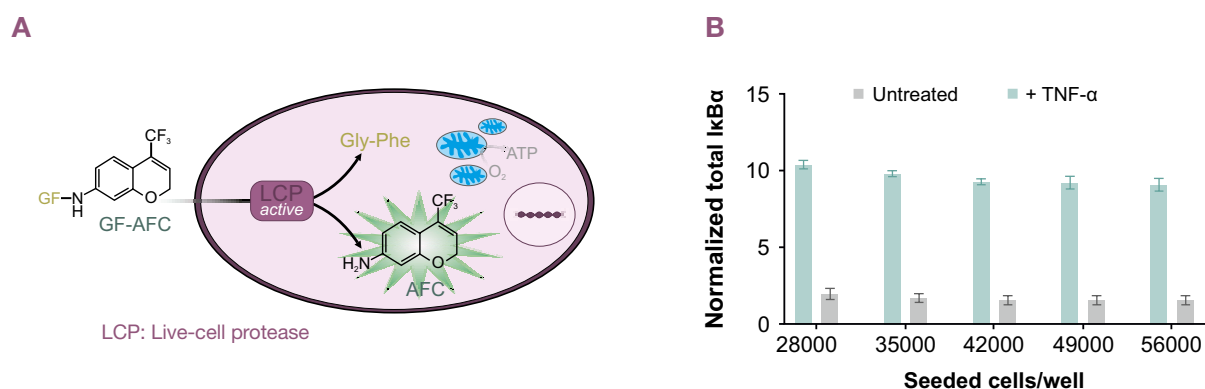
The Lumit[®] Immunoassay Cellular Systems are based on the indirect Lumit[®] detection format in which two primary antibodies are mixed with two matching secondary antibodies that were pre-labeled with either the LgBiT or SmBiT subunit. To date, this format has been extensively validated for cell signaling analysis in cell lysates with a focus on phosphorylation. 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 Digitonin-based lysis buffer. **(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.

Representative Data



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.



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 mix 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.

3. Pre-Validated Lumit® Immunoassays

3.1 Lumit® Immunoassay Cellular Systems (continued)

Pre-validated Targets

A list of application notes for pre-validated targets is available to save you time and labor. Application notes contain target-specific protocols, representative data, as well as information on the commercially available antibodies used.

Cellular Target	Signaling Pathway	Total Protein Protocol	Phosphorylated Protein Protocol	Set ID
AKT	PI3K/mTOR/AKT	Yes	Yes (Ser 473)	Set 1
BCL6	B-cell signaling	Yes	No	Set 1
BTK	B-cell receptor	No	Yes (Tyr 223)	Set 1
c-Jun	JNK	No	Yes (Ser 63)	Set 1
CREB	PKA/CREB	Yes	Yes (Ser 133)	Set 1
ER (Estrogen Receptor)	Estrogen	Yes	No	Set 1
ERK1	ERK	No	Yes (Thr 202)	Set 1
IκBa	NF-κB	Yes	Yes (Ser 32)	Set 1
JNK	JNK	No	Yes (Thr 183/Tyr 185)	Set 1
SMAD1	BMP	Yes	Yes (Ser 463/465)	Set 1
SMAD2	TGF-β	Yes	Yes (Ser 465/467)	Set 2
STAT1	JAK/STAT	Yes	Yes (Tyr 701)	Set 1
STAT1	JAK/STAT	Yes	Yes (Ser 727)	Set 1
STAT2	JAK/STAT	No	Yes (Tyr 690)	Set 1
STAT3	JAK/STAT	Yes	Yes (Tyr 705)	Set 1
4E-BP1	mTor	No	Yes (Ser 65)	Set 2
β-catenin	WNT	Yes	Yes (Thr 41/Ser 45)	Set 2
p65	NF-κB	Yes	Yes (Ser 536)	Set 2
RB (Retinoblastoma Tumor Suppressor)	Cell Cycle	Yes	Yes (Ser 780)	Set 2
RB (Retinoblastoma Tumor Suppressor)	Cell Cycle	Yes	Yes (Ser 807/811)	Set 2

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

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*



GLOMAX® DETECTION SYSTEMS

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

One Instrument. Numerous Applications.

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- Cell viability, cytotoxicity, and apoptosis assays
- Kinetic measurements
- Multiplexing
- Oxidative stress and cell metabolism
- ELISA
- BRET/FRET analysis
- Lumit® Immunoassays

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SYSTEMS

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

4. Build-Your-Own Lumit[®] Immunoassays

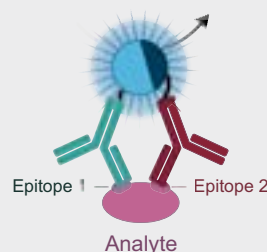
4.1 Lumit[®] Immunoassay Toolbox

Explore in chapter 4 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.

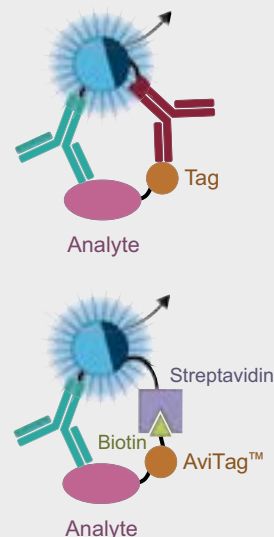
see page 25



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).

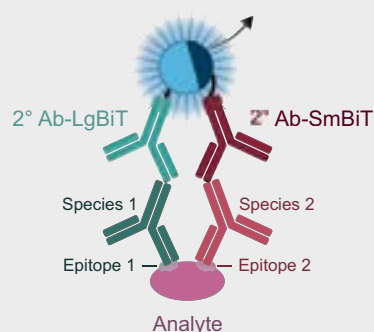
see page 26



Pre-Labeled Secondary Antibodies

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

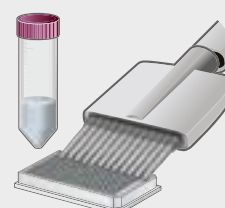
see page 28



Detection Reagents

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

see page 29



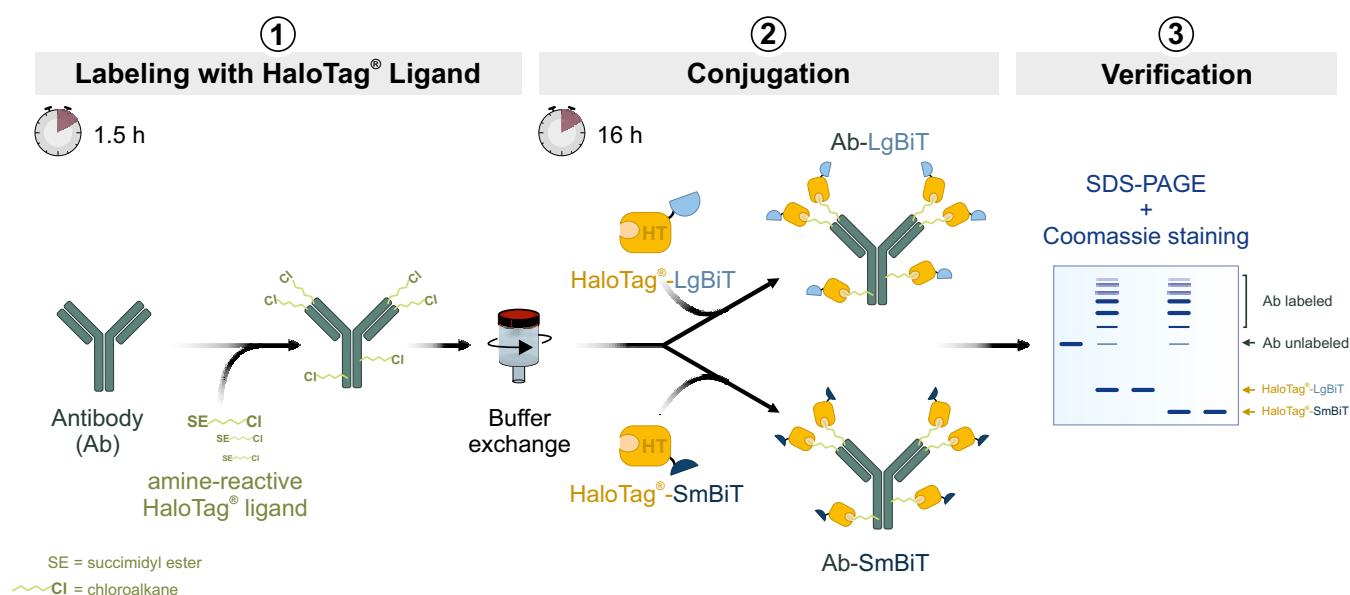
4.2 Lumit® Immunoassay Labeling Kit

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

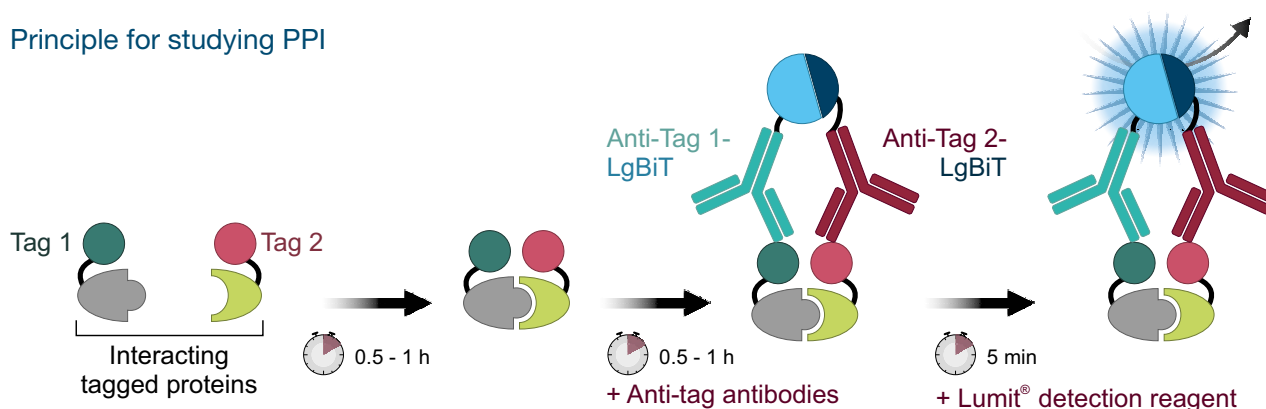
4. Build-Your-Own Lumit[®] Immunoassays

4.3 Lumit[®] Anti-Tag Antibodies / Streptavidin

Description & Application

Anti-Tag Lumit[®] reagents encompass a selection of BiT-labeled antibodies against common protein tags (e.g. His-, Flag[®]-, GST-tag, and human Fc) as well as BiT-labeled streptavidin. These reagents enable the easy setup of biochemical assays to study protein:protein interaction (PPI) and screen for molecules that modulate these interactions. Furthermore, protein:small molecule interactions can be interrogated in a simple, competition-based, and HTS-compatible format.

Principle for studying PPI



Principle & Workflow

For studying protein:protein interactions, two differentially tagged proteins are incubated. Optionally, a compound that modulates the PPI of interest can be added. Subsequently, the BiT-labeled Lumit[®] anti-tag antibodies are added. Upon addition of Lumit[®] detection reagent, the signal is recorded on a plate-reading luminometer. When using AviTag[™]-tagged or biotinylated proteins, one of the BiT-labeled Lumit[®] anti-tag antibodies can be substituted for streptavidin-LgBiT/-SmBiT.

For studying protein:small molecule interactions a biotinylated small molecule (tracer) and a tagged protein are required. The interaction of tracer and target is detected with a BiT-labeled anti-tag antibody and streptavidin-LgBiT/-SmBiT. Target engagement by unlabeled test compounds is evident from a decrease in the luminescent signal due to competitive tracer displacement.



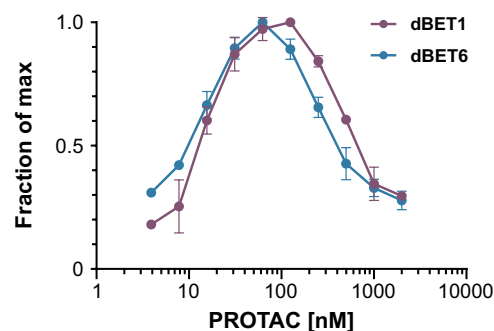
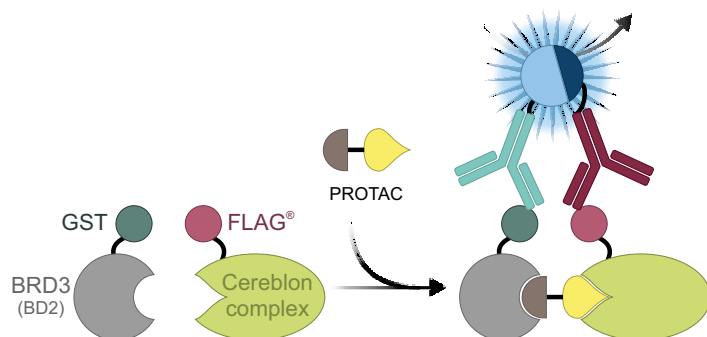
Looking for a source of tagged kinases to quantitatively measure test compound affinity *in vitro*?

Promega offers more than 370 different tagged and purified kinases as part of the Kinase Enzyme Systems, ready-to-use assays to determine kinase activity *in vitro*.

www.promega.com/kinases

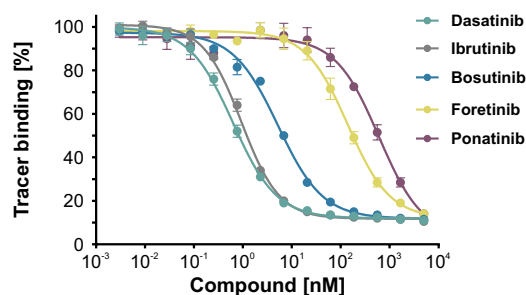
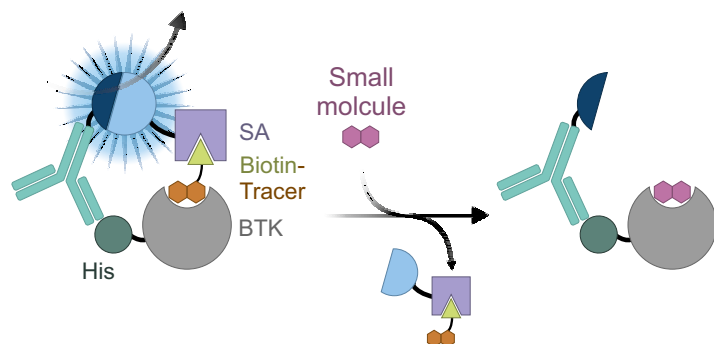


Representative Data



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 BRD3 (BD2) (6.25 nM) and Cereblon (6.25 nM), GST- and FLAG[®]-tagged respectively, were incubated with different concentrations of PROTACs for 60 minutes. Detection was accomplished using Lumit[®] Anti-GST-LgBiT and Lumit[®] Anti-FLAG[®]-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 μ M) for 60 minutes with mild agitation. Samples are incubated for 30 minutes with a mix of Streptavidin-LgBiT and Lumit[®] Anti-6His-SmBiT. The fraction of tracer-bound BTK at equilibrium is detected upon addition of Lumit[®] Immunoassay Detection Reagent A.

Product Box

Lumit[®] Anti-6His-LgBiT and -SmBiT
Cat.# CS332211

Lumit[®] Anti-GST-LgBiT and -SmBiT
Cat.# CS332212

Lumit[®] Anti-Flag[®]-LgBiT and -SmBiT
Cat.# CS332213

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

Lumit[®] Streptavidin-LgBiT and -SmBiT
Cat.# CS332215

Combine with Lumit[®]
Detection Reagent A



4. Build-Your-Own Lumit® Immunoassays

4.4 Lumit® Secondary Antibodies

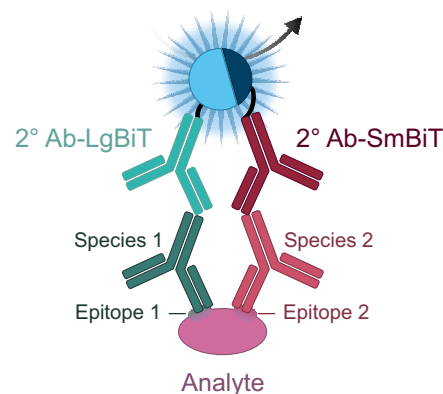
Description & Application

Lumit® Secondary Antibodies are polyclonal BiT-labeled antibodies against IgG supporting the development of an indirect immunoassay format for analyte detection. The polyclonal secondary antibodies are raised in donkey, are immunoaffinity-purified using immobilized antigens, and conjugated to either SmBiT or LgBiT. Species-specific reactivity against heavy and light chains of IgG (mouse, rabbit, and goat) was confirmed.

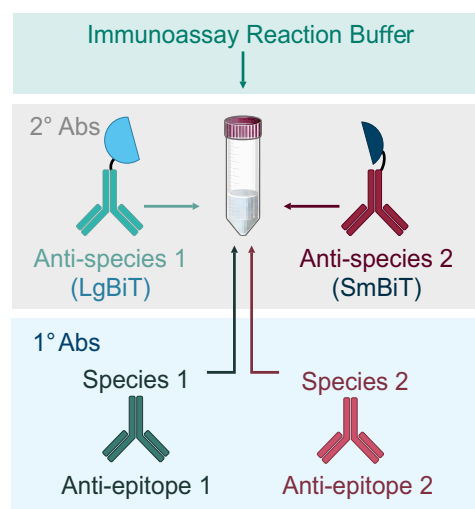
Principle & Workflow

Guidelines for establishing an indirect Lumit® immunoassay for a new target are provided in 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 to three primary antibody pairs. The best combination and concentration to be used in an assay is determined by checkerboard experiments.

Indirect Assay Format



Preparation of Antibody Mix



Product Box

Lumit® Anti-Mouse Ab-LgBiT

Cat.# W1021, W1022

Lumit® Anti-Rabbit Ab-LgBiT

Cat.# W1041, W1042

Lumit® Anti-Goat Ab-LgBiT

Cat.# W1061, W1062

Lumit® Anti-Mouse Ab-SmBiT

Cat.# W1051, W1052

Lumit® Anti-Rabbit Ab-SmBiT

Cat.# W1031, W1032

Lumit® Anti-Goat Ab-SmBiT

Cat.# W1071, W1072

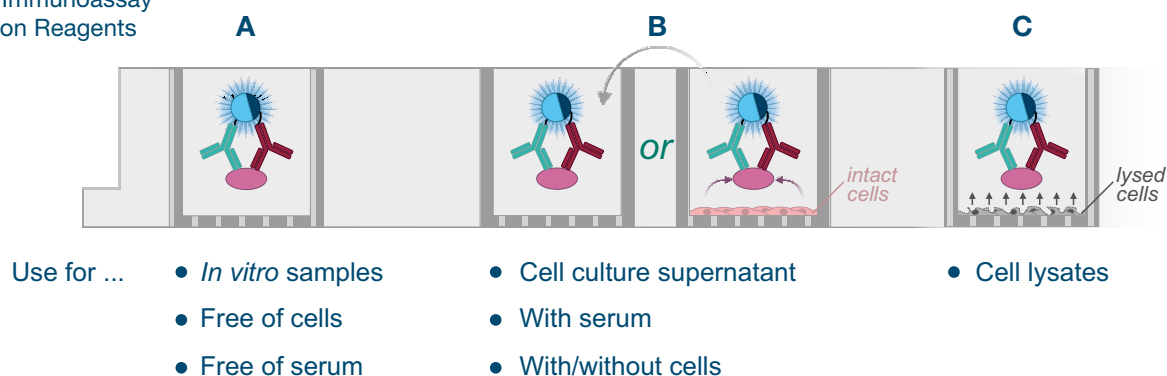


4.4 Lumit® Immunoassay Detection Reagents

Description & Application

Lumit® Immunoassay Detection Reagents support the development of your own Lumit® Immunoassay. There are three different types of reagents (A, B, C) enabling analyte detection under different conditions. Detection Reagent A is used in the absence of cells and serum, while Detection Reagent B is used for cell culture supernatant containing FBS (up to 10 %) and/or in the presence of intact cells. For the quantification of proteins in lysed cells Detection Reagent C is recommended which is part of the Lumit® Immunoassay Lysis and Detection Kit.

Lumit® Immunoassay
Detection Reagents



Principle & Workflow

Guidelines for establishing a Lumit® immunoassay are provided in the technical manuals #TM613, #TM602, and #TM614. In these manuals, detailed descriptions for setting up and optimizing Lumit® immunoassays are supplied including tips & tricks for overcoming matrix effects and suggestions for 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



5. Ordering Information

Pre-Built Lumit® Immunoassays

Immunology

Cytokines / HMGB1

Product	Cat. #	Size
Lumit® IL-1 β Human Immunoassay	W6010	100 assays
	W6012	500 assays
	W6011	1000 assays
Lumit® IL-1 β Mouse Immunoassay	W7010	100 assays
	W7012	500 assays
	W7011	1000 assays
Lumit® IFN- γ Human Immunoassay	W6040	100 assays
	W6042	500 assays
	W6041	1000 assays
Lumit® IL-6 Human Immunoassay	W6030	100 assays
	W6032	500 assays
	W6031	1000 assays
Lumit® IL-2 Human Immunoassay	W6020	100 assays
	W6022	500 assays
	W6021	1000 assays
Lumit® TNF- α Human Immunoassay	W6050	100 assays
	W6052	500 assays
	W6051	1000 assays
Lumit® IL-4 Human Immunoassay	W6060	100 assays
	W6062	500 assays
	W6061	1000 assays
Lumit® IL-10 Human Immunoassay	W6070	100 assays
	W6072	500 assays
	W6071	1000 assays
Lumit® IL-18 Human Immunoassay	CS3291A01*	100 assays
Lumit® HMGB1 Human/Mouse Immunoassay	W6110	100 assays
	W6112	500 assays

Metabolism

Product	Cat. #	Size
Lumit® Insulin Immunoassay Kit	CS3037A05*	100 ¹⁾ –400 ²⁾ assays
	CS3037A01*	500 ¹⁾ –2000 ²⁾ assays
Lumit® Glucagon Immunoassay Kit	W8020	100 ¹⁾ –400 ²⁾ assays
	W8022	500 ¹⁾ –2000 ²⁾ assays

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

¹⁾96-well; ²⁾384-well

Biotherapeutic Development

Product	Cat. #	Size
Lumit® FcRn Binding Immunoassay	W1151	100 assays
	W1152	1000 assays
Lumit® FcγRI Binding Immunoassay	CS3041A01*	100 assays
Lumit® FcγRIIA (H131) Binding Immunoassay	CS3041A02*	100 assays
Lumit® FcγRIIA (R131) Binding Immunoassay	CS3041A03*	100 assays
Lumit® FcγRIIIA (V158) Binding Immunoassay	CS3041A04*	100 assays
Lumit® FcγRIIIA (F158) Binding Immunoassay	CS3041A05*	100 assays

Virology

Product	Cat. #	Size
Lumit® SARS-CoV 2 Spike RBD:hACE2 Immunoassay	CS3163B01*	200 assays
	CS3163B02*	2000 assays
SARS-CoV-2 Spike RBD N501Y (rabbit Fc)	CS3163B01C*	40 µl, 0.5 µM
	CS3163B02C*	400 µl, 0.5 µM
SARS-CoV-2 Spike RBD K417N, E484K, N501Y (rabbit Fc)	CS3163B01D*	40 µl, 0.5 µM
	CS3163B02D*	400 µl, 0.5 µM
SARS-CoV-2 Spike RBD K417T, E484K, N501Y (rabbit Fc)	CS3163B01E*	40 µl, 0.5 µM
	CS3163B02E*	400 µl, 0.5 µM

Pre-Validated Lumit® Immunoassays

Cellular Systems

Product	Cat. #	Size
Lumit® Immunoassay Cellular System – Starter Kit	W1220	200 assays
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

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

5. Ordering Information

Build-Your-Own Lumit® Immunoassays

Labeling & Detection

Product	Cat. #	Size
Lumit® Immunoassay Labeling System	VB2500	1 kit
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-Goat Ab-LgBiT	W1061	30 µl
	W1062	300 µl
Lumit® Anti-Goat Ab-SmBiT	W1071	30 µl
	W1072	300 µl

Labeling & Detection

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

Lumit® Anti-Tag Antibodies / Streptavidin

Product	Cat. #	Size
Lumit® Anti-6His-LgBiT and -SmBiT	CS332211*	20 µl each
• Lumit® Anti-6His-LgBiT		
• Lumit® Anti-6His-SmBiT		
Lumit® Anti-6His-LgBiT	CS332216*	200 µl
Lumit® Anti-6His-SmBiT	CS332217*	200 µl
Lumit® Anti-GST-LgBiT and -SmBiT	CS332212*	20 µl each
• Lumit® Anti-GST-LgBiT		
• Lumit® Anti-GST-SmBiT		
Lumit® Anti-GST-LgBiT	CS332218*	200 µl
Lumit® Anti-GST-SmBiT	CS332219*	200 µl
Lumit® Anti-FLAG®-LgBiT and -SmBiT	CS332213*	20 µl each
• Lumit® Anti-FLAG®-LgBiT		
• Lumit® Anti-FLAG®-SmBiT		
Lumit® Anti-FLAG®-LgBiT	CS332220*	200 µl
Lumit® Anti-FLAG®-SmBiT	CS332221*	200 µl
Lumit® Anti-Human IgG-LgBiT and -SmBiT	CS332214*	20 µl each
• Lumit® Anti-Human IgG-LgBiT		
• Lumit® Anti-Human IgG-SmBiT		
Lumit® Anti-Human IgG-LgBiT	CS332222*	200 µl
Lumit® Anti-Human IgG-SmBiT	CS332223*	200 µl
Lumit® Streptavidin-LgBiT and -SmBiT ¹⁾	CS332215*	20 µl each
• Lumit® Streptavidin-LgBiT		
• Lumit® Streptavidin-SmBiT		
Lumit® Streptavidin-LgBiT	CS332224*	200 µl
Lumit® Streptavidin-SmBiT	CS332225*	200 µl
Lumit® Anti-Mouse Ab-LgBiT and -SmBiT ²⁾	refer to page 32	
Lumit® Anti-Rabbit Ab-LgBiT and -SmBiT ²⁾	refer to page 32	
Lumit® Anti-Goat Ab-LgBiT and -SmBiT ²⁾	refer to page 32	

1) Can be used with AviTag™-tagged proteins or biotinylated proteins/small molecules.

2) Cannot be used with Lumit® anti-6His-BiT system.

* 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.

FLAG is a trademark of Sigma-Aldrich Co. LLC.

AviTag is a trademark of Avidity, LLC.

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

6. References

Build-Your-Own Lumit® Immunoassay

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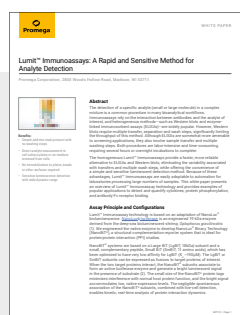
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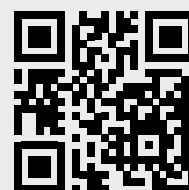
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