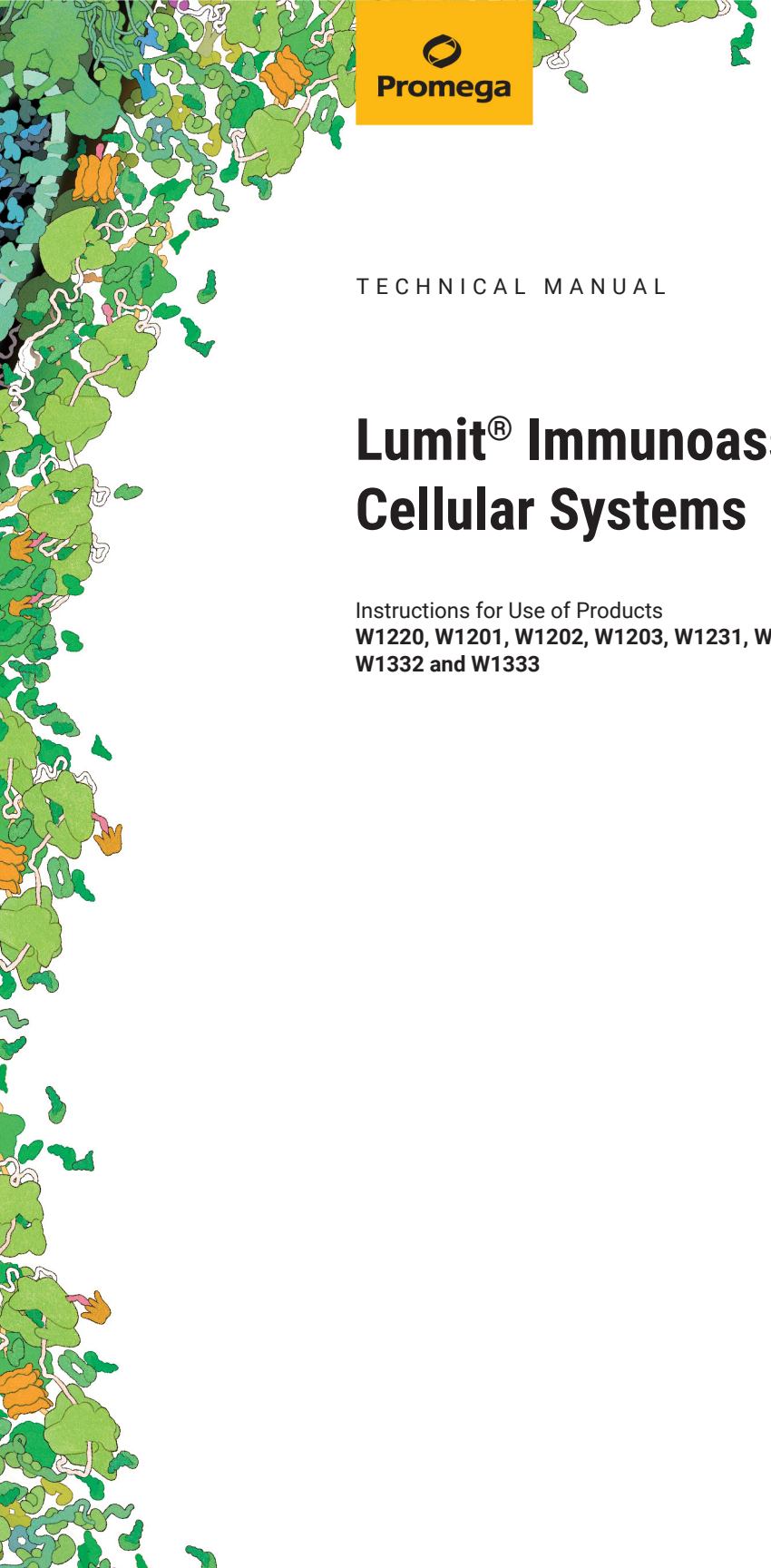

TECHNICAL MANUAL

Lumit® Immunoassay Cellular Systems

Instructions for Use of Products
W1220, W1201, W1202, W1203, W1231, W1232, W1233, W1331, W1332 and W1333

Revised 3/24
TM613

Promega

Lumit® Immunoassay Cellular Systems

All technical literature is available at: www.promega.com/protocols/
Visit the web site to verify that you are using the most current version of this Technical Manual.
E-mail Promega Technical Services if you have questions on use of this system: techserv@promega.com

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1. Description

The Lumit® Immunoassay Cellular System[a–c] is a homogeneous bioluminescent assay that measures levels of target proteins in cell lysates when used with the appropriate primary antibody pairs. It combines immunodetection and NanoLuc® Binary Technology (NanoBiT®). NanoBiT® is a structural complementation reporter ideal for protein:protein interaction (PPI) studies. The NanoBiT® System is composed of two subunits: Large BiT (LgBiT; 18kDa) and Small BiT (SmBiT; 11 amino acid peptide), that can be expressed as recombinant fusions or chemically conjugated to target proteins of interest. The LgBiT and SmBiT subunits have been optimized for stability and minimal self-association due to weak affinity (K_d = 190µM). When two proteins (one tagged with LgBiT, the other tagged with SmBiT) interact, the subunits are brought into proximity to form a functional enzyme that generates luminescence in the presence of its substrate (1).

In the Lumit® Immunoassay Cellular System (Lumit® ICS), NanoBiT® subunits are conjugated to a pair of secondary antibodies against two different species (anti-rabbit, anti-mouse and anti-goat are available separately). Seeded cells are lysed in multiwell plates using a NanoBiT® compatible lysis solution (Digitonin or Lumit® Lysis Buffer II, 10X), and the target protein is detected by adding an antibody mix containing two primary antibodies against the target protein, along with SmBiT- and LgBiT-conjugated secondary antibodies (e.g., Lumit® Anti-Mouse Ab-LgBiT and Lumit® Anti-Rabbit Ab-SmBiT). Binding of the primary/Lumit® secondary antibody complexes to their corresponding epitopes brings NanoBiT® subunits into proximity to form an active NanoLuc® luciferase that generates light in proportion to the amount of target protein (Figure 1). When the primary antibody pair includes a phospho-specific antibody, the luminescence reflects the level of target protein phosphorylation (Figure 1, Panel A). To detect total protein level, the same concept is used except both primary antibodies recognize nonphosphorylated epitopes on the target protein (Figure 1, Panel A). The luminescent signal generated is determined using a luminometer as shown in Figure 2.

The Lumit® Immunoassay Cellular System is solution-based, and, unlike standard immunodetection techniques such as ELISA or Western blot, the protocol does not include washing, liquid transfer or immobilization steps. Therefore, cells are lysed in the same well where antibody binding and luminescence generation occur (Figure 1, Panel B). The assay takes approximately 2 hours to complete in a homogeneous "add-and-read" format. A key advantage of this approach is that it does not require cell engineering. Therefore, the phosphorylation or total amount of an intracellular protein may be detected at native levels in any cell type where it is expressed (2).

In addition, Lumit® secondary antibodies can be adapted to detect any protein of interest, provided the appropriate primary antibodies (from mouse, rabbit or goat) are available. This assay can be performed in a single plate, with no need for medium removal or lysate transfer to new plate. The homogeneous format requires no washing steps, making the assay quicker than Western, ELISA or fluorescence-based methods. The assay works with a small number of cells and there is no immunoprecipitation step for phosphorylated proteins. Detection requires only a luminometer, and can be performed in 96-, 384- or 1536-well plates. A Z´ factor >0.7 has been reported (3).

Additional protocols for many common cellular targets are available. See the application notes at:
www.promega.com/products/immunoassay-elisa/lumit-immunoassays/lumit-immunoassay-cellular-systems/

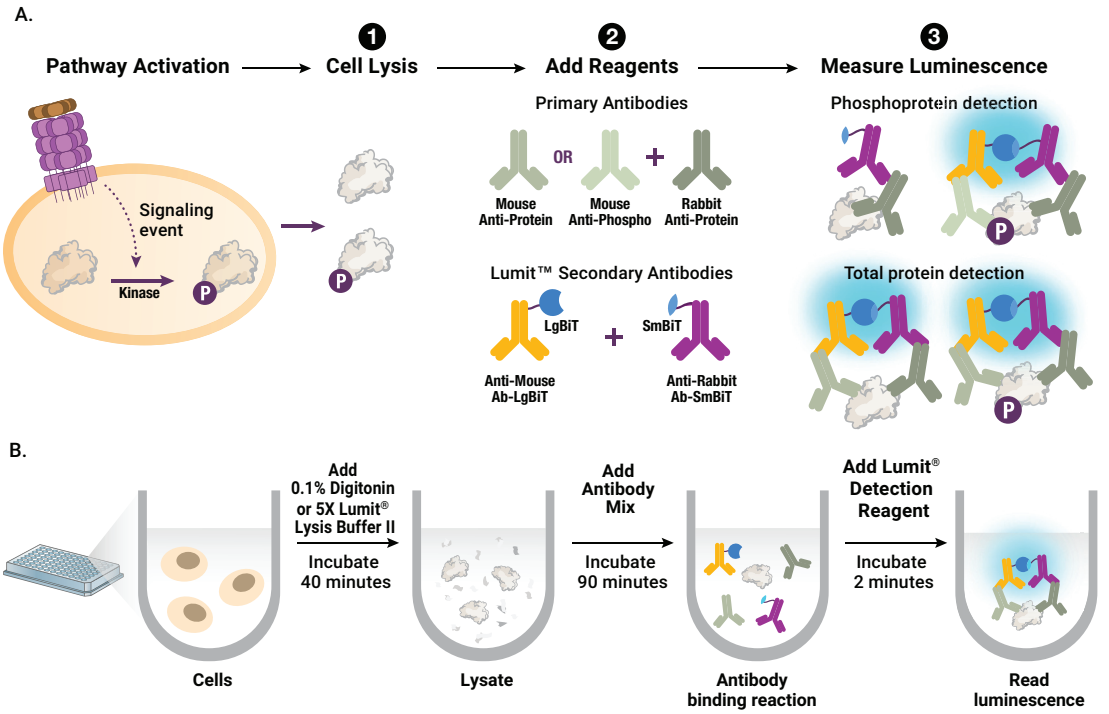

Figure 1. Principle of the Lumit® Immunoassay Cellular System. Panel A. Phosphorylated or total target proteins in lysed cells after stimulation are recognized by each primary antibody pair. The Lumit® secondary antibodies then recognize their cognate primary antibodies, bringing the NanoBiT® subunits into close proximity to form a functional enzyme that generates bright luminescence. **Panel B.** The Lumit® Immunoassay is an endpoint homogeneous assay; cell lysis, antibody binding to the target and luminescence generation all happen in solution without transfer or wash steps.

1. Description (continued)

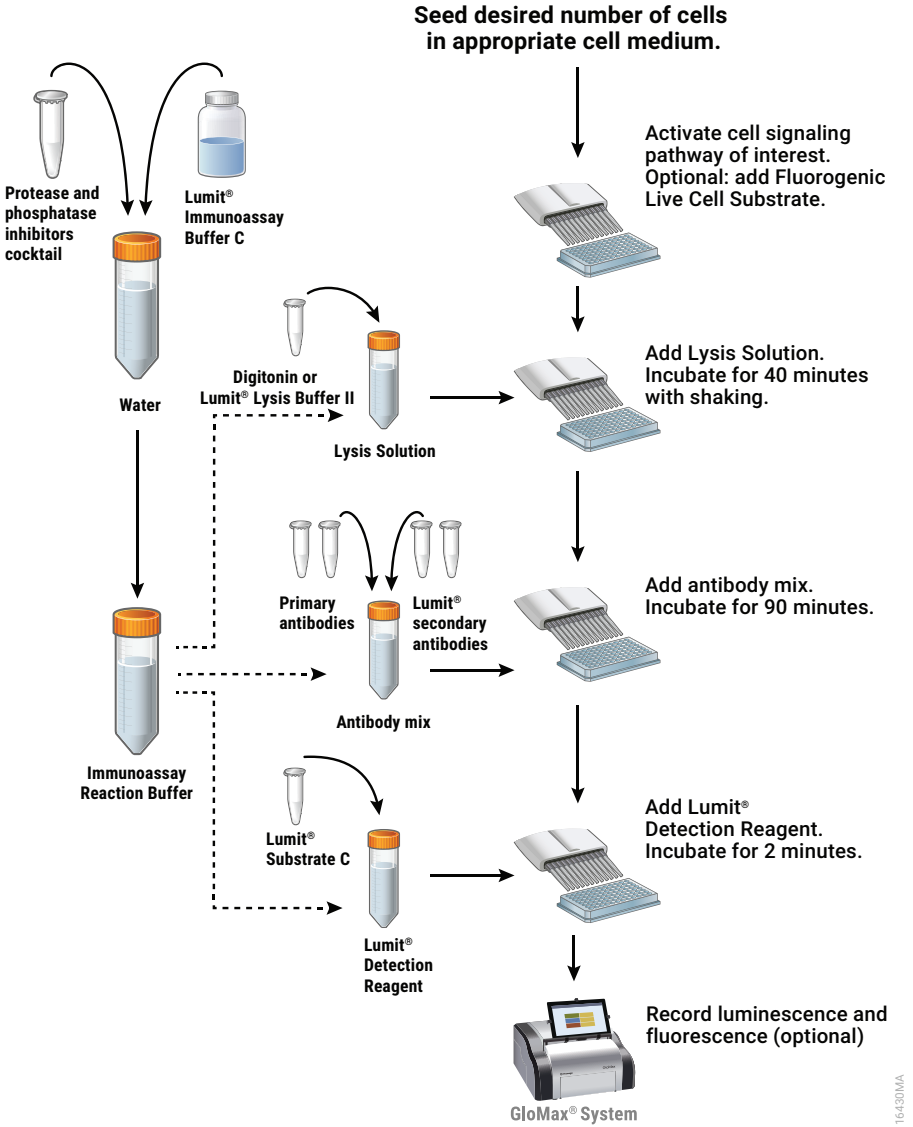

Figure 2. Lumit® Immunoassay Cellular System protocol. "Lysis Solution" refers to lysis reagent prepared with either Digitonin or Lumit® Lysis Buffer II.

Signaling pathway activation leads to a multitude of cellular responses, including enzyme activity modulation, gene expression and protein translocation or degradation. Reversible protein phosphorylation is a common mechanism that transduces the signal from an upstream activation event to downstream cellular responses. The Lumit® Immunoassay Cellular System can be applied to any signaling pathway marker protein or phosphoprotein in which primary antibodies from appropriate species are available (2). An example of detection using two markers of NF-κB pathway regulation is shown in Figure 3.

Notes:

a. The following Lumit® secondary antibodies are currently available: anti-rabbit, anti-mouse and anti-goat. Therefore, the Lumit® Immunoassay Cellular System can be used with target-selective primary antibody pairs from mouse/rabbit, mouse/goat or rabbit/goat.

b. Pairs of primary antibodies from the same species (e.g., mouse/mouse) are **not** compatible with the Lumit® Immunoassay Cellular System.

c. Lumit® Immunoassay Cellular System performance may depend on the affinity of the primary antibodies for the target, the cell lysis solution used and the Lumit® secondary antibodies set used. For example, when testing a rabbit/mouse antibody pair, we recommend using the Lumit® Immunoassay Cellular System - Starter Kit (Cat.# W1220) as it contains both combinations of SmBiT- and LgBiT-conjugated secondary antibodies, as well as both lysis reagents. This kit helps you identify the lysis solution and Lumit® secondary antibody combination that works best for your target protein.

d. Certain detergents are incompatible with Lumit® Immunoassay Cellular Systems due to interference with NanoBiT® complementation (e.g., RIPA). Because of this, we recommend using either Digitonin or Lumit® Lysis Buffer II, 10X, which are provided in the kits.

1. Description (continued)

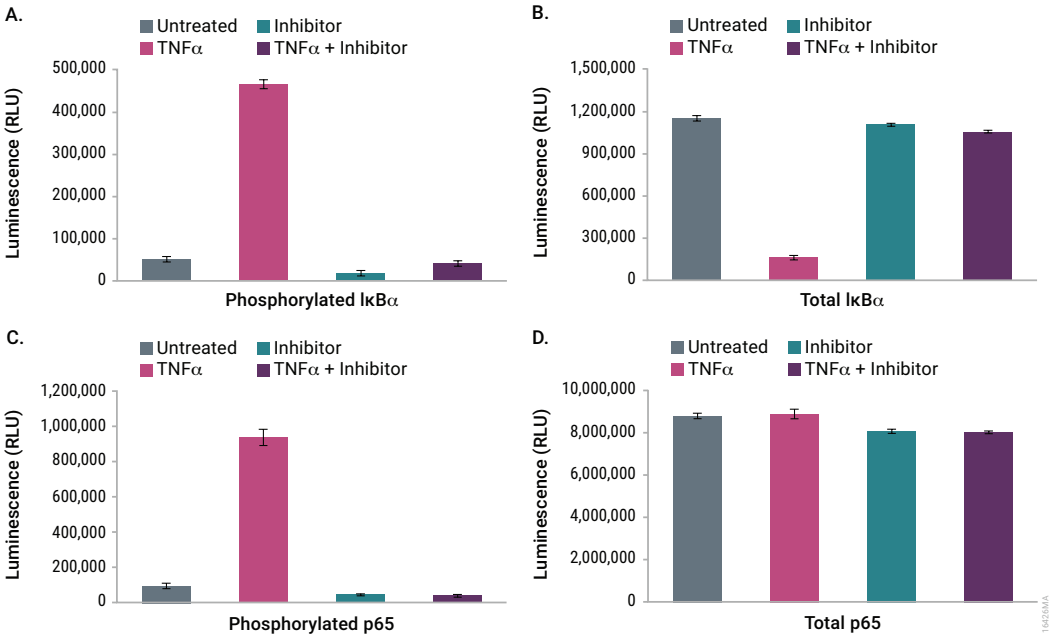

Figure 3. Detecting total and phosphorylated target proteins in NF-κB signaling pathway using the Lumit® Immunoassay Cellular System. Seeded MCF-7 cells (50,000) were pretreated with an IKK complex specific inhibitor, IKK16 (10µM, 1 hour), and then treated with TNFα (20ng/ml) for 30 minutes (IκBα) or 10 minutes (p65). **Panel A.** Cells were pretreated with MG132 (20µM, 1 hour) and then phosphorylated IκBα (S32) was detected using the Lumit® Immunoassay and 150ng/ml of the following antibodies: Mouse anti-IκBα (Cell Signaling Technology Cat.# 4814) and rabbit anti-phospho-IκBα (Cell Signaling Technology Cat.# 2859). **Panel B.** Total IκBα levels were measured using 150ng/ml of the following antibodies: Mouse anti-IκBα (Cell Signaling Technology Cat.# 4814) and rabbit anti-IκBα (Cell Signaling Technology Cat.# 4812). **Panel C.** Phosphorylated p65 (S536) levels were measured using 150ng/ml of the following antibodies: Mouse anti-phospho-p65 (Cell Signaling Technology Cat.# 13346) and rabbit anti-p65 (Cell Signaling Technology Cat.# 8242). **Panel D.** Total p65 levels were measured using the Lumit® Immunoassay and 150ng/ml of the following antibodies: Rabbit anti-p65 (Cell Signaling Technology Cat.# 8242) and mouse anti-p65 (Cell Signaling Technology Cat.# 6956).

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
Lumit® Immunoassay Cellular System - Starter Kit	**200 assays**	**W1220**

This system is sufficient for 200 assays (including 100 assays with the Anti-Mouse Ab-LgBiT/Anti-Rabbit Ab-SmBiT combination and 100 assays with the Anti-Mouse Ab-SmBiT/Anti-Rabbit Ab-LgBiT combination) if performed in 96-well plates using 50µl, 50µl and 25µl of cell lysate, antibody mix and Detection Reagent, respectively, per assay. This system can also be used in 384-well plates using 12.5µl:12.5µl:6.25µl for a total of 800 assays. Includes:

- 2 × 50µl Digitonin, 2%
- 2 × 500µl Lumit® Lysis Buffer II, 10X
- 2 × 1.5ml Lumit® Immunoassay Buffer C, 10X
- 2 × 200µl Lumit® Substrate C
- 30µl Lumit® Anti-Mouse Ab-LgBiT
- 30µl Lumit® Anti-Rabbit Ab-SmBiT
- 30µl Lumit® Anti-Mouse Ab-SmBiT
- 30µl Lumit® Anti-Rabbit Ab-LgBiT
- 1ml Antibody Dilution Buffer
- 10µl Fluorogenic Live Cell Substrate (GF-AFC Substrate), 100mM

PRODUCT	SIZE	CAT.#
Lumit® Immunoassay Cellular System - Set 1	**100 assays**	**W1201**

This system is sufficient for 100 assays if performed in 96-well plates using 50µl, 50µl and 25µl of cell lysate, antibody mix and Detection Reagent, respectively, per assay. This system can also be used in 384-well plates using 12.5µl:12.5µl:6.25µl for a total of 400 assays. Includes:

- 50µl Digitonin, 2%
- 500µl Lumit® Lysis Buffer II, 10X
- 1.5ml Lumit® Immunoassay Buffer C, 10X
- 200µl Lumit® Substrate C
- 30µl Lumit® Anti-Mouse Ab-LgBiT
- 30µl Lumit® Anti-Rabbit Ab-SmBiT
- 1ml Antibody Dilution Buffer
- 10µl Fluorogenic Live Cell Substrate (GF-AFC Substrate), 100mM

2. Product Components and Storage Conditions (continued)

PRODUCT	SIZE	CAT.#
Lumit® Immunoassay Cellular System - Set 1	**1,000 assays**	**W1202**

This system is sufficient for 1,000 assays if performed in 96-well plates using 50µl, 50µl and 25µl of cell lysate, antibody mix and Detection Reagent, respectively, per assay. This system can also be used in 384-well plates using 12.5µl:12.5µl:6.25µl for a total of 4,000 assays. Includes:

- 500µl Digitonin, 2%
- 5ml Lumit® Lysis Buffer II, 10X
- 15ml Lumit® Immunoassay Buffer C, 10X
- 2 × 1ml Lumit® Substrate C
- 300µl Lumit® Anti-Mouse Ab-LgBiT
- 300µl Lumit® Anti-Rabbit Ab-SmBiT
- 1ml Antibody Dilution Buffer
- 2 × 10µl Fluorogenic Live Cell Substrate (GF-AFC Substrate), 100mM

PRODUCT	SIZE	CAT.#
Lumit® Immunoassay Cellular System - Set 1	**10,000 assays**	**W1203**

This system is sufficient for 10,000 assays if performed in 96-well plates using 50µl, 50µl and 25µl of cell lysate, antibody mix and Detection Reagent, respectively, per assay. This system can also be used in 384-well plates using 12.5µl:12.5µl:6.25µl for a total of 40,000 assays. **Note:** Cat.# W1203 is 10 × Cat.# W1202. Includes:

- 10 × 500µl Digitonin, 2%
- 10 × 5ml Lumit® Lysis Buffer II, 10X
- 10 × 15ml Lumit® Immunoassay Buffer C, 10X
- 20 × 1ml Lumit® Substrate C
- 10 × 300µl Lumit® Anti-Mouse Ab-LgBiT
- 10 × 300µl Lumit® Anti-Rabbit Ab-SmBiT
- 10 × 1ml Antibody Dilution Buffer
- 20 × 10µl Fluorogenic Live Cell Substrate (GF-AFC Substrate), 100mM

PRODUCT	SIZE	CAT.#
Lumit® Immunoassay Cellular System - Set 2	**100 assays**	**W1331**

This system is sufficient for 100 assays if performed in 96-well plates using 50µl, 50µl and 25µl of cell lysate, antibody mix and Detection Reagent, respectively, per assay. This system can also be used in 384-well plates using 12.5µl:12.5µl:6.25µl for a total of 400 assays. **Note:** Cat.# W1331 is a combination of Cat.# W1231, W1041 and W1051. Includes:

- 50µl Digitonin, 2%
- 500µl Lumit® Lysis Buffer II, 10X
- 1.5ml Lumit® Immunoassay Buffer C, 10X
- 200µl Lumit® Substrate C
- 30µl Lumit® Anti-Mouse Ab-SmBiT
- 30µl Lumit® Anti-Rabbit Ab-LgBiT
- 1ml Antibody Dilution Buffer
- 10µl Fluorogenic Live Cell Substrate (GF-AFC Substrate), 100mM

PRODUCT	SIZE	CAT.#
Lumit® Immunoassay Cellular System - Set 2	**1,000 assays**	**W1332**

This system is sufficient for 1,000 assays if performed in 96-well plates using 50µl, 50µl and 25µl of cell lysate, antibody mix and Detection Reagent, respectively, per assay. This system can also be used in 384-well plates using 12.5µl:12.5µl:6.25µl for a total of 4,000 assays. **Note:** Cat.# W1332 is a combination of Cat.# W1232, W1042 and W1052. Includes:

- 500µl Digitonin, 2%
- 5ml Lumit® Lysis Buffer II, 10X
- 15ml Lumit® Immunoassay Buffer C, 10X
- 2 × 1ml Lumit® Substrate C
- 300µl Lumit® Anti-Mouse Ab-SmBiT
- 300µl Lumit® Anti-Rabbit Ab-LgBiT
- 1ml Antibody Dilution Buffer
- 2 × 10µl Fluorogenic Live Cell Substrate (GF-AFC Substrate), 100mM

PRODUCT	SIZE	CAT.#
Lumit® Immunoassay Cellular System - Set 2	**10,000 assays**	**W1333**

This system is sufficient for 10,000 assays if performed in 96-well plates using 50µl, 50µl and 25µl of cell lysate, antibody mix and Detection Reagent, respectively, per assay. This system can also be used in 384-well plates using 12.5µl:12.5µl:6.25µl for a total of 40,000 assays. **Note:** Cat.# W1333 is a combination of 10 × Cat.# W1232, 10 × Cat.# W1042 and 10 × Cat.# W1052. Includes:

- 10 × 500µl Digitonin, 2%
- 10 × 5ml Lumit® Lysis Buffer II, 10X
- 10 × 15ml Lumit® Immunoassay Buffer C, 10X
- 20 × 1ml Lumit® Substrate C
- 10 × 300µl Lumit® Anti-Mouse Ab-SmBiT
- 10 × 300µl Lumit® Anti-Rabbit Ab-LgBiT
- 10 × 1ml Antibody Dilution Buffer
- 20 × 10µl Fluorogenic Live Cell Substrate (GF-AFC Substrate), 100mM

PRODUCT	SIZE	CAT.#
Lumit® Immunoassay Lysis and Detection Kit	**100 assays**	**W1231**

This system is sufficient for 100 assays if performed in 96-well plates using 50µl, 50µl and 25µl of cell lysate, antibody mix and Detection Reagent, respectively, per assay. This system can also be used in 384-well plates using 12.5µl:12.5µl:6.25µl for a total of 400 assays. Includes:

- 50µl Digitonin, 2%
- 500µl Lumit® Lysis Buffer II, 10X
- 1.5ml Lumit® Immunoassay Buffer C, 10X
- 200µl Lumit® Substrate C
- 1ml Antibody Dilution Buffer
- 10µl Fluorogenic Live Cell Substrate (GF-AFC Substrate), 100mM

2. Product Components and Storage Conditions (continued)

PRODUCT	SIZE	CAT.#
Lumit® Immunoassay Lysis and Detection Kit	**1,000 assays**	**W1232**

This system is sufficient for 1,000 assays if performed in 96-well plates using 50µl, 50µl and 25µl of cell lysate, antibody mix, and Detection Reagent, respectively, per assay. This system can also be used in 384-well plates using 12.5µl:12.5µl:6.25µl for a total of 4,000 assays. Includes:

- 500µl Digitonin, 2%
- 5ml Lumit® Lysis Buffer II, 10X
- 15ml Lumit® Immunoassay Buffer C, 10X
- 2 × 1ml Lumit® Substrate C
- 1ml Antibody Dilution Buffer
- 2 × 10µl Fluorogenic Live Cell Substrate (GF-AFC Substrate), 100mM

PRODUCT	SIZE	CAT.#
Lumit® Immunoassay Lysis and Detection Kit	**10,000 assays**	**W1233**

This system is sufficient for 10,000 assays if performed in 96-well plates using 50µl, 50µl and 25µl of cell lysate, antibody mix and Detection Reagent, respectively, per assay. This system can also be used in 384-well plates using 12.5µl:12.5µl:6.25µl for a total of 40,000 assays. **Note:** Cat.# W1233 is 10 × Cat.# W1232. Includes:

- 10 × 500µl Digitonin, 2%
- 10 × 5ml Lumit® Lysis Buffer II, 10X
- 10 × 15ml Lumit® Immunoassay Buffer C, 10X
- 20 × 1ml Lumit® Substrate C
- 10 × 1ml Antibody Dilution Buffer
- 20 × 10µl Fluorogenic Live Cell Substrate (GF-AFC Substrate), 100mM

Storage Conditions: Store all components at –30°C to –10°C. Thaw all components, except the antibodies, completely at room temperature before use. Store antibodies at –30°C to –10°C until use. Mix all components thoroughly before use. Store all components at –30°C to –10°C immediately after use. Store Lumit® Substrate C and GF-AFC Substrate in the dark.

Notes:

a. Antibody Dilution Buffer is used to dilute primary antibodies to a working solution after an optimal assay concentration is identified (see Section 6).

b. GF-AFC Substrate is a cell viability substrate for cell number normalization. This is a nonlytic, single-reagent-addition fluorescence assay component that measures the relative number of viable cells in a population. It is based on measurement of a conserved and constitutive protease activity within live cells and therefore serves as a biomarker of cell viability. The substrate enters intact cells, where it is cleaved by the live-cell protease activity to generate a fluorescent signal proportional to the number of living cells. The live-cell protease becomes inactive upon cell lysis. After lysis, the accumulated fluorescence is measured at the end of the Lumit® Immunoassay along with luminescence (Figure 4). Fluorescence can also be read before the lysis step to check for cell viability.

c. See Table 1, Section 4.A, for volume ratios for various plate formats.

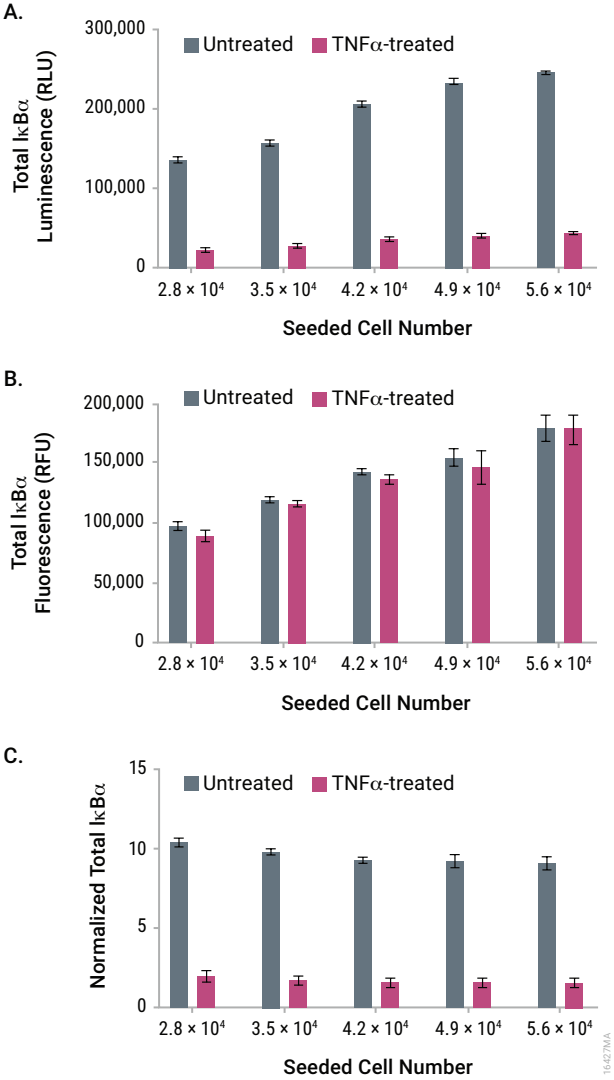

Figure 4. Normalizing luminescence data to cell number per well. Different cell numbers were treated with 50ng/ml TNFα for 30 minutes. GF-AFC Substrate was added to all cells 30 minutes before lysis. The Lumit® Immunoassay was used to detect total IkBα level using 150ng/ml of the following antibodies: Mouse anti-IκBα (Cell Signaling Technology Cat.# 4814) and rabbit anti-IκBα (Cell Signaling Technology Cat.# 4812). Luminescence (**Panel A**) and fluorescence (**Panel B**) were read at the end of the experiment. **Panel C.** Normalized luminescence RLU values to fluorescence RFU values. Luminescence and fluorescence were recorded using the GloMax® Discover System (Cat.# GM3000).

3. General Considerations

Lysis Solution: Detergents commonly used to lyse cells can have a negative effect on NanoBiT® complementation. Digitonin and Lumit® Lysis Buffer II, 10X, have been extensively tested for compatibility with the Lumit® Immunoassay Cellular System. The optimized lysis treatments described in this manual effectively expose intracellular targets to the Lumit® Detection Reagent without affecting NanoBiT® complementation. We have demonstrated the importance of testing both lysis solutions to achieve a working assay, as some protein targets are detectable only with Lumit® Lysis Buffer II, 10X, while some targets are only detectable with Digitonin (Figure 5). We compared the protein level and phosphorylation using different cell disruption protocols, such as detergent-free sonication and other lysis solutions. We obtained similar results using detergent-free sonication and digitonin- or Lumit® Lysis Buffer II, 10X-based cellular lysis. The latter lysis methods have been chosen because of their compatibility with high-throughput formats.

Antibodies: The Lumit® Immunoassay Cellular System is a generic kit that requires the user to provide compatible primary antibodies. For broad target coverage, we offer multiple versions of the Lumit® secondary antibodies in a kit with lysis and detection components or as stand-alone products. To find an optimal primary antibody pair for your target, use either: 1) the Lumit® Immunoassay Cellular System - Starter Kit (Cat.# W1220), when only testing rabbit/mouse antibody pairs; or 2) the Lumit® Immunoassay Lysis and Detection Kit (Cat.# W1231) and any combination of the stand-alone Lumit® Anti-Mouse, Anti-Rabbit or Anti-Goat labeled with LgBiT or SmBiT.

Biological Conditions and Controls: When testing new targets, we recommend optimizing the cell number, as well as any treatment conditions and controls applicable to the biology of the target in question. We do not recommend using a no-cell control with these assays. Cellular debris can quench a small amount of luminescence signal, and so, counterintuitively, Lumit® control wells with no cells may have higher background than Lumit® control wells with cells.

Plates and Instruments: We recommend using standard solid white multiwell plates suitable for luminescence measurements (e.g., Corning Cat.# 3570, 3693 and 3917). Luminescence can be recorded on a variety of plate readers although the relative light units will depend on the instrument. Assay well geometry and small dispensing volumes may affect mixing efficiency. Thus, poor assay homogeneity in individual wells may result in increased reaction noise, reduced signals or both.

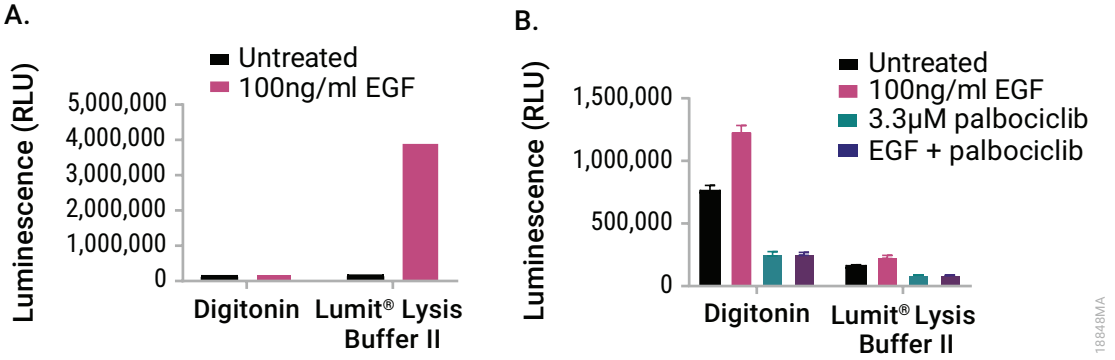

Figure 5. Demonstrating the importance of testing both lysis solutions for developing a Lumit® Immunoassay towards a new target. Panel A. Phosphorylated epidermal growth factor receptor (EGFR) is detected when using Lumit® Lysis Buffer II, 10X, but not Digitonin. A431 cells (25,000) were plated overnight, then stimulated for 5 minutes with 100ng/ml EGF (GIBCO™ Cat.# PHG0311L) prior to the addition of lysis solution. Robust phospho-EGFR detection is seen in cells lysed with Lumit® Lysis Buffer II, but not Digitonin. **Panel B.** Phosphorylation of retinoblastoma (RB) is seen in Digitonin lysed cells, and not cells lysed with Lumit® Lysis Buffer II. MCF7 cells (50,000) were serum starved overnight, then treated with palbociclib (Tocris Cat.# 4786) and or EGF for 24 hours.

4. Preparing for the Lumit® Immunoassay Cellular System

Materials to Be Supplied by the User

- a pair of primary antibodies against the target protein. Pairs can be made of antibodies from rabbit/mouse, mouse/goat or rabbit/goat combinations.
- solid white, multiwell plate (**Note:** Do not use black or clear plates). White plates with clear bottoms can also be used. However, when reading the luminescence signal, use white 3M paper to cover the bottom of the plate.
- multichannel pipette or automated pipetting station
- luminometer capable of reading multiwell plates (e.g., GloMax® Discover System, Cat.# GM3000)
- plate shaker
- protease inhibitor cocktail or protease/phosphatase inhibitor cocktail (e.g., Halt™ Protease and Phosphatase Inhibitor Cocktail, 100X, Thermo Fisher Scientific Cat.# 78440)

4. Preparing for the Lumit® Immunoassay Cellular System (continued)

Below is a quick reference protocol for the Lumit® Immunoassay Cellular System (2:2:1 volume format). For more details regarding cells and reagent preparation and detailed protocols, see Sections 4.A, 4.B and 5.

This protocol is designed for a 96-well plate using a 50µl:50µl:25µl ratio of cell lysate to antibody mix to detection reagent volumes. To use this assay in a high-throughput manner, such as in 384- or 1536-well plates, please refer to Section 7.

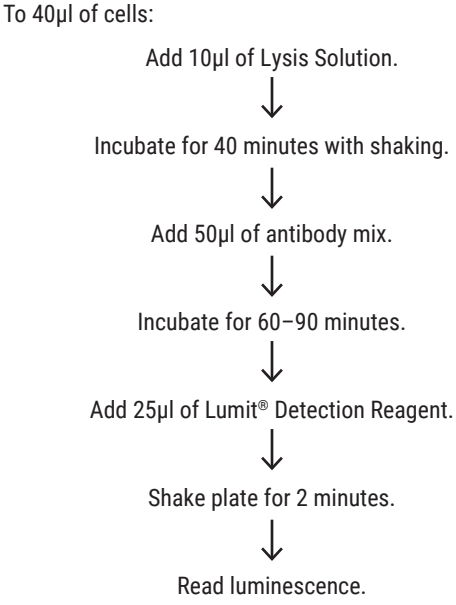

Table 1. Assay Component Volumes for Various Plate Formats.

Plate Type	Cell Lysate (µl)[1]	Antibody Mix (µl)	Substrate (µl)
96-well plate	50	50	25
96-well plate (half volume)	25	25	12.5
384-well plate	12.5	12.5	6.25

[1]Cell lysate volume includes cell medium, treatment and lysis solution.

4.A. Preparing Cells

Adherent Cells

1. Maintain adherent cells in an appropriate growth medium. Split the cells at 70–90% confluency.
2. Count cells and seed them into plates in complete growth medium as follows:

   For 96-well plates, seed 50,000 cells per well.

   For 384-well plates, seed 10,000 cells per well.

   Note: To reach an optimal signal, cell number should be determined experimentally because the signal is dependent on the cell type and signaling pathway. Generally, an optimal signal change can be obtained in a range of 10,000–80,000 cells per well of a 96-well plate.
3. Incubate the plate at the appropriate cell culture conditions overnight.

   Note: If necessary, the cells can be cultured in serum-free medium after the cells are stably adherent on the plate. Determine the length of serum starvation based on the signaling pathway of interest.
4. Replace the medium with 35µl of fresh cell medium.
5. To stimulate and/or inhibit a pathway of interest, treat the cells with 5µl of cell medium containing an appropriate dose of an activator and/or inhibitor. For control wells, add 5µl of cell medium containing vehicle (e.g., DMSO).

   Notes:

   a. Volumes used in Steps 4 and 5 can be adjusted to accommodate different treatments, as long as the final volume before adding lysis solution is 40µl for 96-well and 10µl for 384-well plates.

   b. To measure cell viability and normalize data to viable cell number, add Fluorogenic Live Cell Substrate (GF-AFC Substrate) to the cell medium during treatment (Step 4 or 5) to a final concentration of 50µM in the 40µl volume. The GF-AFC Substrate can be incubated with cells 0.5–3 hours before lysis, and the signal is stable for up to 3 hours.
6. Proceed immediately to the Lumit® Immunoassay Cellular System protocol (Section 5).

Suspension Cells

1. Maintain suspension cells in an appropriate cell growth medium.
2. Count cells and seed them in fresh medium as follows:

   For 96-well plates, seed 100,000 cells per well.

   For 384-well plates, seed 20,000 cells per well.

   Note: To reach an optimal signal, cell number should be determined experimentally because the signal is dependent on cell line and cell signaling pathway. Generally, an optimal signal change can be obtained in a range of 100,000–200,000 cells per well of a 96-well plate.

3. To stimulate and/or inhibit the pathway, treat the cells with 5µl of cell medium containing an appropriate dose of an activator and/or inhibitor. For control wells, add 5µl of cell medium containing vehicle (e.g., DMSO).

   Notes:

   a. Volumes used in Steps 2 and 3 can be adjusted to accommodate different treatments, as long as the final volume before adding lysis solution is 40µl for 96-well and 10µl for 384-well plates.

   b. To measure cell viability and normalize data to viable cell number, add Fluorogenic Live Cell Substrate (GF-AFC Substrate) to the cell medium during treatment (Step 2 or 3) to a final concentration of 50µM in the 40µl volume. The GF-AFC Substrate can be incubated with cells 0.5–3 hours before lysis, and the signal is stable for up to 3 hours.

4. Proceed immediately to the Lumit® Immunoassay Cellular System protocol (Section 5).

4.B. Preparing Reagents

Calculate the required volumes of Immunoassay Reaction Buffer and lysis solution. The assay follows the format of 2:2:1 volume ratios of cell lysate to antibody mix to detection reagent.

Note: The following instructions will prepare enough reagents for ten assay points.

1. **Immunoassay Reaction Buffer:** To make 1.5ml, mix 150µl of Lumit® Immunoassay Buffer C and appropriate volume of protease and phosphatase inhibitors cocktail with up to 1.35ml of water.

2. **Lysis Solution:** Prepare **one** of the following lysis solutions as follows:

   a. Prepare 100µl of Digitonin (0.1%) by mixing 5µl of Digitonin, 2%, and 4X protease/phosphatase inhibitor, then bringing volume to 100µl with Immunoassay Reaction Buffer.

   b. Prepare 100µl of 5X Lumit® Lysis Buffer II by adding 50µl of Lumit® Lysis Buffer II, 10X, to 50µl of water containing 10X protease/phosphatase inhibitors.

3. **Antibody Mix** (to be prepared during lysis step, Step 3, in Section 5): For each batch of ten assays, prepare 500µl of antibody mix by combining 3µl of each of four antibodies as shown in Table 2 with 488µl of Immunoassay Reaction Buffer.

   Note: In most cases, 15ng of each primary antibody per well (150ng/ml final concentration after antibody addition) is optimal. However, we recommend empirically determining optimal primary antibody concentrations (see Section 6). Based on the optimal concentration, an antibody working stock solution can be prepared in Antibody Dilution Buffer and stored at –30°C to –10°C. For example, for a 15ng per assay well optimum, a 50µg/ml antibody stock solution can be prepared and stored for subsequent assays. According to Table 2, 3µl of the antibody stock solution is used to prepare antibody mix for ten assays.

Table 2. Example of Antibody Mix Preparation (in Microliters per Ten Assays).

Component		Volume (µl)
Primary Antibodies	Anti-phospho-epitope antibody	3
	Anti-protein antibody	3
Lumit® Secondary Antibodies	Lumit® Anti-Mouse Antibody-LgBiT	3
	Lumit® Anti-Rabbit Antibody-SmBiT	3
Immunoassay Reaction Buffer		488

4. **Lumit® Detection Reagent** (to be prepared during the antibody binding step in Section 5): Equilibrate Lumit® Substrate C to room temperature. For ten assays, prepare 250µl of fresh Lumit® Detection Reagent by mixing 20µl of Lumit® Substrate C with 230µl of Immunoassay Reaction Buffer (1:12.5 dilution) just before use.

5. Lumit® Immunoassay Cellular System Protocol

The Lumit® Immunoassay Cellular System consists of three solution additions to cells after treatment (Figures 1 and 2). The following protocol does not require removing medium from cells after treatment.

Notes:

a. If your assay requires cell treatment in higher volume, remove cell medium after treatment. Then, resuspend cells in 50µl of 0.02% Digitonin or 1X Lumit® Lysis Buffer II with 1X protease and phosphatase inhibitors and proceed to Step 2.

b. Before performing the Lumit® Immunoassay, prepare cells and reagents as described in Section 4. However, the antibody mix and the Lumit® Detection Reagent should be prepared just before use.

c. We recommend using a set of cell samples without primary antibodies as a control to assess the reagent background level. To calculate net signal, subtract the reagent background values from the sample values.

d. Many cell types have been lysed successfully with the following protocol. You can optimize the lysis steps below for your specific cell type by either extending the incubation time (Step 2) or using a smaller volume with a higher lysis solution concentration (Step 1). Make sure the maximum final concentration of Digitonin is 0.015% and the maximum final concentration of Lumit® Lysis Buffer II is 0.8X after adding antibody mix (Step 4).

1. Add 10µl of lysis solution to each well containing 40µl cells.

   Note: If using Digitonin, the concentration is now 0.02%. If using Lumit® Lysis Buffer II, the concentration is now 1X. Using either of these lysis solutions creates a homogeneous lysate where intracellular target epitopes are exposed for detection by the Lumit® reagents.

2. Shake the plate vigorously at approximately 800rpm for 40 minutes.

3. Prepare antibody mix as described in Section 4.B.

4. Add 50µl of antibody mix to the lysed cells.

5. Shake the plate gently at approximately 400rpm for 2 minutes.
6. Incubate the plate at 23°C for 90 minutes.

   Note: A shorter incubation may be sufficient, but the optimal time should be empirically determined.
7. Prepare Lumit® Detection Reagent as described in Section 4.B.
8. Add 25µl of Lumit® Detection Reagent to the assay wells.
9. Shake the plate gently at approximately 400rpm for 2 minutes.
10. Measure the luminescence with a plate-reading luminometer.

    Note: Instrument settings depend on the manufacturer. An integration time of 0.25–1 second per well should serve as a guideline.
11. **Optional:** If GF-AFC Substrate was used, measure the resulting fluorescence using a fluorometer (380–400nm$_{Ex}$/505nm$_{Em}$) and normalize luminescent RLU values to the fluorescent RFU values.

6. Guidelines for Establishing the Lumit® Immunoassay for a New Target

The Lumit® Immunoassay Cellular System has been used successfully to detect both the phosphorylation and total levels of several signaling proteins in unmodified cells (2). For each validated target, we provide an application note containing representative data, the specific information about primary antibodies used and recommended concentration. The application notes are available on the Lumit® Immunoassay Cellular System web page: **www.promega.com/products/immunoassay-elisa/lumit-immunoassays/lumit-immunoassay-cellular-systems/**

For validated targets, we recommend following the protocol in Section 5 and antibody conditions described in the application notes. To develop a Lumit® Immunoassay for a new target, the following sections describe how to select primary antibodies and perform optimization.

6.A. Primary Antibody Selection

Sensitivity and specificity of the Lumit® Immunoassay depend on the quality of primary antibodies used. It is important to select best-performing antibodies to take full advantage of the Lumit® Immunoassay Cellular System. A good starting point is to select two to three primary antibody pairs (e.g., rabbit/mouse, mouse/goat or rabbit/goat) and use the corresponding Lumit® secondary antibodies with the Lumit® Immunoassay Lysis and Detection Kit. When only testing a rabbit/mouse antibody pair, we recommend using the Lumit® Immunoassay Cellular System - Starter Kit (Cat.# W1220) as it contains both combinations of SmBiT- and LgBiT-conjugated secondary antibodies. While the starter kit will allow you to identify the Lumit® secondary antibody combination that works best for your target protein, additional reagents (Lumit® Cellular - Set 1 or Set 2) are needed the perform the rest of the optimization.

6.B. Selecting A Lysis Solution

Some protein targets can be detected exclusively when lysing with Digitonin, while others are detected exclusively with Lumit® Lysis Buffer II. To determine the optimal lysis solution for your target, we recommend including Digitonin and Lumit® Lysis Buffer II in your initial checkerboard experiment with primary antibodies, as described in Section 6.C. We recommend running the checkerboard experiment in duplicate, with one side of the plate containing cells lysed with Digitonin, and the other side of the plate containing cells lysed with Lumit® Lysis Buffer II (Figure 6, Panel A).

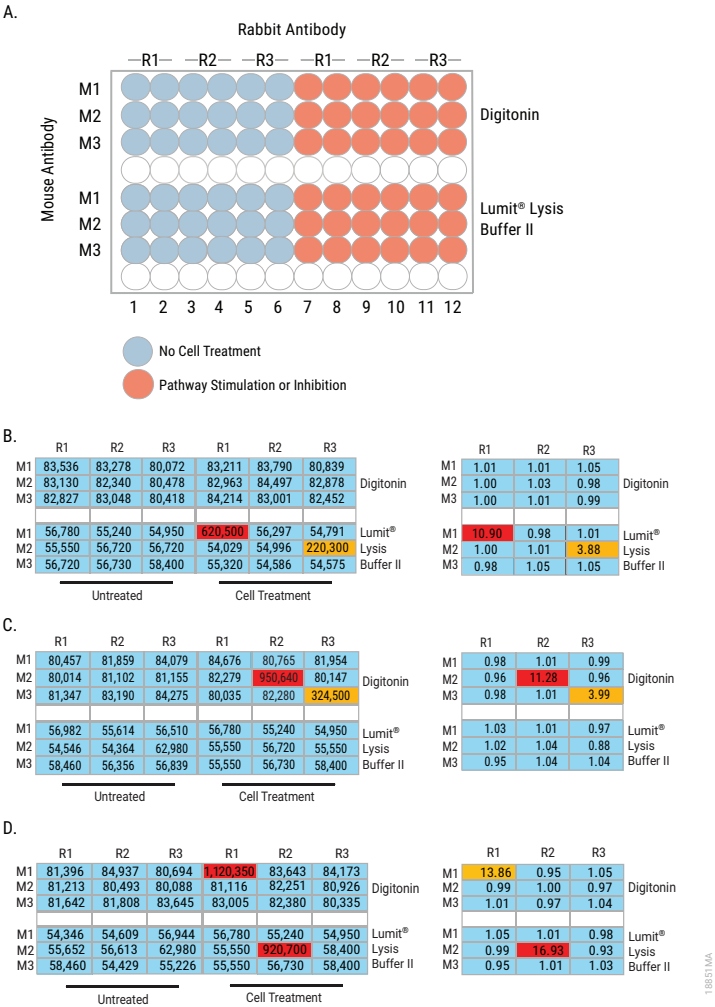

Figure 6. Single antibody concentration checkerboard. Panel A. Plate map demonstrating how to run a checkerboard of three rabbit antibodies (designated R1, R2 and R3) and three mouse antibodies (designated M1, M2 and M3) in duplicate pairs. Two treatment conditions are indicated, and running both Lumit® Lysis Buffer II, 10X, and Digitonin on a single plate is recommended. **Panels B, C and D**. Example data showing possible outcomes when using both lysis solutions to screen for antibodies. Both averaged raw data (left) and treatment over no treatment signal ratio (right) are shown. It is important to take both the raw luminescence and signal-to-background ratio into account when selecting an antibody pair. In **Panel B**, only Lumit® Lysis Buffer II allowed for detection of the target in question and antibody pair M1/R1 is the best choice. In **Panel C**, only Digitonin lysis yielded a functional assay and antibody pair M2/R2 is the best choice. In **Panel D**, both antibody pairs, M1/R1 with Digitonin or M2/R2 with Lumit® Lysis buffer II are good choices for further optimization, such as in the antibody titration checkerboard.

6.C. Selecting the Optimal Primary Antibody Pair in Checkerboard Experiments

To select antibody pairs, we recommend performing a checkerboard experiment with various antibody pairs at a single concentration (e.g., 15ng/well) against the target protein in cells. Include two treatment conditions for your cells to provide high- and low-end signals. Using this approach, select an antibody pair that provides the highest luminescent signal with the largest signal-to-background ratio. In a second checkerboard, titrate each antibody of the selected pair for optimal concentration. Verify selectivity by demonstrating an authentic biological response to inputs that change the target protein levels.

Note: The cells can be nontreated or treated with an activator or inhibitor that will change the target protein level. Sometimes, a treatment that will modulate target protein level in cells is not known; therefore, upper and lower signals in the checkerboard experiments cannot be achieved. In this case, we recommend using a purified recombinant version of the target protein if available.

Single Antibody Concentration Checkerboard

In the example shown in Figure 6, three rabbit and three mouse antibodies against a target protein were used to create a nine-pair checkerboard. Each antibody was used at 150ng/ml final concentration after antibody addition (15ng/well). A modified Lumit® Immunoassay protocol that includes the checkerboard scheme is described here.

1. Prepare 8ml of Immunoassay Reaction Buffer and 400µl each of Digitonin and Lumit® Lysis Buffer II, as described in Section 4.B.
2. Prepare 40µl of cells per well in a 96-well plate as described in Section 4.A following the checkerboard scheme in Figure 6, Panel A.

   Note: If a recombinant version of the target protein is used instead of cells, prepare 1.6ml of 1nM protein in Immunoassay Reaction Buffer. Then add 40µl of Immunoassay Reaction Buffer in the wells marked "No Cell Treatment" and 40µl of target protein solution in the wells marked "Pathway Stimulation or Inhibition".
3. Add 10µl of each lysis solution to the corresponding wells.
4. Shake the plate vigorously at approximately 800rpm for 40 minutes.
5. Prepare 400µl of each primary antibody by diluting 400ng antibody in the appropriate volume of Immunoassay Reaction Buffer.
6. Prepare 1.6ml of Lumit® secondary antibody mix by adding 24µl of Lumit® Anti-Mouse Antibody-LgBiT and 24µl of Lumit® Anti-Rabbit Antibody-SmBiT to 1,552µl of Immunoassay Reaction Buffer.
7. Add 30µl of Immunoassay Reaction Buffer to all Lumit® only control wells.
8. Add 15µl of each rabbit primary antibody to the corresponding wells as shown in Figure 6, Panel A.
9. Add 15µl of each mouse primary antibody to the corresponding wells as shown in Figure 6, Panel A.
10. Add 20µl of Lumit® secondary antibody mix to all wells.
11. Shake the plate gently at approximately 400rpm for 2 minutes.
12. Incubate the plate at 23°C for 90 minutes.

13. Prepare 2ml of Lumit® Detection Reagent as described in Section 4.B.
14. Add 25µl of Lumit® Detection Reagent to all wells.
15. Shake the plate gently at approximately 400rpm for 2 minutes.
16. Measure the luminescence with a plate-reading luminometer.

    Note: Instrument settings depend on the manufacturer. Use an integration time of 0.25–1 second per well as a guideline.
17. Use absolute luminescence readings, as well as signal-to-background ratios, to select an antibody combination for subsequent assays as described in Figure 6. The optimal antibody pair provides a balance between best luminescent signal and signal-to-background ratio. See examples in Figure 6, Panels B, C and D.

    Note: If no antibody pair is selected in this experiment, repeat the checkerboard selection using other available primary antibodies, increase the amount of antibody per well or use the other combination of the Lumit® Antibodies (e.g., Lumit® Anti-Mouse Antibody-SmBiT and Lumit® Anti-Rabbit Antibody-LgBiT). In some instances, the latter combination may perform better.

6.C. Selection of Optimal Primary Antibody Pair in Checkerboard Experiments (continued)

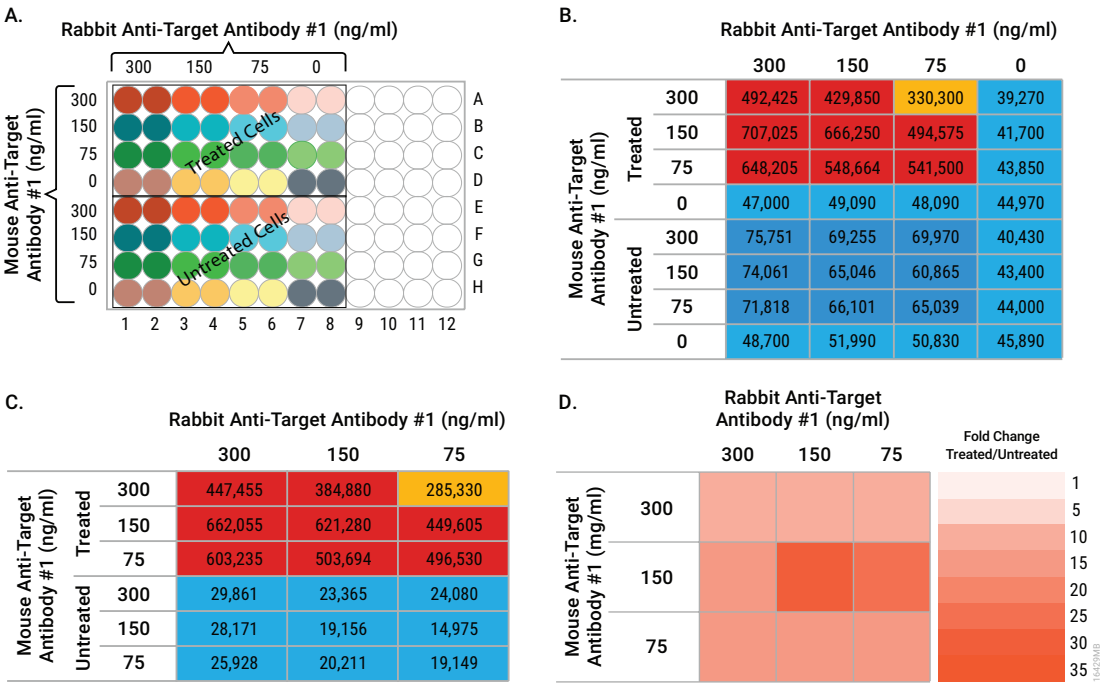

Figure 7. Antibody titration checkerboard experiment. Panel A. Checkerboard experiment design using three dilutions of one rabbit and one mouse antibody selected from the single-dose checkerboard experiment (M1/R1 from Figure 6, Panel B). **Panel B.** Example of average luminescence values obtained using combinations of three dilutions of the rabbit and mouse antibodies for target protein detection. **Panel C.** Net luminescence values after subtraction of the "no primary antibodies" control. **Panel D.** Fold changes (Treated/Untreated) calculated from Panel C and represented in a heat map to determine the best antibody concentration for subsequent experiments.

Antibody Titration Checkerboard

In the example shown in Figure 7, rabbit primary antibody #1 and mouse primary antibody #1 were titrated to determine the optimal concentration of each. Each antibody will be used at 75ng/ml, 150ng/ml and 300ng/ml final concentration after antibody addition (7.5ng, 15ng and 30ng per well). A modified Lumit® Immunoassay protocol that includes the checkerboard scheme is described here.

1. Prepare 7ml of Immunoassay Reaction Buffer and 700µl of Lysis Solution as described in Section 4.

2. Prepare 40µl of cells per well in a 96-well plate as described in Section 4 following the checkerboard scheme in Figure 7, Panel A.

   Note: If a recombinant version of the target protein is to be used instead of cells, add 40µl of Immunoassay Reaction Buffer in the wells marked "Untreated" and 40µl of 1nM target protein solution in the wells marked "Treated".

3. Prepare 300µl of each primary antibody dilution by mixing an appropriate volume of Immunoassay Reaction Buffer with 150ng, 300ng or 600ng antibody for the 75ng/ml, 150ng/ml or 300ng/ml concentrations, respectively.

4. Prepare 1,400µl of Lumit® antibody mix by adding 21µl of Lumit® Anti-Mouse Antibody-LgBiT and 21µl of Lumit® Anti-Rabbit Antibody-SmBiT to 1,358µl of Immunoassay Reaction Buffer.

5. Add 10µl of Lysis Solution to all wells.

6. Shake the plate vigorously at approximately 800rpm for 40 minutes.

7. Add 15µl of each rabbit primary antibody dilution to the corresponding wells as shown in Figure 7, Panel A.

8. Add 15µl of each mouse primary antibody dilution to the corresponding wells as shown in Figure 7, Panel A.

9. Add 15µl of Immunoassay Reaction Buffer to the "0ng antibody" control wells (rows D and H, columns 7 and 8) as shown in Figure 7, Panel A.

10. Add 20µl of Lumit® antibody mix to all wells.

11. Shake the plate gently at approximately 400rpm for 2 minutes.

12. Incubate the plate at 23°C for 90 minutes.

13. Prepare 1.8ml of Lumit® Detection Reagent as described in Section 3.B.

14. Add 25µl of Lumit® Detection Reagent to all wells.

15. Shake the plate gently at approximately 400rpm for 2 minutes.

16. Measure the luminescence with a plate-reading luminometer.

    Note: Instrument settings depend on the manufacturer. An integration time of 0.25–1 second per well should serve as a guideline.

17. Subtract the background value of "no primary antibodies" control (wells D7, D8, H7 and H8; Figure 7, Panel A) from the corresponding experimental samples. Use the normalized luminescence values as well as signal-to-background ratio to select an antibody concentration combination for subsequent assays as described in Figure 7, Panels C and D.

    Note: In this experiment, the optimal antibody concentrations provides a balance between best luminescent signal and signal-to-background ratio.

18. Make primary antibody working stock solutions at the chosen concentration in the Antibody Dilution Buffer and store at –30°C to –10°C. For example, if the optimal amount is found to be 150ng/ml, a 50µg/ml concentrated primary antibody working stock solution can be made and stored for subsequent assays.

7. Guidelines for Miniaturizing Lumit® ICS for High-Throughput Screening

7.A. Scaling Down Reagents for use in 384- or 1536-Well Formats

There are two recommended protocols for scaling down Lumit™ Immunoassays for use in a high-throughput format, ratiometric scaling and user-defined volumes. Ratiometric scaling involves maintaining the recommended 2:2:1 volume ratio of lysate:antibody:substrate. This method requires less optimization but also gives the user less flexibility to choose the volumes they want per well. To complete miniaturization using ratiometric scaling, simply use a fold change to reduce the volume of each step, keeping the recommended 2:2:1 volume ratio.

User-defined volumes allow greater flexibility, but require more effort on your part to optimize. In this scheme, define the volumes you want to use at each step, keeping reagents within the defined concentration limits of the kit components. To complete miniaturization with user-defined volumes, follow the scheme outlined here, staying within the defined ranges listed for each reagent. Use the following recommended reagent volume or concentrations.

1. Lyse cells. Cell lysis recommendations are based on the negative effect that some detergents have on NanoBiT® complementation. Detergent concentrations that are too high can negatively affect light output. If detergent concentrations are too low, not enough lysis will occur for target detection.

   Use either:

   a. Digitonin: 0.01–0.025%, or

   b. Lumit® Lysis Buffer II: 0.8–1.0X

2. Add antibody:

   a. Add 0.1µl/well for Lumit® secondary antibodies.

   b. Titrate primary antibodies, starting at 0.1µl/well from a 50µg/ml stock solution.

3. Add substrate:

   a. ≤7% Lumit® Substrate C Solution to prevent precipitation

   b. ≥1% Substrate per well for best signal results

7.B. Diluting Lumit® Substrate C for Increased Stability

The stability of Lumit® Substrate C is poor when diluted in 1X Immunoassay Reaction Buffer. This is normally not an issue when following the standard protocol and preparing the substrate dilution immediately prior to adding to the plate. However, for instances where the substrate must be diluted more than 10 minutes before adding to the plate (e.g., high-throughput screening), greater stability is needed. To achieve this, prepare a simple dilution of the substrate in ethanol, glycerol and water. Use these guidelines and Table 3 to prepare Lumit® Substrate C dilutions.

- The Lumit® Substrate C concentration in solution should not exceed 7%. The substrate could fall out of solution if the concentration is too high.
- The minimum amount of Lumit® Substrate C in each well of the plate should be ≥1%.
- Always maintain water at 50% in your solution. If you deviate from 7% Lumit® Substrate C, then make up this volume with ethanol and glycerol.
- A simple way to prepare the diluted substrate is to first prepare a solution of 85% ethanol and 15% glycerol. Then, for every 100µl needed, add 50µl of water, 7µl of substrate and 43µl of ethanol and glycerol solution (Table 3).

Table 3. Diluting Substrate for Increased Stability at Room Temperature.

Reagent	Percent in Solution
Lumit® Substrate C	7
85% Ethanol/15% Glycerol	43
Water	50

7.B. Diluting Lumit® Substrate C for Increased Stability (continued)

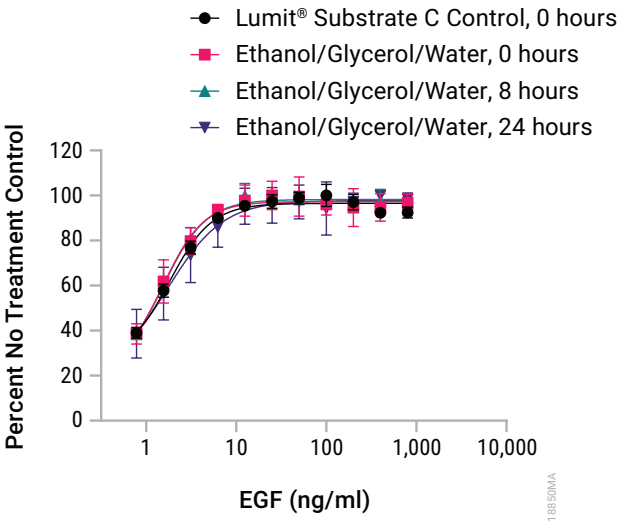

Figure 8. Demonstration of Substrate Stability using p-ERK (Thr202) Assay. Data demonstrating the stability of Lumit® Substrate C when diluted in ethanol/glycerol/water, 8 and 24 hours prior to plate addition. A 0-hour control of Lumit® Substrate C diluted in 1X Immunoassay Reaction buffer was included to demonstrate that diluting substrate in ethanol/glycerol/water has no effect on assay performance. MCF7 cells (50,000) were plated a day prior to running the experiment and allowed to settle overnight. Cells were treated with 100ng/ml EGF or medium control, then shaken for 5 minutes prior to adding Lumit® Lysis Buffer II for cell lysis. Phosphorylated ERK (Thr202) was detected following the procedure in this application note:
www.promega.com/products/immunoassay-elisa/lumitimmunoassays/lumit-immunoassay-cellular-systems/
Plate was shaken for 2 minutes prior to a 1.5-hour incubation with antibodies. Substrate was then added and plate read after a 2-minute shake. Substrates were prepared and incubated at room temperature for the number of hours indicated prior to adding to the plate.

8. References

1. Dixon, A.S. *et al.* (2016) NanoLuc complementation reporter optimized for accurate measurement of protein interactions in cells. *ACS Chem. Biol.* **11**, 400–8.
2. Hwang, B.B. *et al.* (2020) A homogeneous bioluminescent immunoassay approach to probe cellular signaling pathway regulation. *Commun. Biol.* **3**, 8.
3. Zegzouti, H. *et al.* (2020) A versatile bioluminescent immunoassay approach to probe cellular signaling pathway regulation. Poster presented at the 2020 Annual Conference of the Society for Laboratory Automation and Screening (SLAS), available online at: **www.promega.com/resources/scientific-posters/**

9. Related Products

Lumit® Secondary Antibodies[(a)]

Product	Size	Cat.#
Lumit® Anti-Mouse Ab-LgBiT	30µl	W1021
Lumit® Anti-Mouse Ab-LgBiT	300µl	W1022
Lumit® Anti-Mouse Ab-SmBiT	30µl	W1051
Lumit® Anti-Mouse Ab-SmBiT	300µl	W1052
Lumit® Anti-Rabbit Ab-LgBiT	30µl	W1041
Lumit® Anti-Rabbit Ab-LgBiT	300µl	W1042
Lumit® Anti-Rabbit Ab-SmBiT	30µl	W1031
Lumit® Anti-Rabbit Ab-SmBiT	300µl	W1032
Lumit® Anti-Goat Ab-LgBiT	30µl	W1061
Lumit® Anti-Goat Ab-LgBiT	300µl	W1062
Lumit® Anti-Goat Ab-SmBiT	30µl	W1071
Lumit® Anti-Goat Ab-SmBiT	300µl	W1072

9. Related Products (continued)

Kinase Assays

Product	Size	Cat.#
ADP-Glo™ Kinase Assay	400 assays	V6930
NanoBRET® TE Intracellular Kinase Assay, K-3	100 assays	N2600
NanoBRET® TE Intracellular Kinase Assay, K-4	100 assays	N2520
NanoBRET® TE Intracellular Kinase Assay, K-5	100 assays	N2500
NanoBRET® TE Intracellular Kinase Assay, K-8	100 assays	N2620
NanoBRET® TE Intracellular Kinase Assay, K-9	100 assays	N2630
NanoBRET® TE Intracellular Kinase Assay, K-10	100 assays	N2640
NanoBRET® TE Intracellular Kinase Assay, K-11	100 assays	N2650

Instrument

Product	Size	Cat.#
GloMax® Discover System	1 each	GM3000

10. Summary of Changes

The following changes were made to the 3/24 revision of this Technical Manual:

1. Lumit® Lysis Buffer II, 10X, a new kit component, was added in Section 2, as well as to flow diagrams and protocol sections.
2. Lumit® trademark was updated.
3. Limited Use Label License (a) and patent statement (b) were updated.
4. Table 3 was added. Figure 7 was modified. New figures 4, 5, 6 and 8 were added.
5. Section 7 was added and remaining sections renumbered.

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In addition, researcher must either:

(i) use Lumit®-branded luminescent assay reagents (LARs) for all determinations of luminescence activity of this product and its derivatives; or

(ii) contact Promega to obtain a license for use of the product and its derivatives with LARs not manufactured by Promega.

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[b]U.S. Pat. Nos. 9,797,889, 9,797,890, 10,107,800, 11,493,504 and other patents and patents pending.

[c]U.S. Pat. No. 8,809,529, European Pat. No. 2635582 and other patents and patents pending.

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