TECHNICAL MANUAL

Lumit™ Mouse IL-1β Immunoassay

Instructions for Use of Products
W7010, W7011 and W7012

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TM646

Promega

Lumit™ Mouse 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

1. Description 2
2. Product Components and Storage Conditions 4
3. Before You Begin 4
4. Direct (No-Transfer) Protocol for Cultured Cells 6
   - 4.A. Cell Plating and Treatment 6
   - 4.B. Preparing Mouse IL-1β Standard Dilutions 6
   - 4.C. Adding 5X Anti mIL-1β Antibody Mixture to Assay Wells 7
   - 4.D. Adding Lumit™ Detection Reagent B to Assay Wells 8
5. Optional Sample Transfer Protocol 9
   - 5.A. Cell Plating and Treatment 9
   - 5.B. Preparing Mouse IL-1β Standard Dilutions 9
   - 5.C. Adding 2X Anti mIL-1β Antibody Mixture to Sample Wells 10
   - 5.D. Adding Lumit™ Detection Reagent B to Assay Wells 11
6. Calculating Results 12
7. Representative Data 12
8. Troubleshooting 16
9. Appendix 17
   - 9.A. Processed Mouse IL-1β Selectivity Example Data 17
   - 9.B. References 18
   - 9.C. Related Products 19

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1. Description

The Lumit™ Mouse 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 responding to microbial infection or tissue injury (1). IL-1β is processed and released when the inflammasome is activated. The inflammasome is 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™ Mouse 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™ Mouse 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 acids), that have been optimized for stability and minimal association. In this assay, a sample is incubated with a pair of anti-mouse 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. Luminescence generated is directly proportional to the amount of analyte present in the sample.

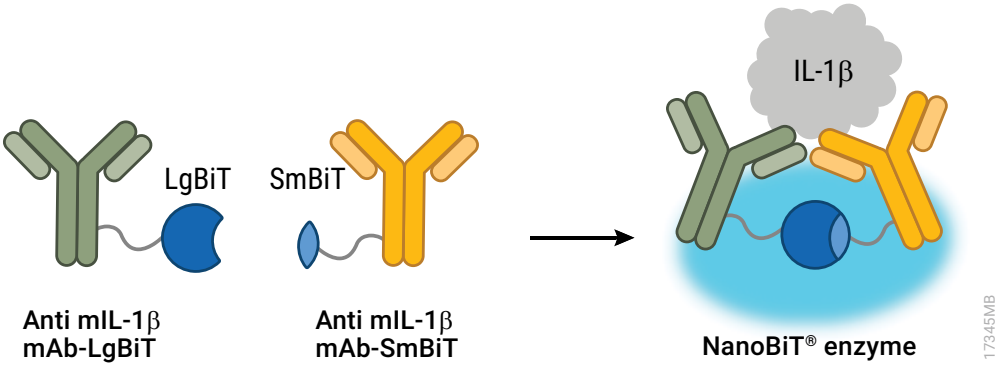

Figure 1. Assay principle. Primary monoclonal antibodies to mouse IL-1β are labeled with SmBiT and LgBiT. In the presence of IL-1β, SmBiT and LgBiT are brought into close proximity, forming the NanoBiT® enzyme. When Lumit™ Detection Reagent B is added, a bright luminescent signal is generated.

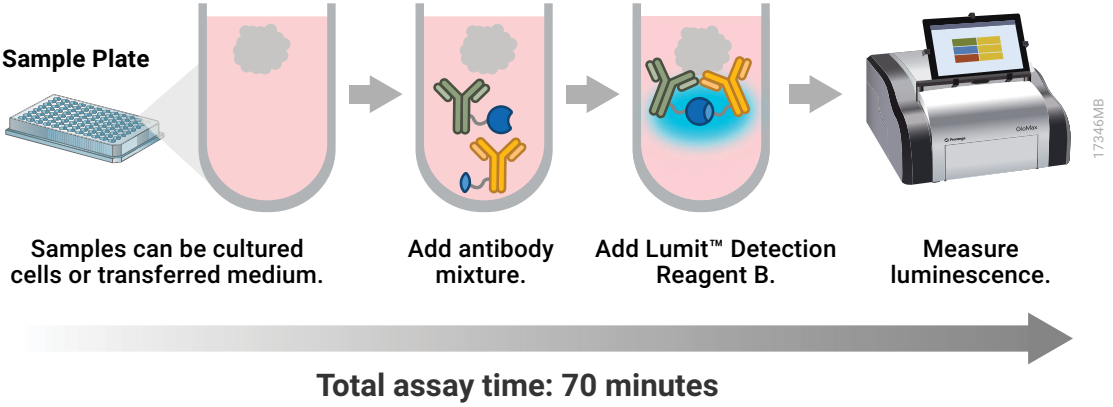

Figure 2. Assay protocol. The Lumit™ IL-1β Immunoassay is performed directly on cells in culture or on medium transferred from the cell culture plate to a new assay plate. The Lumit™ Immunoassay protocol does not require wash steps and is complete in 70 minutes.

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2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT. #
Lumit™ Mouse IL-1β Immunoassay	**100 assays**	**W7010**

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

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

PRODUCT	SIZE	CAT. #
Lumit™ IL-1β Mouse Immunoassay	**1,000 assays**	**W7011**

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

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

PRODUCT	SIZE	CAT. #
Lumit™ IL-1β Mouse Immunoassay	**5 × 100 assays**	**W7012**

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

- 5 × 15µl Anti mIL-1β mAb-SmBiT, 1,000X
- 5 × 15µl Anti mIL-1β mAb-LgBiT, 1,000X
- 25µl Mouse 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 Mouse IL-1β Standard at +2°C to +10°C for up to 1 month. If storing the Mouse 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 mouse IL-1β.

Direct (No-Transfer) Protocol for Cultured Cells (Section 4): Measuring mouse 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): Measuring mouse 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™ Mouse 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	20.0	20.0										
B	5.71	5.71										
C	1.63	1.63										
D	0.47	0.47										
E	0.13	0.13										
F	0.038	0.038										
G	0.011	0.011										
H	0	0										

Materials to Be Supplied by the User

- cells and culture medium [e.g., DMEM GIBCO (Cat.# 11995-065); fetal bovine serum, heat-inactivated, (Sigma Cat.# F4135)]
- 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)

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 microplate. Leave columns 1 and 2 empty. Allow cells to attach if using adherent cells.

   Note: While the broad dynamic range of the assay gives 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 the linear assay constraint, cell number can be increased to meet the detection requirements of low-level cytokine production. See example cell numbers for J774A.1 cells in Section 7, Representative Data, Figures 5–7.

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, add 20µl of 4X treatment agent in culture medium. Cells can be treated overnight or for shorter periods.

4.B. Preparing Mouse IL-1β Standard Dilutions

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

1. Thaw the Mouse IL-1β Standard immediately before use.
2. Briefly mix by flicking tube or pipetting up and down. Quickly centrifuge the tube before opening.
3. Prepare an initial concentration of 20ng/ml mouse IL-1β by diluting Mouse IL-1β Standard (10µg/ml) 1:500 in cell culture medium (typically DMEM + 10% FBS for mouse cells). For example, prepare 500µl by adding 1µl of the Mouse IL-1β Standard to 499µl of culture medium.
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 20ng/ml inital mouse IL-1β dilution (Step 3) to 500µl for the second dilution. Mix and repeat five more times to generate seven standard dilutions with a range of 20ng/ml–11pg/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.

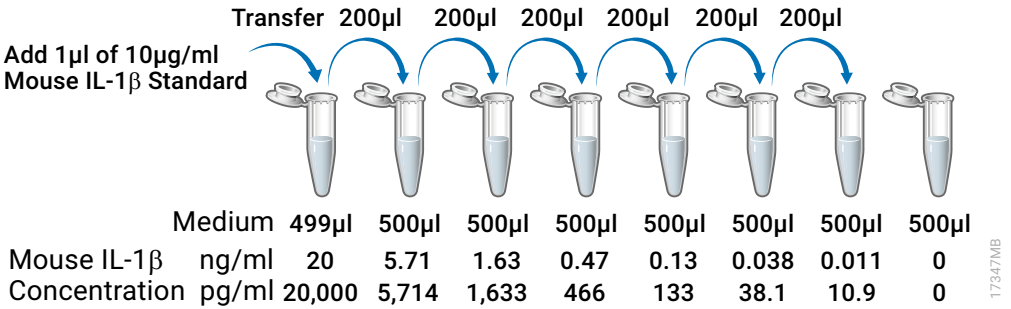

Figure 3. Mouse IL-1β dilution series.

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 Mouse 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 mIL-1β Antibody Mixture to Assay Wells

1. Remove the Anti mIL-1β antibodies from –20°C immediately before use.

   Note: Remove Lumit™ Detection Buffer B from –20°C at the same time and equilibrate to room temperature if not already thawed.

2. Flick tubes to mix and briefly centrifuge the Anti mIL-1β antibodies 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 mIL-1β mAb-SmBiT	12µl
Anti mIL-1β mAb-LgBiT	12µl

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

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

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

4.C. Adding 5X Anti mIL-1β Antibody Mixture to Assay Wells (continued)

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 mIL-1β antibody mixture (Section 4.C), prepare the Lumit™ Detection Reagent B.

1. Equilibrate the required volume of Lumit™ Detection Buffer B to ambient temperature.
2. Remove the Lumit™ Substrate B from –20°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. To assay a complete 96- or 384-well 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:

1. The 1,000 assay size Lumit™ Mouse IL-1β Immunoassay (Cat.# W7011) 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.# W7011 is used for assaying 10 plates individually, mix 2,375µl of Lumit™ Detection Buffer B + 125µl of Lumit™ Detection Substrate B for each plate.
2. 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 the procedure for transferring medium from treated cells to a separate assay plate, leaving the cells and remaining medium for additional uses. Transfer 50µl of culture medium/well from treated cells to a separate white 96-well assay plate, leaving the first two columns open for the IL-1β standard dilutions.

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.

   Note: 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 for J774A.1 cells in Section 7, Representative Data, Figures 5–7.

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 Mouse IL-1β Standard Dilutions

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

1. Thaw the Mouse IL-1β Standard immediately before use.
2. Mix by flicking the tube or pipetting up and down. Briefly centrifuge the tube before opening.
3. Prepare an initial concentration of 20ng/ml mouse IL-1β by diluting Mouse IL-1β Standard (10µg/ml) 1:500 in cell culture medium (typically DMEM + 10% FBS for mouse cells). For example, prepare 500µl by adding 1µl of the Mouse IL-1β Standard to 499µl 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.B. Preparing Mouse IL-1β Standard Dilutions (continued)

5. Prepare 3.5-fold serial dilutions of standard. Transfer 200µl from the 20ng/ml stock to 500µl for the second dilution. Mix and repeat five more times to generate seven standard dilutions with a range of 20ng/ml–11pg/ml. The last well or chamber should contain only culture medium as the background control.

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

6. After the cell treatment is complete, add the standard dilutions and background control in duplicate to two columns in the assay plate (see 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 Mouse 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 mIL-1β Antibody Mixture to Sample Wells

1. Remove the Anti mIL-1β antibodies from –20°C immediately before use.

   Note: Remove Lumit™ Detection Buffer B from –20°C at the same time and equilibrate to room temperature if not already thawed.

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

3. Immediately prior to use, prepare a 2X antibody mixture by diluting both antibodies 1:500 dilution 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 mIL-1β mAb-SmBiT	12µl
Anti mIL-1β mAb-LgBiT	12µl

4. Add the 2X Anti mIL-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 mIL-1β antibody mixture to 50µl/well of medium or IL-1β standard dilutions.

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

5. Incubate for 60–90 minutes at room temperature.

   Note: To incubate at room temperature, a HEPES-containing culture medium may provide additional buffering capacity outside of a CO_2 incubator. The plates may also be incubated at 37°C in a CO_2 incubator.

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

While medium is incubating with the Anti mIL-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 –20°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:

1. The 1,000 assay size Lumit™ Mouse IL-1β Immunoassay (Cat.# W7011) 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.# W7011 is used for assaying 10 plates individually, mix 2,375µl of Lumit™ Detection Buffer B + 125µl of Lumit™ Detection Substrate B for each plate.
2. 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 5X 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

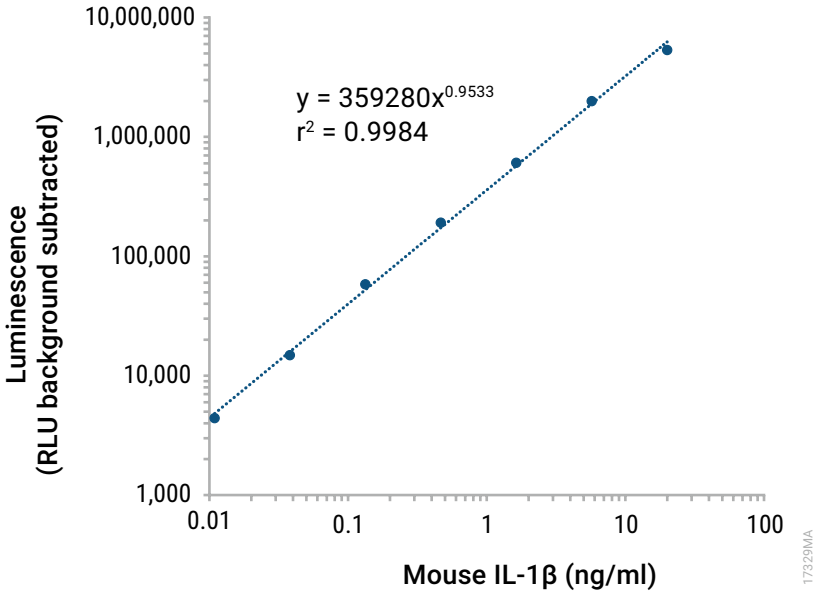

Figure 4. Standard curve for the Lumit™ Mouse IL-1β Immunoassay. This is a representative standard curve, but it should not be used for interpolation of unknowns. Generate a standard curve on each assay plate to interpolate experimental samples.

Table 1. Intra-Assay Precision. Mouse IL-1β Standard (10µg/ml) was diluted to 5ng/ml followed by tenfold serial dilutions to 0.5ng/ml and 0.05ng/ml. These three concentrations 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.

	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)	5	0.5	0.05	5	0.5	0.05	5	0.5	0.05	5	0.5	0.05
Mean (ng/ml)	5.37	0.49	0.05	5.39	0.50	0.05	5.35	0.53	0.05	5.36	0.50	0.05
Standard Deviation	0.35	0.033	0.003	0.34	0.034	0.003	0.33	0.036	0.004	0.37	0.033	0.003
Percent CV	6.6	6.8	6.3	6.3	6.8	6.4	6.2	6.8	9.3	6.8	6.6	6.6
Average Percent of Expected	107	98	102	108	100	95	107	105	95	107	99	98
Percent Range	97–124	87–110	89–114	97–123	89–113	83–106	97–122	94–118	79–114	96–124	89–111	85–110

7. Representative Data (continued)

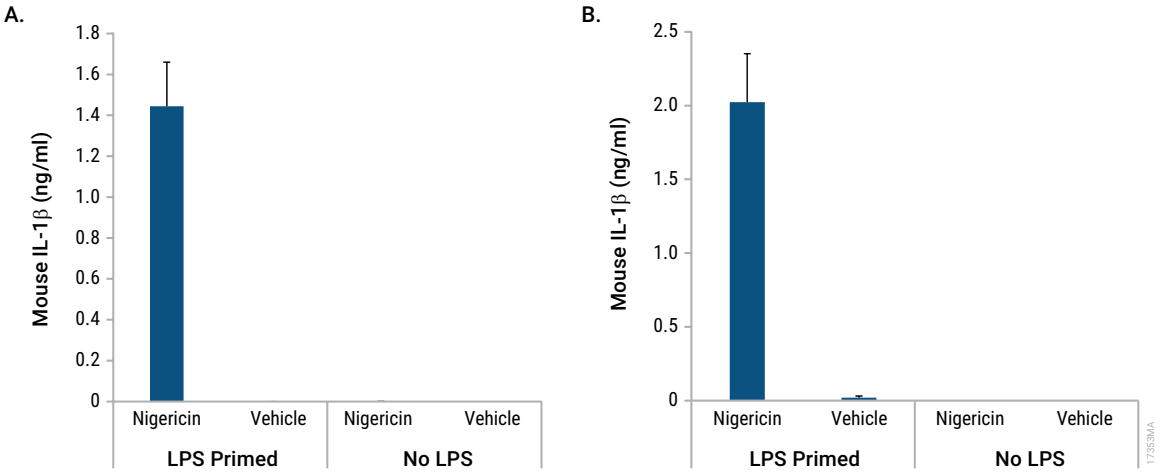

Figure 5. Lumit™ Detection of IL-1β released from Mouse J774A.1 macrophages in 96- and 384-well plates. Panel A. J774A.1 cells were plated in DMEM + 10% FBS at 50,000 cells/well in 96-well plates and primed with 500ng/ml LPS for 4 hours or left unprimed. Nigericin (20µM) was used to activate the inflammasome and IL-1β release. After 2 hours of nigericin or vehicle treatment, Anti mIL-1β mAb-SmBiT and Anti mIL-1β mAb-LgBiT were added to the cells at 5X, incubated for 1 hour, followed by addition of Lumit™ Detection Reagent B. **Panel B.** J774A.1 cells were plated in DMEM + 10% FBS at 10,000 cells/well in 384-well plates and primed with 500ng/ml LPS overnight or left unprimed. Nigericin (20µM) was used to activate the inflammasome and IL-1β release. After 1 hour of nigericin or vehicle treatment, Anti mIL-1β mAb-SmBiT and Anti mIL-1β mAb-LgBiT were added to the cells at 5X, incubated for 1 hour, followed by addition of Lumit™ Detection Reagent B.

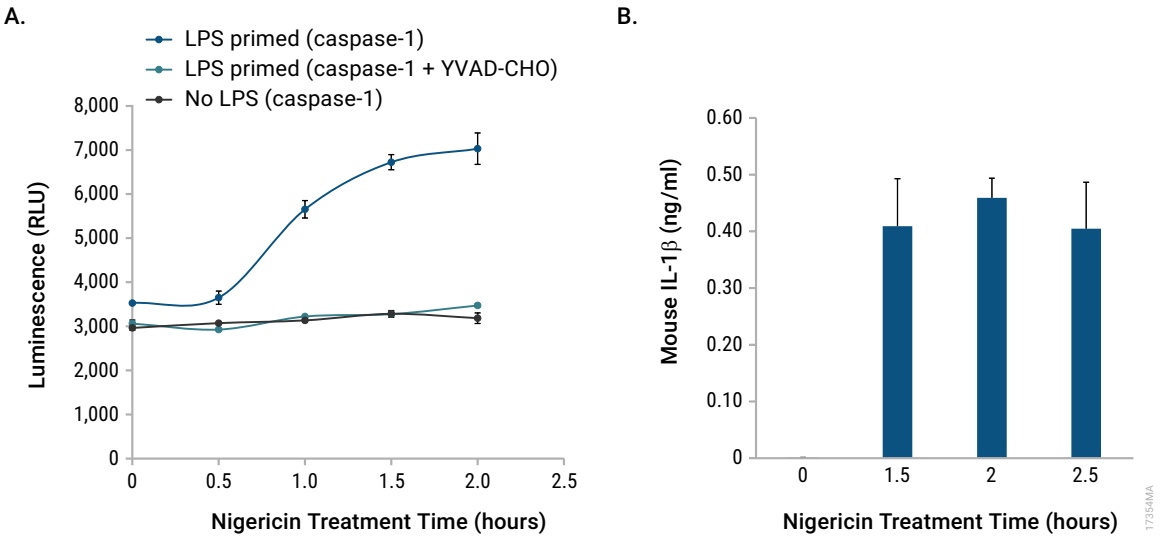

Figure 6. Lumit™ Mouse IL-1β Immunoassay and Caspase-Glo® 1 Inflammasome Assay multiplex. J774A.1 mouse macrophages were plated in DMEM + 10% FBS at 50,000 cells/well in 96-well plates and primed with 500ng/ml LPS for 3.5 hours or left unprimed. Nigericin (20µM) was used to induce the inflammasome and IL-1β release. After various times of nigericin treatment, half of the culture medium from the cells was transferred to another plate and monitored for active caspase-1 with the Caspase-Glo® 1 Inflammasome Assay (**Panel A**; 9). The 2X Lumit™ antibody mixture was added to the remaining cells and culture medium and incubated for 1 hour, followed by addition of the Lumit™ Detection Reagent B (**Panel B**). Nigericin induces both caspase-1 activation and IL-1β release in J774A.1 cells primed with LPS.

7. Representative Data (continued)

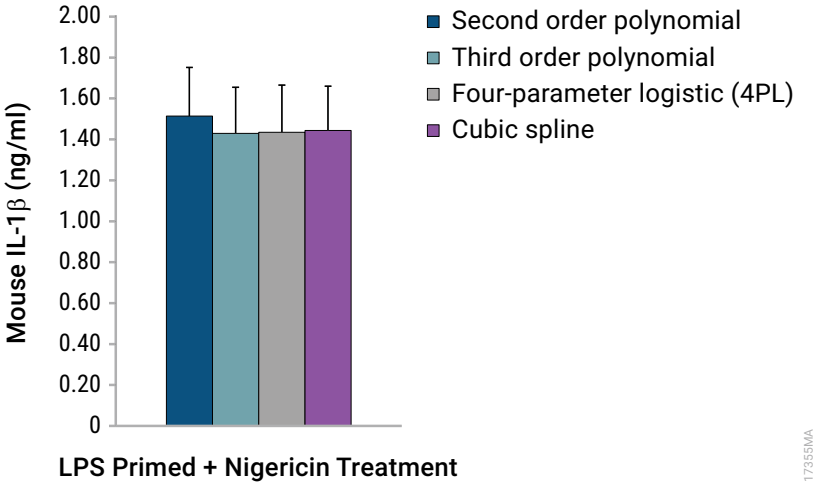

Figure 7. Different methods of interpolating from standard dilutions for quantifying IL-1β released. J774A.1 cells were plated in DMEM + 10% FBS at 50,000 cells/well in 96-well plates and primed with 500ng/ml LPS for 4 hours (see Figure 5, Panel A). Nigericin (20µM) was used to induce the inflammasome and IL-1β release. Three different non-linear regression methods and the cubic spline method (GraphPad® Prism 8) were used to quantify the amount of IL-1β released by interpolating from the standard curve.

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 mouse cells are used with the Lumit™ Mouse IL-1β Immunoassay. The Lumit™ Mouse IL-1β Immunoassay will not detect IL-1β from other species. If there is any question whether the experimental mouse cell type expresses IL-1β, use J774A.1 cells as a positive control.
Mouse IL-1β standard dilutions are 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 standard contaminates the background control.

Symptom	Causes and Comments
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 Mouse IL-1β Selectivity Example Data

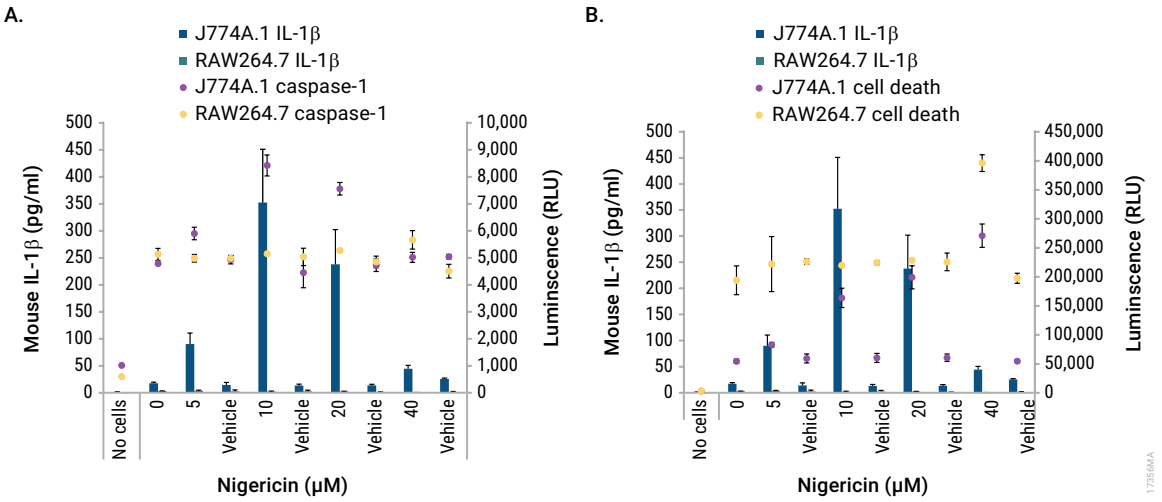

Figure 8. Selectivity for processed mouse IL-1β. This assay is specific for mature, processed IL-1β. This was assessed using mouse macrophage cell lines, J774A.1 and RAW 264.7. Both cell lines were plated at 50,000 cells/well and LPS-primed overnight followed by treatment with a titration of nigericin or vehicle for 2 hours. RAW264.7 cells do not express apoptosis-associated speck-like protein containing a C-terminal caspase recruitment domain (ASC), a necessary component of the NLRP3 inflammasome. Consequently, these cells do not activate caspase-1 nor process pro-IL-1β, although proIL-1β is upregulated with LPS-priming of these cells (10). The Lumit™ Mouse IL-1β Immunoassay detected released IL-1β from the J774A.1 cells, but not from the RAW 264.7 cells as expected. Likewise, caspase-1 activity measured with the Caspase-Glo® 1 Inflammasome Assay was detected only in J774A.1 cells but not RAW264.7 cells (**Panel A**). Caspase-1 activation was consistent with IL-1β release; low at 5μM nigericin, highest at 10 and 20μM nigericin and barely detected at 40μM nigericin (**Panel B**). The cell death assay, CytoTox-Fluor™ Cytotoxicity Assay, demonstrated increasing cell death in the J774A.1 cells between 5–40μM nigericin and significant cell death in the RAW264.7 cells at 40μM. The Lumit™ Mouse IL-1β Immunoassay is specific for mature IL-1β and does not detect proIL-1β as evidenced by not detecting any IL-1β in RAW264.7 cells treated with 40μM nigericin and exhibiting significant cell death. Interestingly, with 40μM nigericin treatment, cell death occurred in both cell lines that appeared inflammasome-independent and nonpyroptotic.

9.B. References

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9.C. Related Products

Lumit™ Immunoassays

Product	Size	Cat.#
Lumit™ Human IL-1β Immunoassay*	100 assays	W6010
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.

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9.C. Related Products (continued)

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.

[a]U.S. Pat. Nos. 9,797,889; 9,797,890; 10,107,800; and other patents and patents pending.

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