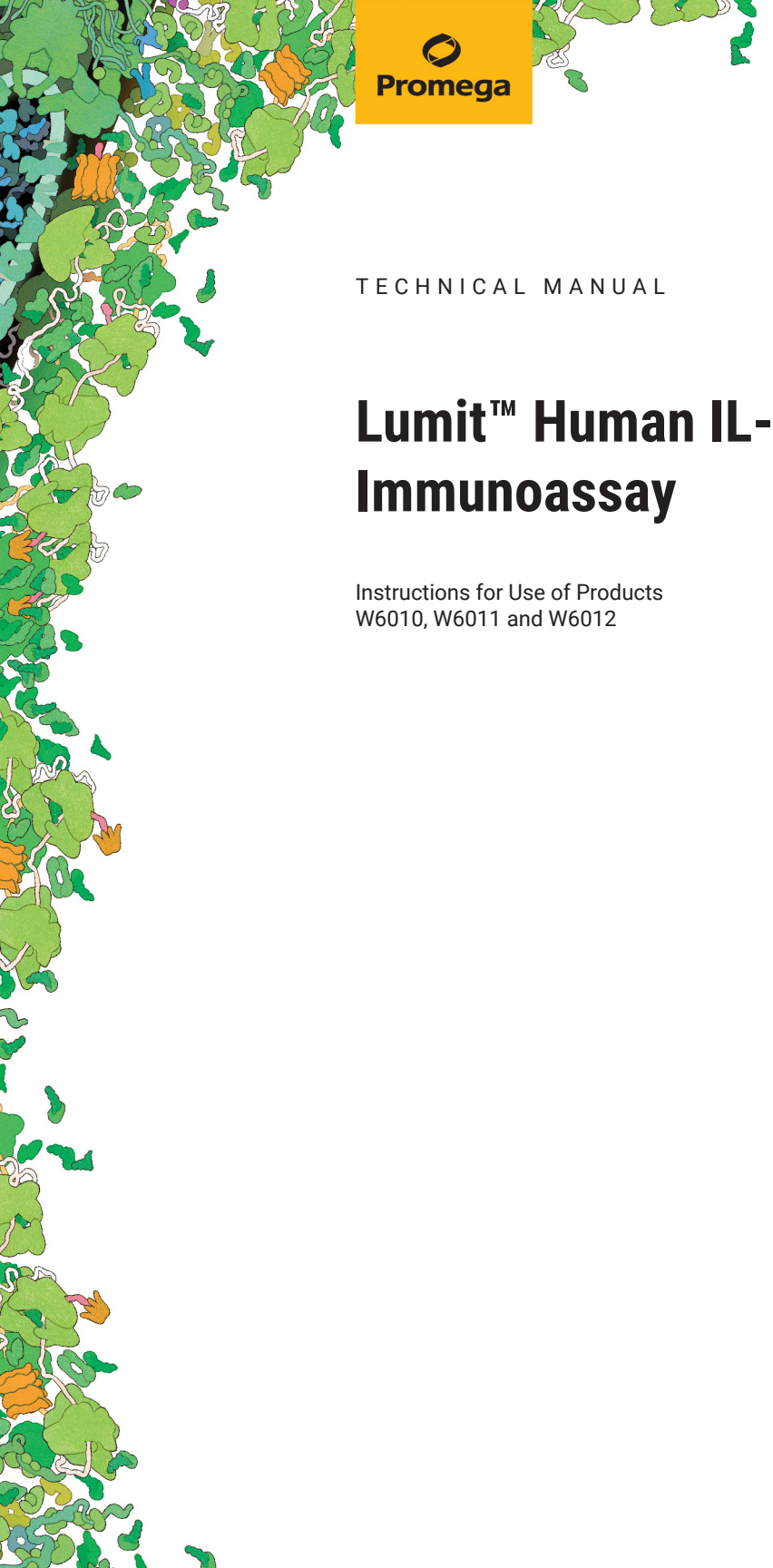

TECHNICAL MANUAL

Lumit™ Human IL-1β Immunoassay

Instructions for Use of Products
W6010, W6011 and W6012

Revised 7/22
TM645

Promega

Lumit™ Human IL-1β Immunoassay

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™ Human IL-1β Immunoassay[a,b] is a homogeneous, bioluminescent assay for detecting interleukin-1β (IL-1β) released from cells without the need for sample transfers or wash steps. IL-1β is critical to innate immunity as a key proinflammatory cytokine that responds to microbial infection or tissue injury (1). IL-1β is processed and released subsequent to formation of the inflammasome, a multiprotein complex assembled in response to pathogens and other damage-associated molecular patterns or triggers. Mature, active IL-1β is processed from an inactive precursor, proIL-1β, by caspase-1, a key component of the inflammasome (2). Caspase-1 is recruited to the inflammasome and subsequently activated via proximity-induced autoactivation (3,4). Different inflammasomes assemble with different pattern recognition receptors (PRRs) in response to various pathogen-associated molecular patterns (PAMPS) or damage-associated molecular patterns (DAMPs), but a common denominator is activation of caspase-1 followed by processing and release of active IL-1β and IL-18 (5). Released IL-1β signals through the IL-1 receptor 1 (IL-1R1) and the coreceptor IL-1R3 (also named receptor accessory protein—IL-1RAcP) expressed on numerous cell types (1). IL-1β is associated with acute and chronic inflammation, including what is termed cytokine storm, a poorly understood but dangerous phenomenon involving hyperactivation of the innate immune system.

Assay Principle

The Lumit™ Human IL-1β Immunoassay has been developed for use with cell culture samples. Lumit™ reagents can be dispensed directly into microplate wells containing cells and culture medium. Alternatively, medium from cell wells can be transferred to a separate plate for analysis. Assay performance with additional sample types must be determined by the user.

The Lumit™ Human IL-1β Immunoassay is based on NanoLuc® Binary Technology (NanoBiT®). NanoBiT is a luminescent structural complementation system designed for biomolecular interaction studies (6,7). The 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 spontaneous association. In this assay, a sample is incubated with a pair of anti-human IL-1β monoclonal antibodies covalently labeled with SmBiT or LgBiT. When the labeled antibodies recognize and bind to released IL-1β, the complementary LgBiTs and SmBiTs are brought into proximity, thereby reconstituting NanoBiT® enzyme and generating luminescence in the presence of the Lumit™ substrate (Figure 1). Luminescence generated is directly proportional to the amount of analyte present in the sample.

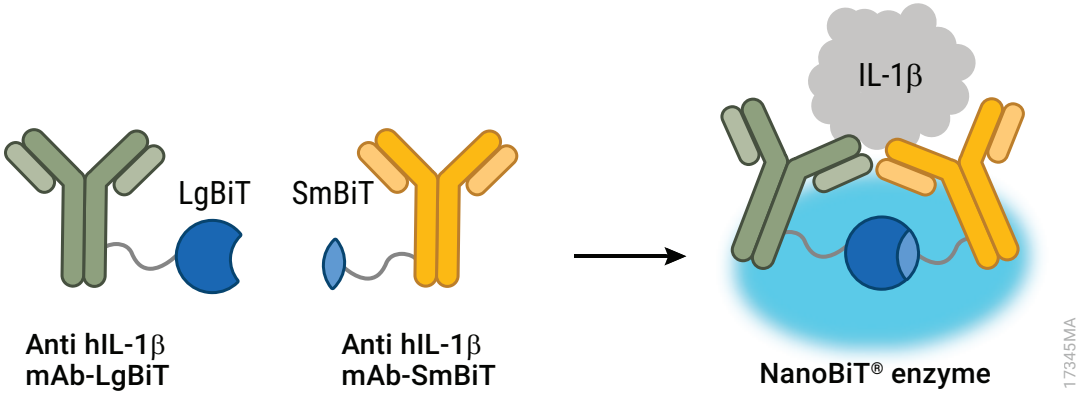

Figure 1. Assay principle. Primary monoclonal antibodies to human IL-1β are labeled with SmBiT and LgBiT. In the presence of IL-1β, SmBiT and LgBiT are brought into close proximity, forming the NanoBiT® enzyme. When Lumit™ Detection Reagent B is added, a bright luminescent signal is generated.

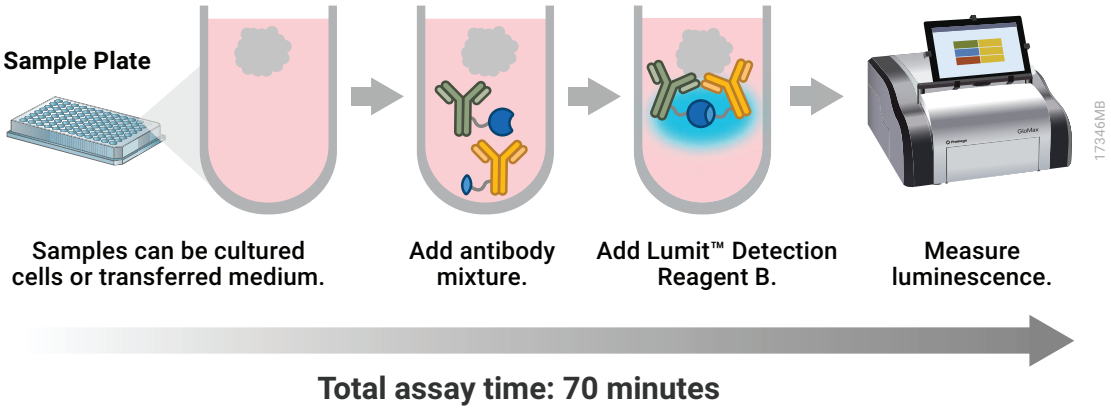

Figure 2. Assay protocol. The Lumit™ IL-1β Immunoassay is performed directly on cells in culture or on medium transferred from the cell culture plate to a new assay plate. The Lumit™ Immunoassay protocol does not require wash steps and is complete in 70 minutes.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
Lumit™ Human IL-1β Immunoassay	**100 assays**	**W6010**

Sufficient for 100 assays in 96-well plates; volumes can be adjusted for alternate plate sizes. Includes:

- 15µl Anti hIL-1β mAb-SmBiT, 1,000X
- 15µl Anti hIL-1β mAb-LgBiT, 1,000X
- 25µl Human IL-1β Standard
- 160µl Lumit™ Detection Substrate B
- 3.2ml Lumit™ Detection Buffer B

PRODUCT	SIZE	CAT.#
Lumit™ Human IL-1β Immunoassay	**1,000 assays**	**W6011**

Sufficient for 1,000 assays in 96-well plates; volumes can be adjusted for alternate plate sizes. Includes:

- 150µl Anti hIL-1β mAb-SmBiT, 1,000X
- 150µl Anti hIL-1β mAb-LgBiT, 1,000X
- 25µl Human IL-1β Standard
- 1.25ml Lumit™ Detection Substrate B
- 25ml Lumit™ Detection Buffer B

PRODUCT	SIZE	CAT.#
Lumit™ Human IL-1β Immunoassay	**5 × 100 assays**	**W6012**

Sufficient for 500 assays in 96-well plates; volumes can be adjusted for alternate plate sizes. Includes:

- 5 × 15µl Anti hIL-1β mAb-SmBiT, 1,000X
- 5 × 15µl Anti hIL-1β mAb-LgBiT, 1,000X
- 25µl Human IL-1β Standard
- 5 × 160µl Lumit™ Detection Substrate B
- 5 × 3.2ml Lumit™ Detection Buffer B

Storage Conditions: Store all components at –30°C to –10°C. Once thawed, store Human IL-1β Standard at +2°C to +10°C for up to 1 month. If storing the Human IL-1β Standard for more than 1 month after thawing, dispense into aliquots and store at –30°C to –10°C. Store Lumit™ Detection Buffer B at room temperature once thawed.

3. Before You Begin

There are two protocols for measuring human IL-1β.

Direct (No-Transfer) Protocol for Cultured Cells (Section 4): Measure human IL-1β directly in cell culture wells. Add 20µl of a 5X antibody mixture to 80µl of cells or IL-1β standard dilutions in culture medium, and incubate for 60–90 minutes. Following incubation, add 25µl of Lumit™ Detection Reagent B and record luminescence.

Sample Transfer Protocol (Section 5): Measure human IL-1β in medium samples transferred from treated cell wells. Transfer 50µl of culture medium from cell wells to a separate assay plate. Add 50µl of a 2X antibody mixture to 50µl of transferred sample or standard dilutions, and incubate for 60–90 minutes. Following incubation, add 25µl of Lumit™ Detection Reagent B and record luminescence.

Note: Assay volumes are scalable and can be adjusted based on sample sizes. The protocols below list common volumes for 96- and 384-well plates. Other volumes may be used, maintaining the final antibody concentration of 1:1,000 in total volume, and Lumit™ Detection Reagent B added at a 5X concentration (1:100 final dilution).

Reagent Preparation and Storage

Prepare the Lumit™ Human IL-1β standard curve, Lumit™ antibody mixture and Lumit™ Detection Reagent B on the day of use. Do **not** reuse the diluted IL-1β standard curve, the Lumit™ antibody mixture or the Lumit™ Detection Reagent B.

Use personal protective equipment and follow your institution's safety guidelines and disposal requirements when working with biohazardous materials such as cells and cell culture reagents.

Plate Map for Both Protocols

	IL-1β Standard Curve (ng/ml)		Test Samples									
	1	2	3	4	5	6	7	8	9	10	11	12
A	40.0	40.0										
B	11.43	11.43										
C	3.27	3.27										
D	0.93	0.93										
E	0.27	0.27										
F	0.076	0.076										
G	0.022	0.022										
H	0	0										

Materials to Be Supplied by the User

- cells and culture medium [We recommend a HEPES-containing RPMI 1640 medium (e.g., GIBCO Cat.# A10491-010) supplemented with 10% fetal bovine serum (FBS; e.g., Sigma Cat.# F4135 heat-inactivated FBS)]
- white, multiwell tissue culture plates (solid white or white with clear bottom) compatible with a luminometer [e.g., 96-well Corning® (Cat.# 3903)]
- multichannel pipette or automated pipetting station
- dilution tubes or multi-chamber, dilution reservoir (e.g., Dilux® D-1002)
- reagent reservoir trays (e.g., Corning® Costar Cat.# 07200127)
- plate shaker for mixing multiwell plates
- luminometer capable of reading multiwell plates (e.g., GloMax® Discover System, Cat.# GM3000)

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4. Direct (No-Transfer) Protocol for Cultured Cells

This protocol describes how to detect IL-1β released directly in assay wells containing cells and culture medium. For quantitation purposes, a calibration curve is generated using an IL-1β standard diluted in culture medium.

4.A. Cell Plating and Treatment

1. Plate cells into a 96- or 384-well, white (or white with clear bottom), tissue culture plate. Leave columns 1 and 2 empty. Allow cells to attach if using adherent cells.

   Notes:

   a. While the broad dynamic range of the assay offers considerable flexibility for cell number, the optimal number of cells dispensed per well for a specific cell model should be empirically determined. Ensure the maximum level of cytokine released does not exceed the linear range of the detection chemistry. Within that constraint, cell number can be increased to meet the detection requirements of low-level cytokine production. See example cell numbers used for THP-1 cells and PBMCs in Section 7, Representative Data, Figures 5–8.

   b. If differentiated THP-1 cells are being used, we recommend differentiating with 20nM phorbol myristate acetate (PMA) for 2–3 days. Then replace the medium before treatment. Pro-IL-1β from cell turnover during differentiation can interfere with the assay.

2. Treat cells by adding a volume of test agent to each well such that the total volume is as follows:

   96-well plate: 80µl per well.

   384-well plate: 20µl per well.

   For example, if 60µl of cells are plated per well in a 96-well plate, add 20µl of 4X treatment agent in culture medium. Cells can be treated overnight or for shorter periods (e.g., differentiated THP-1 cells treated for 4 hours with LPS will release significant amounts of IL-1β).

4.B. Preparing Human IL-1β Standard Dilution

Shortly before completing cell treatments, prepare IL-1β dilutions.

1. Thaw the Human IL-1β Standard immediately before use.
2. Briefly mix by flicking tube or pipetting. Quickly centrifuge the tube before opening.
3. Prepare an initial concentration of 40ng/ml human IL-1β by diluting Human IL-1β Standard (10µg/ml) 1:250 in cell culture medium (typically HEPES-containing RPMI 1640 + 10% heat-inactivated FBS for human cells). For example, prepare 500µl of 40ng/ml human IL-1β by adding 2µl of the Human IL-1β Standard stock to 498µl of culture medium (see Figure 3).
4. Set up seven tubes (or seven chambers in a dilution reservoir) with 500µl of culture medium in each.
5. Prepare 3.5-fold serial dilutions of standard. Transfer 200µl from the 40ng/ml initial human IL-1β dilution (Step 3) to 500µl of culture medium for the second dilution. Mix and repeat five more times to generate seven standard dilutions with a range of 40ng/ml–22pg/ml. The last well or chamber should contain only culture medium as the background control.

   Note: Change pipette tips between each dilution step to avoid analyte carryover. The linear range of the assay is large, so carryover from high to low concentrations can compromise the standard curve.

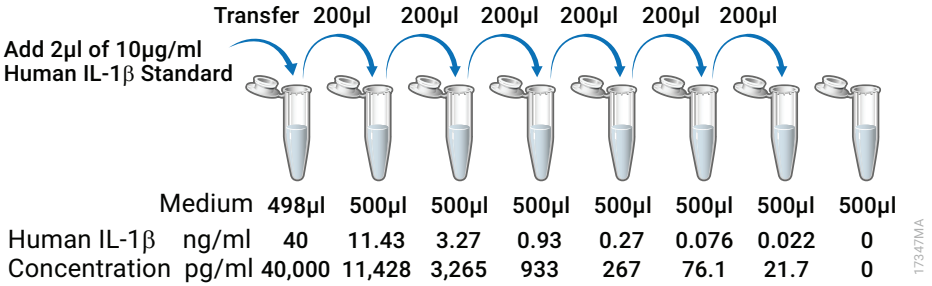

Figure 3. Human IL-β1 dilution series.

6. After the cell treatment is complete, add the standard dilutions and background control in duplicate to two columns in the assay plate (see the plate map in Section 3, Before You Begin).

   96-well plate: Dispense 80µl per well.

   384-well plate: Dispense 20µl per well.

   Note: Extra Human IL-1β Standard (10µg/ml) can be stored at 4°C for 1 month. If storing the standard for more than 1 month, dispense into aliquots and store at –30°C to –10°C. Avoid multiple freeze-thaw cycles.

4.C. Adding 5X Anti hIL-1β Antibody Mixture to Assay Wells

1. Remove the Anti hIL-1β antibodies from –30°C to –10°C immediately before use.

   Note: Remove Lumit™ Buffer B from –30°C to –10°C at the same time and equilibrate to room temperature if not already thawed.

2. Flick tubes to mix and briefly centrifuge the Anti hIL-1β antibody tubes before opening.

3. Immediately prior to use, prepare a 5X antibody mixture by diluting both antibodies 1:200 into a single volume of culture medium. Pipet to mix the antibody solution. To assay a complete 96- or 384-well plate, including some excess reagent volume, prepare the 5X antibody mixture as follows:

Reagent	Volume
culture medium	2.4ml
Anti-hIL-1β mAb-SmBiT	12µl
Anti-hIL-1β mAb-LgBiT	12µl

4. Add the 5X Anti hIL-1β antibody mixture to wells containing cultured cells or standard dilutions, carefully avoiding cross contamination between wells. Change pipette tips between rows to avoid cross contamination.

   96-well plate: Dispense 20µl/well of 5X Anti hIL-1β antibody mixture to 80µl/well of cells or IL-1β standard dilutions.

   384-well plate: Dispense 5µl/well of 5X Anti hIL-1β antibody mixture.

5. **Optional:** Briefly mix with a plate shaker (e.g., 10 seconds at 250–350 rpm).

6. Incubate for 60–90 minutes at 37°C in a 5% CO_2 humidified incubator.

4.D. Adding Lumit™ Detection Reagent B to Assay Wells

While cells are incubating with the Anti hIL-1β antibody mixture (Section 4.C), prepare the Lumit™ Detection Reagent B.

1. Equilibrate the required volume of Lumit™ Dilution Buffer B to ambient temperature.
2. Remove the Lumit™ Substrate B from –30°C to –10°C storage, and mix. If the Lumit™ Substrate B has collected in the cap or on the sides of the tube, briefly centrifuge.
3. Prepare a 20-fold dilution of Lumit™ Detection Substrate B in room temperature Lumit™ Detection Buffer B to create enough volume of Lumit™ Detection Reagent B for the number of wells to be assayed. To assay a 96- or 384-well assay plate, including some excess reagent volume, prepare 5X Lumit™ Detection Reagent B as follows:

Reagent	Volume
Lumit™ Detection Buffer B	3,040µl
Lumit™ Detection Substrate B	160µl

Notes:

a. The 1,000 assay size Lumit™ Human IL-1β Immunoassay (Cat.# W6011) contains 25ml of Detection Buffer B and 1.25ml of Detection Substrate B. There is sufficient overfill to prepare Lumit™ Detection Reagent B for analyzing 5 or 10 plates at once. If Cat.# W6011 is used for assaying 10 plates individually, mix 2,375µl of Lumit™ Detection Buffer B + 125µl of Lumit™ Detection Substrate B for each plate.

b. Once reconstituted, the Lumit™ Detection Reagent B will lose 10% activity in approximately 3 hours at 20°C. At 4°C, the reconstituted reagent will lose 10% activity in approximately 7 hours.

4. Equilibrate assay plate with cells to room temperature for 10–15 minutes.
5. Add room temperature 5X Lumit™ Detection Reagent B to each assay well of the plate.

   96-well plate: Dispense 25µl per well.

   384-well plate: Dispense 6.25µl per well.
6. Briefly mix with a plate shaker (e.g., 10 seconds at 300–500 rpm).
7. Incubate 3–5 minutes.
8. Read luminescence.

   Note: Assay signal is stable with a half-life of approximately 2 hours, compatible with batch processing of multiple assay plates. We recommend incorporating standard controls on each assay plate for normalization.

5. Optional Sample Transfer Protocol

This protocol describes transfer of sample medium from treated cell wells into a separate assay plate for subsequent cytokine detection, leaving the cells and remaining medium for additional uses. For quantitation purposes, a dilution series of Human IL-1β Standard prepared in culture medium is used to generate a calibration curve.

5.A. Cell Plating and Treatment

1. Plate cells into a 96- or 384-well tissue culture plate. Leave columns 1 and 2 empty. Allow cells to attach if using adherent cells.

   Notes:

   a. The optimal number of cells dispensed per well for a specific cell model should be empirically determined to ensure the maximum level of cytokine released does not exceed the linear range of the detection chemistry. Within that constraint, cell number can be increased to meet the detection requirements of low-level cytokine production. See example cell numbers used for THP-1 cells and PBMCs in Section 7, Representative Data, Figures 5–8.

   b. If differentiated THP-1 cells are being used, we recommend differentiating with 20nM phorbol myristate acetate (PMA) for 2–3 days. Then replace the medium before treatment. Pro-IL-1β from cell turnover during differentiation can interfere with the assay.

2. Treat cells by adding a volume of test agent to each well. The final treatment volume is flexible. Typical volumes are as follows:

   96-well plate: 100µl per well.

   384-well plate: 25µl per well.

3. After cell treatment is complete, transfer cell medium from each well to the corresponding wells of a separate white assay plate.

   96-well plate: Transfer 50µl per well.

   384-well plate: Transfer 12.5µl per well.

   Note: You can transfer lower volumes if analyte concentrations are sufficient for detection, but sample volume should be diluted with culture medium prior to assay. Account for the sample dilution factor when determining the actual concentration of released cytokine in treated cell wells.

   96-well plate: Dilute to a final volume of 50µl.

   384-well plate: Dilute to a final volume of 12.5µl.

5.B. Preparing Human IL-1β Standard Dilutions

Shortly before completing cell treatments, prepare IL-1β dilutions.

1. Thaw the Human IL-1β Standard immediately before use.
2. Mix by flicking the tube or pipetting. Briefly centrifuge the tube before opening.

5.B. Preparing Human IL-1β Standard Dilutions (continued)

3. Prepare an initial concentration of 40ng/ml human IL-1β by diluting Human IL-1β Standard (10µg/ml) 1:250 in cell culture medium (typically HEPES-containing RPMI 1640 + 10% heat-inactivated FBS for human cells). For example, prepare 500µl of 40ng/ml human IL-1β by adding 2µl of the Human IL-1β Standard stock to 498µl of culture medium. (See Figure 3.)
4. Set up seven tubes (or seven chambers in a dilution reservoir) with 500µl of culture medium in each.
5. Proceed with 3.5-fold serial dilutions of standard. Transfer 200µl from the 40ng/ml stock to 500µl of culture medium for the second dilution. Mix and repeat five times to generate seven standard dilutions with a range of 40ng/ml–22pg/ml. The last well or chamber should contain only culture medium as the background control.

   Note: Change pipette tips between preparing each standard dilution to avoid analyte carryover. The linear range of the assay is very large, so carryover from high to low concentrations can compromise the standard curve.
6. After transferring the culture medium from the treated cell wells to a separate assay plate, add the standard dilutions and background control in duplicate to two columns in the transfer plate (see the plate map in Section 3, Before You Begin).

   96-well plate: Dispense 50µl per well.

   384-well plate: Dispense 12.5µl per well.

   Note: Extra Human IL-1β Standard (10µg/ml) can be stored at 4°C for 1 month. If storing the standard for more than 1 month, dispense into aliquots and store at –30°C to –10°C. Avoid multiple freeze-thaw cycles.

5.C. Adding 2X Anti hIL-1β Antibody Mixture to Sample Wells

1. Remove the Anti hIL-1β antibodies from –30°C to –10°C immediately before use.

   Note: Remove Lumit™ Buffer B from –30°C to –10°C at the same time and equilibrate to room temperature if not already thawed.
2. Flick tubes to mix and briefly centrifuge the Anti hIL-1β antibody tubes before opening.
3. Immediately prior to use, prepare a 2X antibody mixture by diluting both antibodies 1:500 into a single volume of culture medium. Pipet to mix the antibody solution. To assay a complete 96- or 384-well plate, including some excess reagent volume, prepare the 2X antibody mixture as follows:

Reagent	Volume
culture medium	6ml
Anti-hIL-1β mAb-SmBiT	12µl
Anti-hIL-1β mAb-LgBiT	12µl

4. Add the 2X Anti hIL-1β antibody mixture to transferred culture medium or IL-1β standard dilutions, carefully avoiding cross contamination between wells. Change pipette tips between rows to avoid cross contamination.

   96-well plate: Dispense 50µl/well of 2X Anti hIL-1β antibody mixture to 50µl/well of medium or standard dilutions.

   384-well plate: Dispense 12.5µl/well of 2X Anti hIL-1β antibody mixture.

5. **Optional:** Briefly mix with a plate shaker (e.g., 10 seconds at 250–350 rpm).
6. Incubate for 60–90 minutes at room temperature.

   Note: To incubate at room temperature, a HEPES-containing RPMI 1640 medium will provide best results. Without HEPES, the buffering capacity outside of a CO_2 incubator is limited. The plates can also be incubated at 37°C in a CO_2 incubator.

5.D. Adding Lumit™ Detection Reagent B to Sample Wells

While medium is incubating with the Anti hIL-1β antibody mixture (Section 5.C), prepare the Lumit™ Detection Reagent B.

1. Equilibrate the required volume of Lumit™ Dilution Buffer B to ambient temperature.
2. Remove the Lumit™ Substrate B from –30°C to –10°C storage and mix. If the Lumit™ Substrate B has collected in the cap or on the sides of the tube, briefly centrifuge.
3. Prepare a 20-fold dilution of Lumit™ Detection Substrate B into room temperature Lumit™ Detection Buffer B to create enough volume of Lumit™ Detection Reagent B for the number of wells to be assayed. For a 96- or 384-well assay plate, including some excess reagent volume, prepare 5X Lumit™ Detection Reagent B as follows:

Reagent	Volume
Lumit™ Detection Buffer B	3,040µl
Lumit™ Detection Substrate B	160µl

Notes:

a. The 1,000 assay size Lumit™ Human IL-1β Immunoassay (Cat.# W6011) contains 25ml of Detection Buffer B and 1.25ml of Detection Substrate B. There is sufficient overfill to prepare Lumit™ Detection Reagent B for analyzing 5 or 10 plates at once. If Cat.# W6011 is used for assaying 10 plates individually, mix 2,375µl of Lumit™ Detection Buffer B + 125µl of Lumit™ Detection Substrate B for each plate.

b. Once reconstituted, the Lumit™ Detection Reagent B will lose 10% activity in approximately 3 hours at 20°C. At 4°C, the reconstituted reagent will lose 10% activity in approximately 7 hours.

4. Equilibrate assay plate to room temperature for 10–15 minutes.
5. Add room temperature Lumit™ Detection Reagent B to each well of the plate.

   96-well plate: Dispense 25µl per well.

   384-well plate: Dispense 6.25µl per well.
6. Briefly mix with a plate shaker (e.g., 10 seconds at 300–500 rpm).
7. Incubate 3–5 minutes.
8. Read luminescence.

   Note: Assay signal is stable with a half-life of approximately 2 hours, compatible with batch processing of multiple assay plates. We recommend incorporating standard controls on each assay plate for normalization.

6. Calculating Results

Create a standard curve for the known cytokine concentrations using software (e.g., GraphPad® Prism) capable of nonlinear regression analysis or cubic spline curve fitting.

Subsequently, interpolate the concentration of cytokine in various cell samples. The broad dynamic range of the Lumit™ standard curve that closely approaches linearity is well-suited for second or third order polynomial regression curve fitting, as well as cubic spline curve fitting. Four-parameter logistic (4PL) curve fitting is also commonly used, but may not be ideal since the broad, linear dynamic range for the Lumit™ standard curve is not well-suited for sigmoidal curve fitting (8).

Alternatively, while somewhat less accurate, a Log-Log plot of average RLU (background-subtracted) vs. cytokine standard concentrations can be fit with the Power trendline in Microsoft Excel® (see Section 7) and subsequently used for interpolation of the concentration of cytokine release in various cell samples.

7. Representative Data

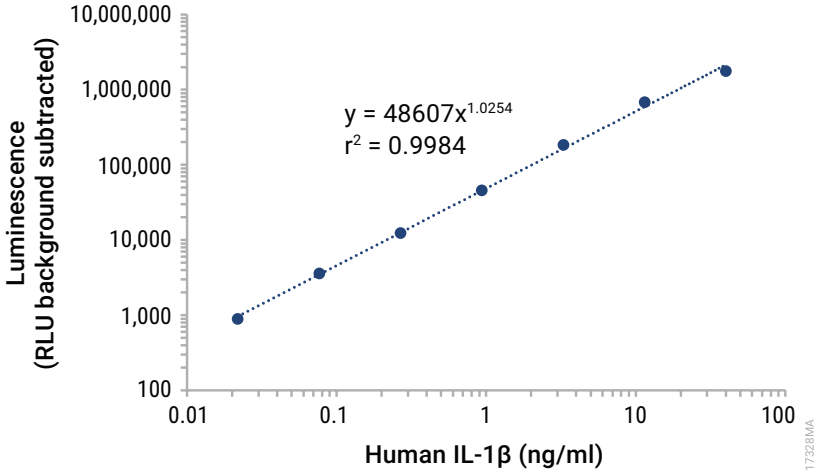

Figure 4. Standard curve for the Lumit™ Human IL-1β Immunoassay. This is a representative standard curve, but it should not be used for interpolation of unknowns. Generate a standard curve on each assay plate to interpolate experimental samples.

Table 1. Intra-Assay Precision. Three samples of known concentrations of human IL-1β were tested 20 times on one plate to assess intra-assay precision. A standard curve was used to interpolate the IL-1β quantities in each well by the various methods noted using GraphPad® Prism software.

	Cubic spline			4PL			Second order polynomial (quadratic)			Third order polynomial (cubic)		
n	20	20	20	20	20	20	20	20	20	20	20	20
Expected (ng/ml)	20	2.0	0.2	20	2.0	0.2	20	2.0	0.2	20	2.0	0.2
Mean (ng/ml)	19.86	2.05	0.22	19.47	1.99	0.23	19.54	1.90	0.23	18.99	1.96	0.22
Standard Deviation	1.19	0.07	0.01	1.13	0.07	0.02	1.13	0.07	0.01	1.05	0.07	0.02
Percent CV	6.0	3.3	6.4	5.8	3.5	6.7	5.8	3.6	5.8	5.5	3.6	6.5
Average Percent of Expected	99	102	112	97	99	116	98	95	115	95	98	109
Percent Range	84–107	96–108	102–126	83–104	93–105	103–131	84–105	89–101	105–129	82–101	92–104	98–123

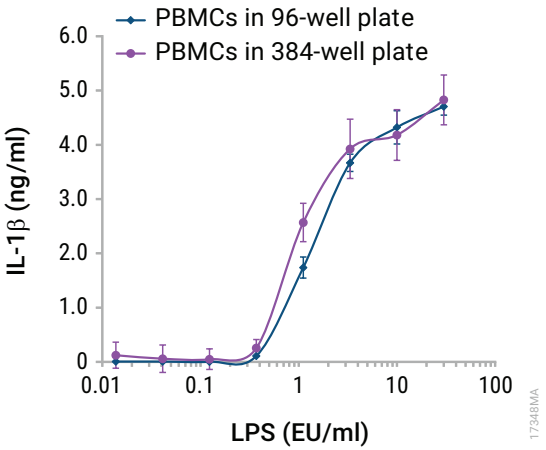

Figure 5. Lumit™ Detection of IL-1β released from human peripheral blood mononuclear cells (PBMCs) in 96- and 384-well plates. PBMCs from four-donor pools (BioIVT) were plated in RPMI 1640 + 10% heat-inactivated FBS at 55,000 cells/well in 96-well plates and 16,500 cells/well in 384-well plates and treated with a titration of lipopolysaccharide (LPS) overnight (9). The next day, the 5X Anti hIL-1β antibody mixture was dispensed to the cell wells and incubated for 1 hour before Lumit™ Detection Reagent B was added.

7. Representative Data (continued)

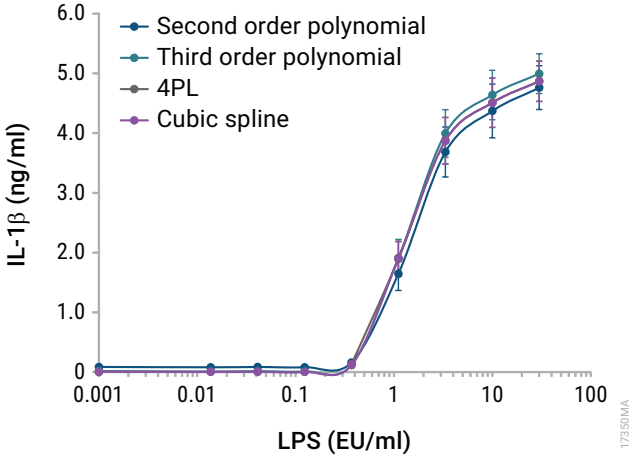

Figure 6. Different interpolation methods for quantifying IL-1β released using a standard curve. PBMCs from four-donor pools (BioIVT) were plated in RPMI 1640 + 10% heat-inactivated FBS at 55,000 cells/well in 96-well plates and treated with a titration of LPS overnight. The next day, the 5X Anti hIL-1β antibody mixture was added to the cell wells and incubated for 1 hour before Lumit™ Detection Reagent B was added. Three different nonlinear regression methods and the cubic spline method (GraphPad® Prism 8) were used to interpolate the amount of IL-1β released from the standard curve.

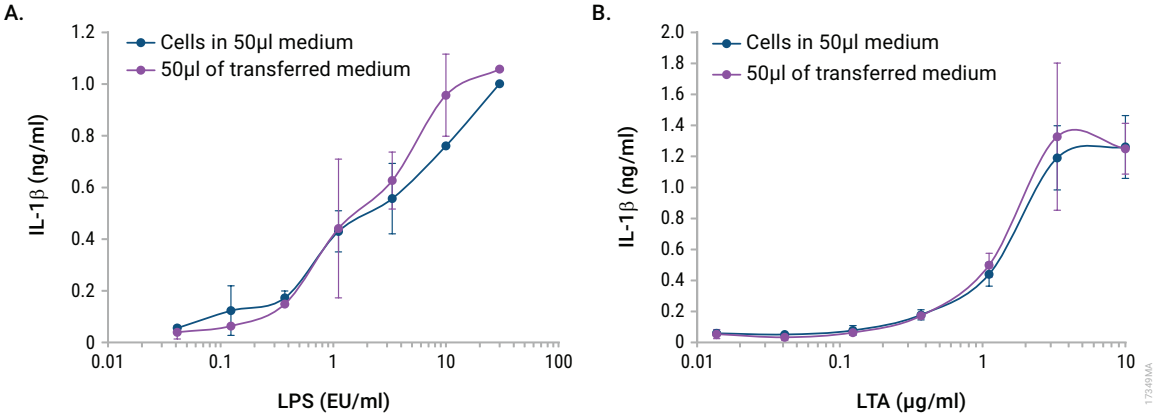

Figure 7. Detecting IL-1β released from human THP-1 cells. THP-1 cells were plated in RPMI 1640 + 10% heat-inactivated FBS at 50,000 cells/well in 96-well plates and differentiated with 20nM PMA for 3 days. After 3 days, the culture medium was removed, and replaced with titrations of LPS (**Panel A)** or LTA (lipoteichoic acid; **Panel B**) in fresh culture medium. The cells were treated for 4.5 hours followed by transfer of half of the culture medium (50µl) from each well to a second assay plate. The 2X Anti hIL-1β antibody mixture was added to both assay plates. The assay plate containing cells plus the remaining culture medium was incubated in 5% CO_2 at 37°C, and the transferred culture medium plate was incubated at room temperature, both for 1 hour. After incubation with the Anti hIL-1β antibody mixture, Lumit™ Detection Reagent B was added. Luminescence was recorded and the amount of IL-1β interpolated from the standard dilutions curve.

7. Representative Data (continued)

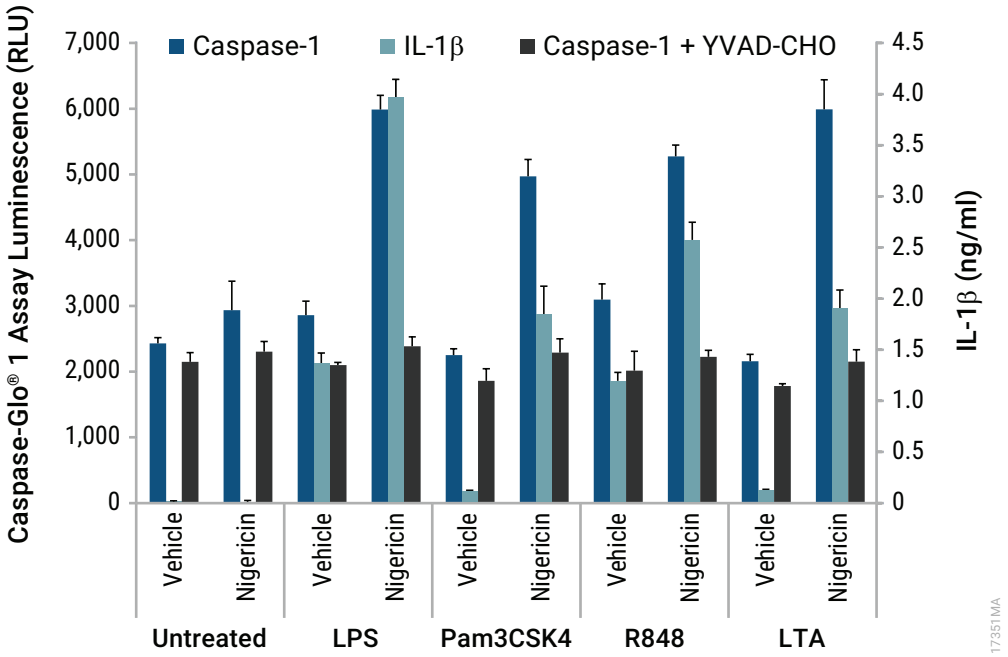

Figure 8. Multiplexing the Lumit™ Human IL-1β Immunoassay with the Caspase-Glo® 1 Inflammasome Assay. PBMCs were plated in RPMI 1640 + 10% heat-inactivated FBS at ~90,000 cells/well in 96-well plates and treated overnight with different TLR-agonists, including 2EU/ml LPS, 100ng/ml Pam3CSK4, 2µg/ml R848 and 10µg/ml LTA. The next day, the potassium ionophore, nigericin (20µM), was added to half of the wells and vehicle to the other half. After 2 hours of nigericin treatment, half of the culture medium from the cells was transferred and assayed for caspase-1 with the Caspase-Glo® 1 Inflammasome Assay. The YVAD-CHO caspase-1 inhibitor is added to half of the Caspase-Glo® 1 Reagent to demonstrate specificity for caspase-1 (10). The 2X Lumit™ antibody mixture was added to the remaining cells and culture medium and incubated for 1 hour, followed by adding Lumit™ Detection Reagent B. LPS and R848 alone stimulate significant IL-1β release from PBMCs via the alternative NLRP3 inflammasome pathway. Luminescence was recorded and the amount of IL-1β interpolated from the standard dilutions curve. Nigericin increases both caspase-1 activation and IL-1β release in PBMCs treated with TLR-agonists via the canonical NLRP3 inflammasome pathway.

8. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: **www.promega.com**. E-mail: **techserv@promega.com**

Symptom	Causes and Comments
No signal from treated cells.	Make sure that human cells are used with the Lumit™ Human IL-1β Immunoassay. The Lumit™ Human IL-1β Immunoassay will not detect IL-1β from other species. To confirm that the human cell type used expresses IL-1β, use treated PBMCs or treated, differentiated THP-1 cells as a positive control.
Human IL-1β standard curve is not linear.	The 3.5-fold dilutions should be carefully created without carryover from a higher concentration. We recommend changing pipette tips after each dilution step to prevent carryover. The sensitivity of the assay and the broad linear range (>3 logs) means that any carryover will disrupt the linear range. Also, make sure that no IL-1β contaminates the background control.
The relative light units (RLUs) for the standard curve are variable.	There may be some variation in RLUs due to culture conditions, temperature, etc., but as long as the standard curve is run on the same plate as the test samples under the same conditions, released IL-1β can be accurately quantitated.

9. Appendix

9.A. Processed Human IL-1β Selectivity Example Data

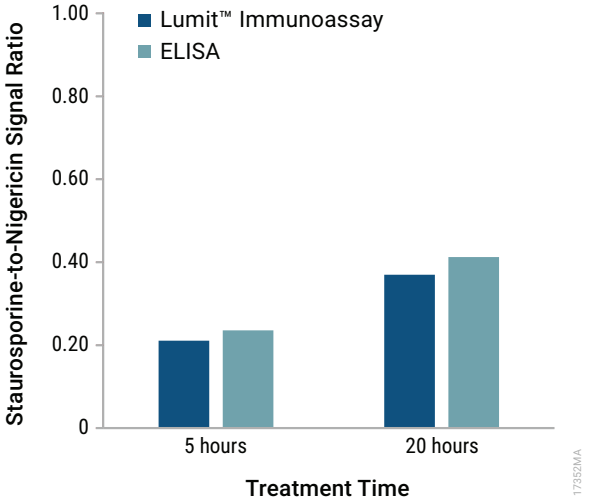

Figure 8. Selectivity for processed human IL-1β. The assay is selective for mature, processed IL-1β, but there is some cross-reactivity with human proIL-1β. To assess the effect of cross-reactivity in a physiological setting, THP-1 cells were differentiated with 20nM PMA to upregulate proIL-1β. The cells were then treated with nigericin or staurosporine for 5 or 20 hours to induce pyroptosis or apoptosis, respectively. Then, the IL-1β levels were measured using the Lumit™ Human IL-1β Immunoassay or a commercially available ELISA. Nigericin causes inflammasome activation and processing of proIL-1β into mature IL-1β, whereas staurosporine does not induce the inflammasome nor processing of proIL-1β (10). The ratio of staurosporine-to-nigericin signal gives some indication of cross-reactivity in a physiological setting and is shown at 5 and 20 hours of treatment. The ratios were similar for the Lumit™ Human IL-1β Immunoassay and the ELISA (with reported cross-reactivity for proIL-1β to be 15.4%), indicating that both assays have similar cross-reactivities to proIL-1β. The differentiated, apoptotic THP-1 cells represent an atypical case where all the proIL-1β has been released.

We recommend confirming inflammasome activation with the Caspase-Glo® 1 Inflammasome Assay (see Section 9.B).

9.B. Multiplexing Assays for Testing Inflammasome Inhibition Example Data

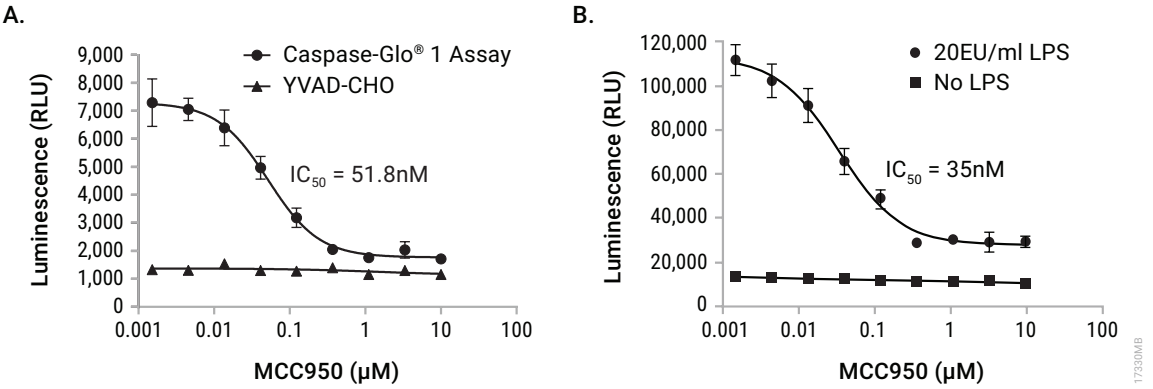

Figure 9. Inflammasome inhibitor testing by multiplexing the Lumit™ Human IL-1β Immunoassay and Caspase-Glo® 1 Inflammasome Assay. The add-and-read format of the Lumit™ Human IL-1β Immunoassay enables high-throughput screening for modulation of IL-1β release. The NLRP3 inflammasome inhibitor, MCC950, prevents caspase-1 activation and release of mature IL-1β (11,12). A titration of MCC950 was added to differentiated THP-1 cells (5 × 10^4 cells/well) followed by LPS (20EU/ml) or vehicle. Half of the culture medium (50µl) was removed after treating cells with LPS for 3 hours and tested with the Caspase-Glo® 1 Inflammasome Assay (**Panel A**). The Lumit™ Human IL-1β Immunoassay was performed on the remaining cells and medium after 5 hours of treatment (**Panel B**).

9.C. References

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5. Sharma, D. and Kanneganti, T.D. (2016) The cell biology of inflammasomes: Mechanisms of inflammasome activation and regulation. *J. Cell Biol*. **213**, 617–29.

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9.C. References (continued)

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10. O'Brien, M. *et al*. (2017) A bioluminescent caspase-1 activity assay rapidly monitors inflammasome activation in cells. *J. Immunol. Methods* **447**, 1–13.

11. Coll, R.C. *et al*. (2015) A small-molecule inhibitor of the NLRP3 inflammasome for the treatment of inflammatory diseases. *Nat. Med.* **21**, 248–55.

12. Gritsenko, A. *et al*. (2020) Priming is dispensable for NLRP3 inflammasome activation in human monocytes in vitro. *Front. Immunol.* **11**, 1–14.

9.D. Related Products

Lumit™ Immunoassays

Product	Size	Cat.#
Lumit™ Mouse IL-1β Immunoassay*	100 assays	W7010
Lumit™ Immunoassay Labeling Kit	1 each	VB2500
Lumit Detection Reagent B*	100 assays	VB4050
Lumit™ Immunoassay Cellular Systems–Starter Kit	200 assays	W1220
Lumit™ FcRn Binding Immunoassay*	100 assays	W1151

*Additional sizes available.

Inflammasome Assay

Product	Size	Cat.#
Caspase-Glo® 1 Inflammasome Assay	10ml	G9951

Additional sizes available.

Cell Viability Assays

Product	Size	Cat.#
CellTiter-Glo® 2.0 Cell Viability Assay	10ml	G9241
RealTime-Glo™ MT Cell Viability Assay	100 reactions	G9711
CellTiter-Fluor™ Cell Viability Assay	10ml	G6080

Additional sizes available.

Cytotoxicity Assays

Product	Size	Cat.#
LDH-Glo™ Cytotoxicity Assay	10ml	J2380
CytoTox-Glo™ Cytotoxicity Assay	10ml	G9290
CellTox™ Green Cytotoxicity Assay	10ml	G8741

Additional sizes available.

Apoptosis Assays

Product	Size	Cat.#
Caspase-Glo® 3/7 Assay System	2.5ml	G8090
Caspase-Glo® 8 Assay System	2.5ml	G8200
Caspase-Glo® 9 Assay System	2.5ml	G8210
RealTime-Glo™ Annexin V Apoptosis and Necrosis Assay	100 assays	JA1011

Additional sizes available.

Energy Metabolism and Oxidative Stress Assays

Product	Size	Cat.#
NAD/NADH-Glo™ Assay	10ml	G9071
NADP/NADPH-Glo™ Assay	10ml	G9081
ROS-Glo™ H_2O_2 Assay	10ml	G8820
GSH/GSSG-Glo™ Assay	10ml	V6611
Lactate-Glo™ Assay	5ml	J5021
Glucose-Glo™ Assay	5ml	J6021
Glutamine/Glutamate-Glo™ Assay	5ml	J8021
Glycerol-Glo™ Assay	5ml	J3150
Triglyceride-Glo™ Assay	5ml	J3160
Cholesterol/Cholesterol Ester-Glo™ Assay	5ml	J3190

Additional sizes available.

10. Summary of Changes

The following changes were made to the 7/22 revision of this document:

1. The 384-well plate amount in Section 5.B., Step 6 was updated to 12.5µl per well.
2. “Notes” in Sections 4 and 5 were changed from list by numbers to list by letters.
3. Font and cover image were updated.