TECHNICAL MANUAL

Lumit™ Immunoassay Detection Reagents

Instructions for Use of Products
VB2010, VB2020, VB2030, VB4050 and VB4060

Revised 8/20
TM614

Promega

Lumit™ Immunoassay Detection Reagents

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

1. Description

The Lumit™ Immunoassay is based on NanoLuc® Binary Technology (NanoBiT). NanoBiT is a structural complementation system designed for biomolecular interaction studies (1). The NanoBiT® system is composed of two subunits, Large BiT (LgBiT; 18kDa) and Small BiT (SmBiT; 11 amino acid peptide), that have been optimized for stability and minimal self-association due to weak affinity (190µM). In Lumit™ Immunoassays, antibodies are chemically labeled with SmBiT and LgBiT subunits. Upon binding of the labeled antibodies to an analyte, the subunits come into close proximity, reassemble into a functional luciferase enzyme and generate a luminescent signal in the presence of substrate (Figure 1). Other immunoassay formats, including competition and indirect immunoassays, can also be developed using this technology (2).

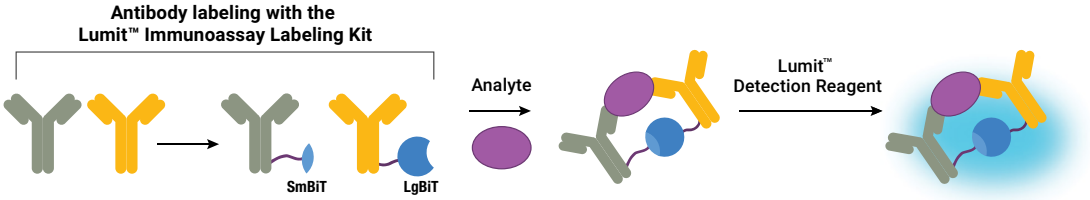

Figure 1. Schematic of Lumit™ Immunoassay.

The Lumit™ Immunoassay Labeling Kit and Lumit™ Immunoassay Detection Reagents[a, b] enable you to develop Lumit™ Immunoassays customized to your specific application. The following protocol is for use with Lumit™ Immunoassay Detection Reagents. For the Lumit™ Immunoassay Labeling Kit, refer to the *Lumit™ Immunoassay Labeling Kit Technical Manual* #TM602.

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2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
Lumit™ Immunoassay Detection Reagent A	**500 assays**	**VB2010**

Not for Medical Diagnostic Use. Includes:

- 200µl Lumit™ Detection Substrate A
- 10ml Lumit™ Immunoassay Buffer A, 10X

PRODUCT	SIZE	CAT.#
Lumit™ Immunoassay Detection Reagent A	**5,000 assays**	**VB2020**

Not for Medical Diagnostic Use. Includes:

- 2 × 1ml Lumit™ Detection Substrate A
- 100ml Lumit™ Immunoassay Buffer A, 10X

PRODUCT	SIZE	CAT.#
Lumit™ Immunoassay Detection Reagent A	**50,000 assays**	**VB2030**

Not for Medical Diagnostic Use. Includes:

- 5 × 4ml Lumit™ Detection Substrate A
- 10 × 100ml Lumit™ Immunoassay Buffer A, 10X

PRODUCT	SIZE	CAT.#
Lumit™ Immunoassay Detection Reagent B	**100 assays**	**VB4050**

Not for Medical Diagnostic Use. Includes:

- 160µl Lumit™ Detection Substrate B
- 3.2ml Lumit™ Immunoassay Buffer B

PRODUCT	SIZE	CAT.#
Lumit™ Immunoassay Detection Reagent B	**1,000 assays**	**VB4060**

Not for Medical Diagnostic Use. Includes:

- 1.25ml Lumit™ Detection Substrate B
- 25ml Lumit™ Immunoassay Buffer B

Notes: Thaw the Lumit™ Immunoassay Buffer A and B before use. Thawed buffer can be stored at 2°C to 10°C. The number of assays that can be performed with the reagents may change depending on the type of assay. See application-specific suggestions in Section 3.

Storage Conditions: Store all components at –30°C to –10°C.

3. Guidelines for Use of Lumit™ Immunoassay Detection Reagents

General Considerations

- Lumit™ Immunoassay Detection Reagents are designed for use with Lumit™ Immunoassay technology. Please refer to the *Lumit™ Immunoassay Labeling Kit Technical Manual*, #TM602 to learn more about the development and use of Lumit™ Immunoassays.
- The Lumit™ Immunoassay Detection Reagents should be made fresh for each experiment. We do not recommend long-term storage of the reagent at any temperature.
- Lumit™ Immunoassay performance will depend on the reagent, quality of antibodies, sample type, sample matrix, etc. Optimization is required for each application.
- Protocols provided below are for a standard 96-well white plate. The assay can be scaled down to a 96-well half-area white plate or up to a 384-well white plate.
- Reagents are compatible with all plate-reading luminometers. Relative Light Unit (RLU) readings will vary with the sensitivity and settings of each instrument. The use of different instruments will affect the magnitude of RLUs and might affect the assay window for test samples. Figure 2 lists the sensitivity and dynamic range of some common commercial luminometers.
- Detergents, large excess of proteins and other matrices impact the assay and will decrease the light output. If needed, RLUs can be increased by increasing the amount of Lumit™ Detection Substrate in the detection reagent.

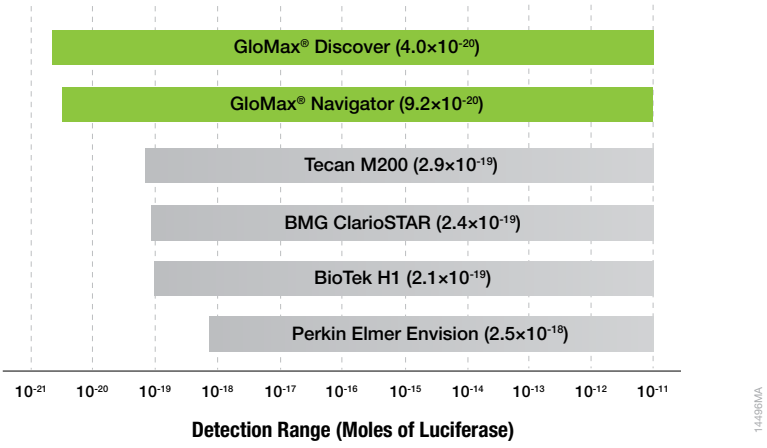

Figure 2. The detection range of various luminometers.

3.A. Lumit™ Immunoassay Detection Reagent A

Before you begin, dilute Lumit™ Immunoassay Buffer A to 1X by combining 90ml of PBS and 10ml of Lumit™ Immunoassay Buffer A, 10X.

Materials to Be Supplied by the User

- white 96-well plate
- multichannel pipette or automated pipetting station
- luminometer capable of reading multiwell plates (e.g., GloMax® Discover System)
- reagent reservoir (e.g., Thermo Scientific Cat.# 8093-11)
- plate shaker
- plate sealer

Biochemical Assays

Biochemical assays are performed to detect and quantify analytes in vitro. To minimize matrix effects, you will need to optimize the assay performance in the sample matrix. We suggest optimizing sample volume and sample dilution. Absolute light output and signal/background ratio can be tailored by changing the concentration of Lumit™ Detection Substrate in the well.

1. Identify the antibodies specific for the analyte of interest, label them with SmBiT and LgBiT, and determine the optimum concentrations to be used as provided in the *Lumit™ Immunoassay Labeling Kit Technical Manual*, #TM602.
2. Prepare 2ml of antibody-SmBiT and 2ml of antibody-LgBiT. Make the solutions at concentrations determined from Step 1 using 1X Lumit™ Immunoassay Buffer A.
3. Add 20µl of each antibody-SmBiT and antibody-LgBiT to the wells.

   Note: For simplicity, a master mix can be prepared by mixing 2ml each of antibody-SmBiT and antibody-LgBiT, and adding 40µl of master mix to each well. Master mix should be prepared fresh just before use.
4. Add 20µl of sample.
5. Mix gently and incubate for 30–120 minutes at room temperature.
6. Prepare fresh Lumit™ Detection Reagent A by mixing 40µl of the Lumit™ Detection Substrate A with 1,960µl of the 1X Lumit™ Dilution Buffer A (50-fold dilution).

   Note: To increase the RLU, Lumit™ Detection Reagent can be prepared at 25-fold or 12.5-fold dilution.
7. Add 20µl of diluted Lumit™ Detection Reagent A to each well.
8. Wait 3 minutes and measure luminescence using a plate-reading luminometer.

   Note: The luminescence intensity will decay gradually, due to depletion of the furimazine substrate. The signal depletion can be significant at very high analyte concentration, in which case the experimental condition should be modified by increasing the substrate concentration or reading the plate immediately after addition of the substrate.

Cell Lysate Assays

Intracellular proteins and post-translation modifications can be detected in cell lysate using Lumit™ Immunoassays. However, the Lumit™ Immunoassay is very sensitive to detergents and will not work with commonly used lysis reagents containing high concentrations of detergents (e.g., RIPA buffer). A suggested protocol is provided below for using Lumit™ Immunoassay in cell lysates.

Note: An optimized detection module containing detergent (digitonin), buffers and detection reagent is available (Cat.# W1231). This module also includes reagents for measuring cell viability to normalizing data to viable cell number.

1. Identify the antibodies specific for the analyte of interest, label them with SmBiT and LgBiT, and determine the optimum concentration to be used as provided in the *Lumit™ Immunoassay Labeling Kit Technical Manual*, #TM602.
2. Maintain the cells in a white 96-well plate and grow them to 70–90% confluency (50,000–200,000 cells per well is suggested).
3. Change the media, add 35µl of fresh medium and treat the cells by adding the appropriate amount of activator/inhibitor in 5µl of medium. The total volume is 40µl in the well.
4. Add 10µl of 0.1% digitonin (concentration in the well 0.02%).
5. Incubate for 20 minutes with shaking.
6. Prepare 2.5ml of antibody-SmBiT and 2.5ml of antibody-LgBiT at concentrations determined from Step 1. Prepare using 1X Lumit™ Immunoassay Buffer A.
7. Make a master mix by mixing 2.5ml each of antibody-SmBiT and antibody-LgBiT. Master mix should be prepared fresh just before use.
8. Add 50µl of the master mix to the wells (final digitonin concentration in the well is 0.01%).
9. Incubate for 60–90 minutes at room temperature.
10. Prepare fresh Lumit™ Detection Reagent A by mixing 200µl of Lumit™ Detection Substrate A with 2,300µl of 1X Lumit™ Dilution Buffer A (12.5-fold dilution).

    Notes:

    a) With the standard protocol, 200µl of Lumit™ Detection Substrate A will be used for one 96-well plate.

    b) It may be possible to dilute Lumit™ Detection Substrate A (1:25 or 1:50 fold) if sufficient signal is generated and/or a sensitive luminometer is used.
11. Add 25µl of Lumit™ Immunoassay Detection Reagent A to each well.
12. Wait 3 minutes and measure luminescence using a plate-reading luminometer.

    Note: The luminescence intensity will decay gradually, due to depletion of the furimazine substrate. The signal depletion can be significant at very high analyte concentration, in which case the experimental condition should be modified by increasing the substrate concentration or reading the plate immediately after addition of the substrate.

3.B. Lumit™ Immunoassay Detection Reagent B

Lumit™ Immunoassay Detection Reagent B is specifically designed for detection of analytes in cell culture media containing fetal bovine serum (FBS), and for detection of analyte directly in cell culture wells containing media and cells.

Materials to Be Supplied by the User

- white 96-well plate
- multichannel pipette or automated pipetting station
- luminometer capable of reading multiwell plates (e.g., GloMax® Discover System)
- reagent reservoir (e.g., Thermo Scientific Cat.# 8093-11)
- plate shaker
- plate sealer

Cell Supernatant Transfer Assays

This protocol describes the procedure for transferring medium from treated cells to a separate assay plate, leaving the cells and remaining medium intact for additional uses. For quantitation purposes, a dilution series of analyte prepared in the cell culture medium can be used for generating a calibration curve.

1. Identify the antibodies specific for the analyte of interest, label them with SmBiT and LgBiT, and determine the optimum concentrations to be used as provided in the *Lumit™ Immunoassay Labeling Kit Technical Manual*, #TM602.
2. Plate the cells in a white 96-well plate.

   Note: The optimal number of cells to plate per assay well for a particular cell model should be empirically determined to ensure that expected maximum levels of analyte produced will not exceed the linear dynamic range of the assay detection chemistry. Within that constraint, cell number can be increased to meet the desired sensitivity for lower-level analyte production.
3. Treat the cells by adding the desired concentration(s) of activator/inhibitor to the assay wells.
4. Transfer 50µl of cell supernatants (and calibration standards) to empty assay wells in a white 96-well plate.

   Notes:

   a. Prepare calibration standards in cell medium.

   b. Samples may have to be diluted if the analyte concentration exceeds the linear dynamic range of the assay.
5. Prepare 2.5ml of antibody-SmBiT and 2.5ml of antibody-LgBiT, at concentrations determined from Step 1 using cell culture medium.
6. Prepare a master mix by mixing 2.5ml each of antibody-SmBiT and antibody-LgBiT, and adding 50µl of master mix to each well. Master mix should be prepared fresh just before use.
7. Mix gently, then incubate for 30–120 minutes at room temperature.
8. Prepare fresh Lumit™ Immunoassay Detection Reagent B by mixing 160µl of Lumit™ Detection Substrate B into 3,040µl of Lumit™ Detection Buffer B (20-fold dilution).

9. Add 25µl of Lumit™ Immunoassay Detection Reagent B to each well.
10. Wait 3 minutes and measure luminescence using a plate-reading luminometer.

    Note: The luminescence intensity will decay gradually, due to depletion of the furimazine substrate. The signal depletion can be significant at very high analyte concentration, in which case the experimental condition should be modified by increasing the substrate concentration or reading the plate immediately after addition of the substrate.

Cell Culture Direct (No-Transfer) Assays

This protocol describes the procedure for detection of analyte directly in cell culture wells containing both cells and cell supernatant, without the need for sample transfer. For quantitation purposes, a dilution series of analyte prepared in the cell culture medium can be used for generating a calibration curve.

1. Identify the antibodies specific for the analyte of interest, label them with SmBiT and LgBiT, and determine the optimum concentrations to be used as provided in the *Lumit™ Immunoassay Labeling Kit Technical Manual*, #TM602.
2. Plate the cells in a white 96-well plate.

    Note: The optimal number of cells to plate per assay well for a particular cell model should be empirically determined to ensure that expected maximum levels of analyte produced will not exceed the linear dynamic range of the assay detection chemistry. Within that constraint, cell number can be increased to meet the desired sensitivity for lower level analyte production.
3. Treat the cells by adding the desired concentration(s) of activator/inhibitor to the assay wells. The additional volume of treatment should be such that the resultant total culture volume is 80µl, including the volume of the previously plated cells.
4. Prepare 1ml of 10X antibody-SmBiT and 1ml of 10X antibody-LgBiT, at concentrations determined from Step 1 using cell culture medium.
5. Prepare a master mix by mixing 1ml each of antibody-SmBiT and antibody-LgBiT, and adding 20µl of master mix to each well. Master mix should be prepared fresh just before use.
6. Mix gently, then incubate for 30–120 minutes at 37°C in a 5% CO_2 humidified incubator.
7. Prepare fresh Lumit™ Immunoassay Detection Reagent B by mixing 160µl of Lumit™ Detection Substrate B into 3,040µl of Lumit™ Detection Buffer B (20-fold dilution).
9. Add 25µl of Lumit™ Immunoassay Detection Reagent B to each well.
10. Wait 3 minutes and measure luminescence using a plate-reading luminometer.

    Note: The luminescence intensity will decay gradually, due to depletion of the furimazine substrate. The signal depletion can be significant at very high analyte concentration, in which case the experimental condition should be modified by increasing the substrate concentration or reading the plate immediately after addition of the substrate.

4. References

1. Dixon, AS. *et al.* (2016) NanoLuc complementation reporter optimized for accurate measurement of protein interactions in cells. *ACS Chem Biol.* **11(2)**, 400–8.
2. Hwang, B. *et al.* (2020) A homogeneous bioluminescent immunoassay approach to probe cellular signaling pathway regulation. *Commun. Biol.* **3**, 8.

5. Related Products

Lumit™ Immunoassay Labeling System

Product	Size	Cat.#
Lumit™ Immunoassay Labeling Kit	1 each	VB2500

Not for Medical Diagnostic Use.

Lumit™ Immunoassay Cellular System

Product	Size	Cat.#
Lumit™ Immunoassay Cellular System–Starter Kit	200 assays	W1220
Lumit™ Immunoassay Cellular System–Set 1	100 assays	W1201
Lumit™ Immunoassay Cellular System–Set 1	1,000 assays	W1202
Lumit™ Immunoassay Cellular System–Set 1	10,000 assays	W1203
Lumit™ Immunoassay Cellular System–Set 2	100 assays	W1331
Lumit™ Immunoassay Cellular System–Set 2	1,000 assays	W1332
Lumit™ Immunoassay Cellular System–Set 2	10,000 assays	W1333
Lumit™ Immunoassay Lysis and Detection Kit	100 assays	W1231
Lumit™ Immunoassay Lysis and Detection Kit	1,000 assays	W1232
Lumit™ Immunoassay Lysis and Detection Kit	10,000 assays	W1233

Not for Medical Diagnostic Use.

Luminometers

Product	Size	Cat.#
GloMax® Discover System	1 each	GM3000
GloMax® Navigator System	1 each	GM2000
GloMax® Explorer System	1 each	GM3500

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

6. Summary of Change

The following change was made to the 8/20 revision of this document:

Updated Materials to Be Supplied by the User in Sections 3.A and 3.B.