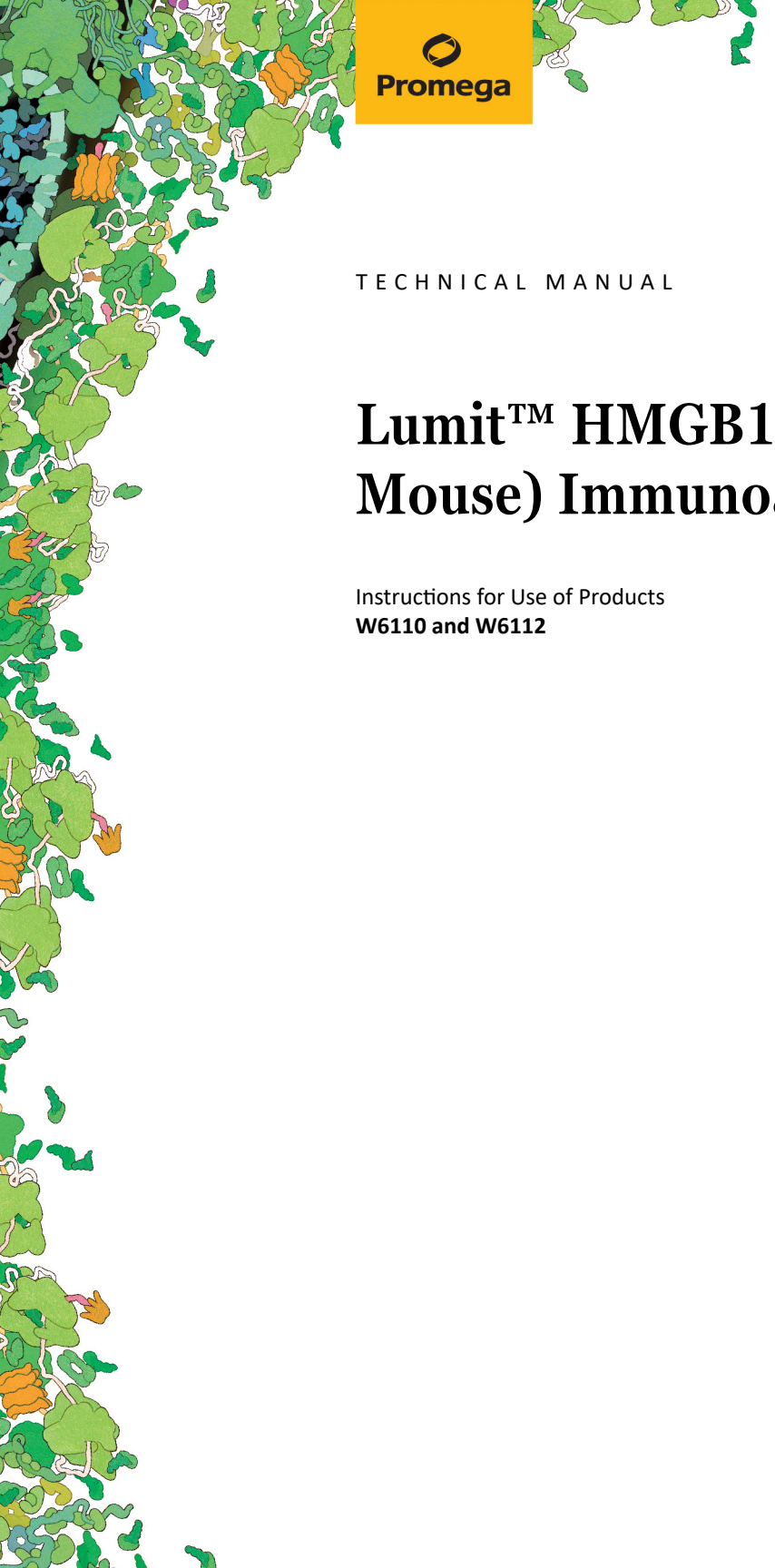

TECHNICAL MANUAL

Lumit™ HMGB1 (Human/ Mouse) Immunoassay

Instructions for Use of Products
W6110 and W6112

4/22
TM681

Promega

Lumit™ HMGB1 (Human/Mouse) 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™ HMGB1 (Human/Mouse) Immunoassay[a,b] is a homogeneous bioluminescent assay for detecting High Mobility Group Box 1 (HMGB1) protein released from cells. The assay can be used without the need for sample transfers or wash steps. HMGB1 is a highly abundant and evolutionarily conserved protein that has many important biological activities in intracellular and extracellular environments. HMGB1 typically resides with DNA and histones inside the nucleus to regulate transcription and help order and shape chromatin structure. When HMGB1 is translocated to the cytoplasm, it can help mediate autophagy. During a process called Immunogenic Cell Death (ICD), tumor or oncolytic virus-infected cells release HMGB1 via either active or passive mechanisms in response to ICD-inducing treatments. Once outside the dying tumor cell, HMGB1 can serve as a Damage-Associated Molecular Pattern (DAMP) molecule, which can help stimulate recruitment of dendritic cells to the tumor bed (1,2).

Assay Principle

The Lumit™ HMGB1 (Human/Mouse) Immunoassay has been developed for use with cell culture samples. Lumit™ reagents can be dispensed directly into plate wells containing cells and culture medium. Alternatively, users may transfer cell supernatants. Assay performance with additional sample types must be determined by the user. The immunoassay detects both human and mouse HMGB1.

The Lumit™ HMGB1 (Human/Mouse) Immunoassay is based on NanoLuc® Binary Technology (NanoBiT®). NanoBiT is a luminescent structural complementation system designed for biomolecular interaction studies (3,4). 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 HMGB1 monoclonal antibodies covalently labeled with SmBiT or LgBiT. When the labeled antibodies recognize and bind to released HMGB1, 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.

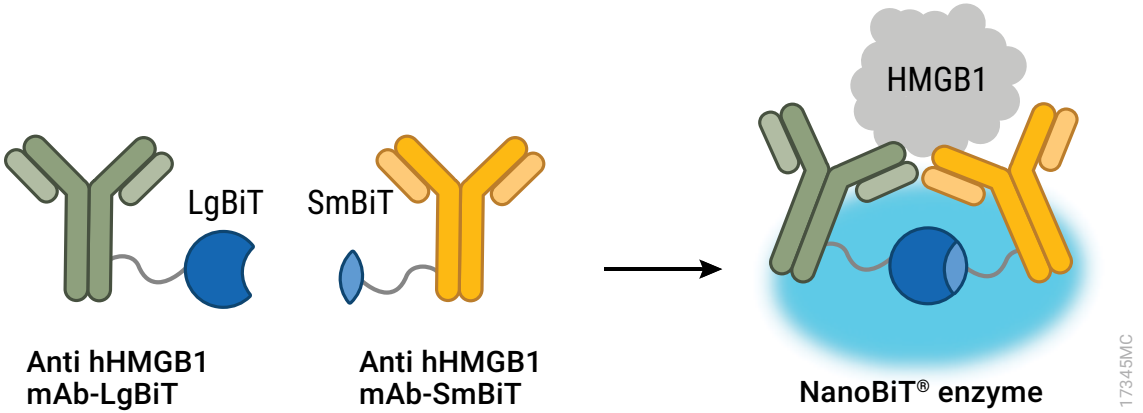

Figure 1. Assay principle. Primary monoclonal antibodies to human HMGB1 are labeled with SmBiT and LgBiT. In the presence of HMGB1, SmBiT and LgBiT are brought into close proximity, forming the NanoBiT® enzyme. When Lumit™ Detection Reagent B is added, a bright luminescent signal is generated.

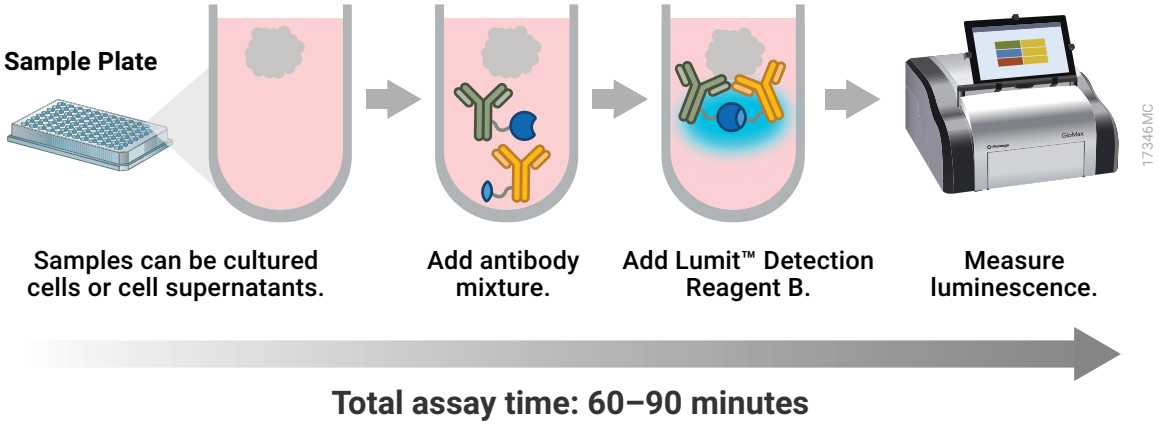

Figure 2. Assay protocol. The Lumit™ HMGB1 Immunoassay is performed directly on cells in culture or on cell supernatants transferred to an assay plate. The Lumit™ Immunoassay protocol does not require wash steps and is complete in 60–90 minutes.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
Lumit™ HMGB1 (Human/Mouse) Immunoassay	**100 assays**	**W6110**

Sufficient for 100 assays in 96-well plates. Includes:

- 50µl Anti-hHMGB1 mAb-SmBiT, 250X
- 50µl Anti-hHMGB1 mAb-LgBiT, 250X
- 20µl HMGB1 (Human) Positive Control
- 160µl Lumit™ Detection Substrate B
- 3.2ml Lumit™ Detection Buffer B

PRODUCT	SIZE	CAT.#
Lumit™ HMGB1 (Human/Mouse) Immunoassay	**5 × 100 assays**	**W6112**

Sufficient for 500 assays in 96-well plates. Includes:

- 5 × 50µl Anti-hHMGB1 mAb-SmBiT, 250X
- 5 × 50µl Anti-hHMGB1 mAb-LgBiT, 250X
- 20µl HMGB1 (Human) Positive Control
- 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 HMGB1 (Human) Positive Control at +2°C to +10°C for up to 1 month. If storing the HMGB1 (Human) Positive Control 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. For long term storage of Lumit™ Detection Substrate B, protect from light.

Note: The assay kit is provided with HMGB1 (Human) Positive Control that is intended to be used as a positive control protein. Recombinant mouse HMGB1 protein, obtained separately from commercial vendors, can also be used as a positive control protein.

3. Before You Begin

The Direct (No-Transfer) Protocol detailed in Section 4 is optimized for detecting HMGB1 (human or mouse) directly in cell culture wells. Add 20µl of a 5X antibody mixture to 80µl of cells or HMGB1 (Human) Positive Control dilutions in culture medium and incubate for 60–90 minutes. Following incubation, add 25µl of Lumit™ Detection Reagent B and record luminescence.

Notes:

a. The protocol in Section 4 lists common volumes for 96-well plates. Other volumes may be used, maintaining the final antibody concentration of 1:250 in total volume, and Lumit™ Detection Reagent B added at a 5X concentration (1:100 final dilution).

b. We recommend using standard tissue culture medium supplemented with 5–10% fetal bovine serum (FBS). Lesser concentrations of FBS may produce higher background (nonanalyte-mediated antibody pairing) and higher replicate variability. The use of medium without phenol red may increase assay sensitivity and reduce inner-filter effects from luminescence quenching.

c. If detecting released HMGB1 in transferred cell supernatants, see Section 8.D for important considerations.

Reagent Preparation and Storage

Prepare the HMGB1 (Human) Positive Control dilution series, Lumit™ antibody mixture and Lumit™ Detection Reagent B on the day of use. Do not reuse the HMGB1 positive control dilution series, 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.

3. Before You Begin (continued)

Plate Map for Both Protocols

	HMGB1 Positive Control Dilution Series (ng/ml)*		Test samples									
	1	2	3	4	5	6	7	8	9	10	11	12
A	1,000	1,000										
B	400	400										
C	160	160										
D	64	64										
E	25.6	25.6										
F	10.2	10.2										
G	4.1	4.1										
H	0	0										

*__Note:__ The indicated HMGB1 (Human) Positive Control dilution series listed above and described in the subsequent protocol directions are only a recommendation.

Materials to Be Supplied by the User

- cells (human- or mouse-derived)
- culture medium [We recommend standard tissue culture medium supplemented with 10% fetal bovine serum (FBS)]
- **optional:** recombinant mouse HMGB1 protein
- hemocytometer and trypan blue
- white, multiwell tissue culture plates (solid white or white with clear bottom) compatible with a luminometer [e.g., 96-well Corning® (Cat.# 3917)]
- multichannel pipette or automated pipetting station
- dilution tubes or multi-chamber, dilution reservoir (e.g., Dilux® Cat.# D-1002)
- reagent reservoir trays (e.g., Midwest Scientific Cat.# RR25)
- plate shaker for mixing multiwell plates
- luminometer capable of reading multiwell plates (e.g., GloMax® Discover System, Cat.# GM3000)
- positive control HMGB1-release inducers (e.g., doxorubicin, idarubicin or mitoxantrone)

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

This protocol describes the detection of HMGB1 directly in assay wells containing cells and culture medium. For the purposes of a positive control, HMGB1 (Human) Positive Control protein prepared in culture medium is used to generate a positive control dilution series.

Note: Alternatively, if detecting HMGB1 in cell supernatants, dispense samples at 80µl per well into a 96-well assay plate and proceed to Section 4.B. See Section 8.D for important considerations when using cell supernatants.

4.A. Cell Plating and Treatment

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

   Note: While the broad dynamic range of the assay offers 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 HMGB1 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 HMGB1 production.

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.

   For example, if 40µl of cells are plated per well in a 96-well plate, add 40µl of 2X treatment agent in culture medium. Incubation time of cells with test agents needs to be empirically determined to observe optimal release of HMGB1. In the examples in this protocol, cells are typically treated overnight (18–24 hours), or longer depending on stimuli, to observe release of HMGB1 above an untreated control.

4.B. Preparing Recombinant, Human HMGB1 Positive Control Dilution Series

Shortly before completing cell treatments, prepare HMGB1 (Human) Positive Control dilutions in the identical culture medium used for cell samples.

Note: If using multiple cell models each requiring different culture medium, separate positive control dilution series must be generated in each medium used in the study.

1. Immediately before use, thaw the HMGB1 (Human) Positive Control at room temperature for approximately 15 minutes.
2. Briefly centrifuge the tube before opening, then mix by pipetting.
3. Prepare an initial concentration of 1,000ng/ml human HMGB1 by diluting HMGB1 (Human) Positive Control (500µg/ml) 1:500 in prewarmed cell culture medium (typically standard tissue culture medium + 10% FBS for human cells). For example, prepare 1,500µl of 1,000ng/ml human HMGB1 by adding 3µl of the HMGB1 (Human) Positive Control stock to 1,497µl of culture medium (see Figure 3).
4. Set up seven tubes (or seven chambers in a dilution reservoir) with 600µl of culture medium in each.

4.B. Preparing Recombinant, Human HMGB1 Positive Control Dilution Series (continued)

5. Prepare 2.5-fold serial dilutions of positive control. Transfer 400µl from the 1,000ng/ml initial human HMGB1 dilution (Step 3) to 600µl of culture medium for the second dilution. Mix and repeat five more times to generate seven positive control dilutions with a range of 1,000–4.1ng/ml. The last well or chamber should contain only culture medium as the background control.

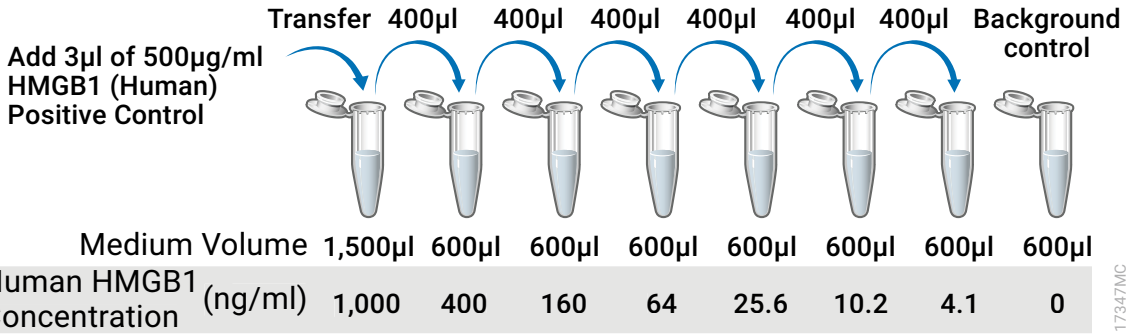

Figure 3. HMGB1 (Human) Positive Control dilution series. This dilution series is only a recommendation.

6. After the cell treatment exposure is complete, add the positive control 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.

   Note: Extra HMGB1 (Human) Positive Control (500µg/ml) can be stored at 4°C for 1 month. If storing the positive control for longer than 1 month, dispense into aliquots and store at –30°C to –10°C. Avoid multiple freeze-thaw cycles.

4.C. Adding 5X Anti-hHMGB1 Antibody Mixture to Assay Wells

Note: If using multiple cell models each requiring different culture medium, separate 5X Anti-hHMGB1 antibody mixtures must be generated in each medium used in the study.

1. Remove the Anti-hHMGB1 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. Briefly centrifuge the tubes before opening then mix by pipetting.

3. Immediately prior to use, prepare a 5X antibody mixture by diluting both antibodies 1:50 into a single volume of prewarmed culture medium. Pipet to mix the antibody solution. To assay a complete 96-well plate, including some excess reagent volume, prepare the 5X antibody mixture as follows:

Reagent	Volume
culture medium	2.4ml
Anti-hHMGB1 mAb-SmBiT	50µl
Anti-hHMGB1 mAb-LgBiT	50µl

4. Add the 5X Anti-hHMGB1 antibody mixture to wells containing cultured cells or HMGB1 (Human) Positive Control dilutions, carefully avoiding cross contamination between wells by changing pipette tips when moving from high to low analyte levels.

   96-well plate: Dispense 20µl/well of 5X Anti-hHMGB1 antibody mixture to 80µl/well of cells or HMGB1 (Human) Positive Control dilutions.

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

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

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

While cells are incubating with the Anti-hHMGB1 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™ Detection Substrate B from –20°C storage, and mix. If the Lumit™ Detection Substrate B has collected in the cap or on the sides of the tube, briefly centrifuge.
3. Prepare a 1:20 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-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

Note: 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. After the incubation in Section 4.C, Step 6, add room temperature 5X Lumit™ Detection Reagent B to each assay well of the plate with a multichannel pipette.

   96-well plate: Dispense 25µl per well.

5. Briefly mix with a plate shaker (e.g., 10 seconds at 300–500rpm).
6. Incubate 3–5 minutes.
7. 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 HMGB1 positive controls on each assay plate for normalization.

4.E. Optional: Multiplexing with the CellTiter-Glo® Luminescent Cell Viability Assay for Cell Viability Analysis

Note: The optional CellTiter-Glo® Luminescent Cell Viability Assay needs to be performed after the Lumit™ HMGB1 (Human/Mouse) Immunoassay (Sections 4.A–4.D).

1. Obtain the CellTiter-Glo® Luminescent Cell Viability Assay (Cat.# G7570) and prepare as described in the *CellTiter-Glo® Luminescent Cell Viability Assay Technical Manual* #TB288. Refer to Figure 7 for example data.
2. After performing the Lumit™ HMGB1 (Human/Mouse) Immunoassay, add 100µl of CellTiter-Glo® Reagent per well to each well containing cells (treated and untreated).
3. Mix using an orbital shaker at 300–500rpm for 30 seconds.
4. Measure luminescence after 10 minutes.

5. Analyzing Results

The data resulting from use of the Lumit™ HMGB1 (Human/Mouse) Immunoassay can be handled in two principal ways: 1) a comparison of induction potential versus an uninduced cell control or by 2) interpolating HMGB1 release values from the positive control curve. Either way, it is important to first establish the performance of the assay with the positive control dilution series.

Note: The HMGB1 (Human) Positive Control is meant for research use only and is not calibrated against a separate HMGB1 standard. Slight lot-to-lot variation in the positive control may lead to slight variation in quantified analyte amounts.

Positive Control Dilution and Curve: This data is handled by averaging luminescence (RLU) readings from replicate wells for each concentration of HMGB1 (Human) Positive Control as well as for the background controls. The average background control RLU is then subtracted from the average RLU for each HMGB1 (Human) Positive Control concentration. This data can be plotted using software (e.g., GraphPad® Prism) capable of simple linear regression analysis. Alternatively, a Log-Log plot of average RLU (background-subtracted) vs. HMGB1 Positive Control concentrations can be fit with the Power trendline in Microsoft Excel® (see Section 6). Although the overall intensity (RLU) may vary between different vendors of luminometers, successful deployment of the Lumit™ HMGB1 (Human/Mouse) Immunoassay will produce a linear relationship (r^2 >0.95) with the recombinant antigen provided.

Experimental Induction versus Uninduced Control: Most assay users will find the relative increase in HMGB1 release versus an untreated control to be the most straightforward and unencumbered manner of treating the data sets. Like the positive control dilution series, the average luminescence from the background control is subtracted from induced and uninduced replicate wells. The data is then plotted as a function luminescence vs. concentration. The induction ratio at any concentration can be calculated by dividing that value by the uninduced control.

Interpolation from Positive Control Dilution Series: This mode of analysis is possible if sufficient motivation exists but contains a number of practical caveats. For best results, phenol red-free medium should be used to mitigate inner-filter effects known to occur through differential cell growth between treated and untreated cells during a typical exposure period. Further, it may be necessary to collect and use conditioned medium to create the positive control dilution series that is best matched for determination of unknowns, although this conditioned medium will contain basal levels of HMGB1. The user should expect additional sample matrix model refinement to employ this format.

Like the positive control dilution series, the average luminescence from the background control is subtracted from induced and uninduced replicate wells. For interpolation analysis, second- or third-order polynomial regression, cubic spline or four-parameter logistic (4PL) curve fitting is commonly used.

6. Representative Data

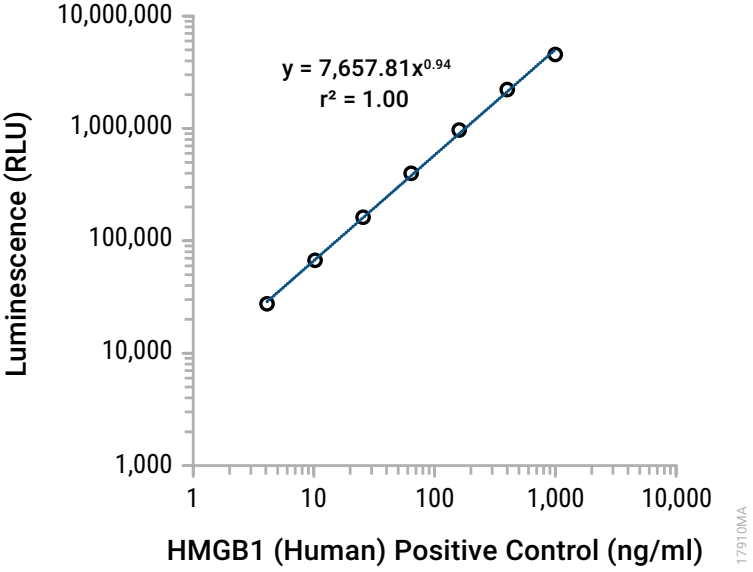

Figure 4. Positive control dilution series for the Lumit™ HMGB1 (Human/Mouse) Immunoassay. This is representative data obtained with the HMGB1 (Human) Positive Control dilution series and should not be used for calculation of unknowns. Generate a positive control dilution series on each assay plate as a positive control and for normalization purposes. **Note:** Luminescent values have the background subtracted.

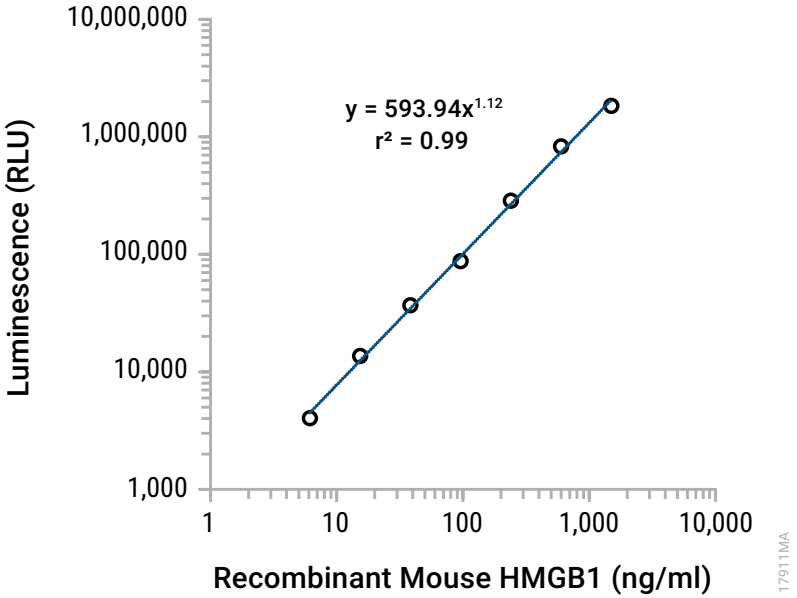

Figure 5. The Lumit™ HMGB1 (Human/Mouse) Immunoassay using recombinant mouse HMGB1 protein. This is representative data obtained with a recombinant mouse HMGB1 protein dilution series and should not be used for calculation of unknowns. Generate a positive control dilution series on each assay plate as a positive control and for normalization purposes. **Note:** Luminescent values have the background subtracted.

6. Representative Data (continued)

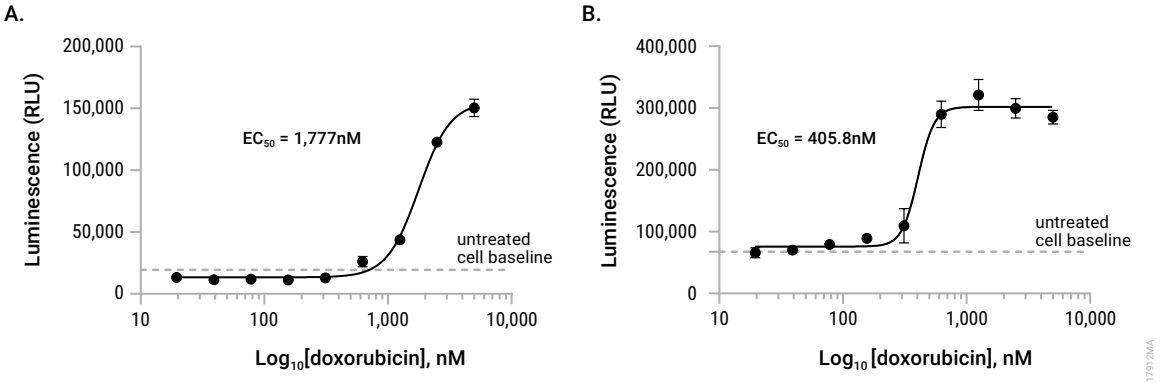

Figure 6. Biological release of HMGB1 in cell-based ICD models. Human U2OS cells (**Panel A**) and mouse EL4 cells (**Panel B**) were treated with doxorubicin for 24 hours. The Lumit™ HMGB1 (Human/Mouse) Immunoassay was performed directly on cell culture samples to measure detectable HMGB1 produced from treated cells.

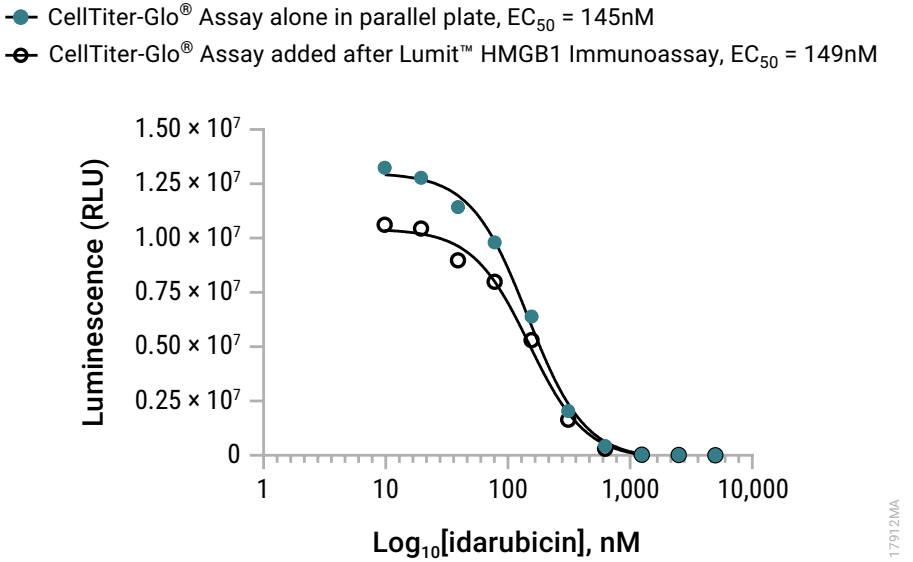

Figure 7. A same-well multiplex with the CellTiter-Glo® Luminescent Cell Viability Assay reduces effort and saves resources. Mouse EL4 cells were challenged with serial dilutions of idarubicin in parallel plates for 24 hours. At the end of the exposure, the Lumit™ HMGB1 Human/Mouse Immunoassay reagent was added to one plate and data collected (data not shown). Next, the CellTiter-Glo® Reagent was prepared and added directly to both plates. Luminescence was collected and plotted for both data sets.

7. 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 with Positive Control	Technical issue with conducting the assay or luminometer set up.
No signal from treated cells	If a positive luminescence relationship is observed in the HMGB1 (Human) Positive Control dilution series, there are three possibilities for no signal from cells: • A measurable HMGB1 release event did not occur with test compound. We encourage the use of reference ICD inducers such as mitoxantrone and doxorubicin as control toxins. • HMGB1 antigen released into the extracellular environment may have degraded as a function of time. We recommend employing the CellTiter-Glo® Assay to determine the extent of cytotoxicity or test at an earlier time point. • A nonhuman or nonmouse cell line was used. We have not tested other species and cannot verify the performance of the assay with those lines.
Human HMGB1 dilution series is not linear	The 2.5-fold serial dilution series of the positive control listed in the Plate Map in Section 3 and described in the Protocols in Figure 3 is listed as a recommendation. There may be slight variability based on the type of medium, the type of luminometer used, user-to-user variability, etc. Use the recommended positive control dilution series as a starting point and then reoptimize if necessary.
The relative light units (RLUs) for the positive control dilution series are variable between assays	There may be some variation in RLUs due to culture conditions, temperature, etc., but as long as the positive control is used on the same plate as the test samples under the same conditions, released HMGB1 can be accurately measured.

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8. Appendix

8.A. Cell Models and Standard Operating Procedure

The Lumit™ HMGB1 (Human/Mouse) Immunoassay is a highly sensitive, homogeneous assay intended to measure experimentally released HMGB1. As with any assay that measures parameters associated with cell health, carefully validate a model (cell number and exposure period) whereby untreated cells maintain optimal viability over the time course. Special attention should be taken to gently passage sensitive cell types that are susceptible to mechanical or enzymatic dispersion. For these cell lines, we recommend an acclimation period of at least 6 hours after passage, or a medium exchange prior to dosing. Failure to optimize cell health parameters may lead to increases in HMGB1 levels in untreated wells and a general overestimation of compound potency.

8.B. Temperature

The enzymatic activity of the complemented NanoBiT® Luciferase enzyme is temperature dependent. Strive to maintain a constant 37°C environment over the test exposure period and a constant room temperature environment for the antibody/detection step incubation period. Try to reduce thermal gradients which may adversely affect replicate variability.

8.C. Spectral Interferences

Test article or control compounds: Some reference ICD inducers, such as doxorubicin and mitoxantrone, absorb light in the visible spectrum and can quench luminescence in a dose-dependent manner. Test compounds with visible color may similarly affect luminescence. Data sets may benefit from normalization to basal release values for each dose determined immediately after adding a quenching compound.

Phenol red-containing medium: Test articles and control compounds that provoke the ICD phenotype often cause differential cell growth within the dilution series, which may slightly alter pH. In extreme cases, this “inner-filter” may alter luminescence intensity and skew comparisons to a calibration curve. Therefore, the best practice is to use phenol red-free medium when possible. Most common media (for example, RPMI 1640, DMEM, MEM, DMEM/F-12, McCoy’s 5A, Opti-MEM, etc.) are readily available without phenol red through vendors such as GIBCO/Thermo Fisher Scientific or others.

8.D. Considerations for the Optional Sample Transfer Assay Protocol

1. Supernatant samples should be collected and tested within 24 hours of the expected peak release of HMGB1. If this is not possible, supernatant samples may be stored at 4°C for up to 48 hours with minimal HMGB1 protein degradation. **Do not** store the treated cell supernatant samples at –20°C as this temperature, the freeze/thaw cycle or both are detrimental to detecting released HMGB1 protein.
2. When transferring the cell medium with an attachment-dependent cell line, transfer the cell medium supernatant (if interested in only detecting released HMGB1), being careful not to disrupt the attached cell layer. It is possible, that over the course of test agent treatment, some cells may have died and lifted off the attached cell layer. Thus, transferring dead cells and detecting HMGB1 in membrane-compromised cells is possible.
3. With nonadherent cell lines, transferring samples of cell medium supernatant without cells (if interested in only detecting released HMGB1) may be easier in a larger format. For example, the treatment of nonadherent cells could be performed in larger volumes in a 12- or 24-well plate. After the compound treatment exposure period, perform the transfer from sample wells to tubes and then centrifuge the cells. Treated medium supernatant samples can then be collected and transferred to the 96-well assay plate for released HMGB1 detection.
4. In general, detecting HMGB1 in transferred cell supernatants will produce similar dose response profiles and EC_{50} values when compared to the direct assay method but will produce reduced luminescent signal as compared to that obtained with the direct assay protocol. This reduction in overall luminescence may result in a lower dynamic range.

8.E. 3D Cell Models

The protocol described in Section 4 can be used without modification for microtissues and spheroids. Porous extracellular matrices (Matrigel®, etc.) allow uniform reagent access, but may cause optical interferences. You can expect a reduction in luminescence values when using a black microplate with rounded bottoms.

8.F. References

1. Wang, Y.J. *et al.* (2018) Immunogenic effects of chemotherapy-induced tumor cell death. *Genes Dis*. **5**, 194–203.
2. Magna, M. and Pisetsky, D.S. (2014) The role of HMGB1 in the pathogenesis of inflammatory and autoimmune diseases. *Mol. Med*. **20**, 138–46.
3. Dixon, A.S. *et al.* (2016) NanoLuc complementation reporter optimized for accurate measurement of protein interactions in cells. *ACS Chem. Biol.* **11**, 400–8.
4. Hwang, B. *et al.* (2020) A homogeneous bioluminescent immunoassay to probe cellular signaling pathway regulation. *Commun. Biol.* **3**, 8.

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8.G. Related Products

Lumit™ Immunoassays

Product	Size	Cat.#
Lumit™ FcRn Binding Immunoassay	100 assays	W1151
Lumit™ HMGB1 (Human/Mouse) Immunoassay	100 assays	W6110
Lumit™ Human IL-1β Immunoassay	100 assays	W6010
Lumit™ Mouse IL-1β Immunoassay	100 assays	W7010
Lumit™ IFNγ (Human) Immunoassay	100 assays	W6040
Lumit™ IL-2 (Human) Immunoassay	100 assays	W6020
Lumit™ IL-4 (Human) Immunoassay	100 assays	W6060
Lumit™ IL-6 (Human) Immunoassay	100 assays	W6030
Lumit™ IL-10 (Human) Immunoassay	100 assays	W6070
Lumit™ TNF-α (Human) Immunoassay	100 assays	W6050

Additional sizes available.

Lumit™ Immunoassay Reagents

Product	Size	Cat.#
Lumit™ Immunoassay Labeling Kit	1 each	VB2500
Lumit Detection Reagent B*	100 assays	VB4050
Lumit™ Immunoassay Cellular Systems–Starter Kit	200 assays	W1220

*Additional sizes available.

Inflammation Assays

Product	Size	Cat.#
Caspase-Glo® 1 Inflammasome Assay	10ml	G9951
RealTime-Glo™ Extracellular ATP Assay	200 assays	GA5010

Additional sizes available.

Cell Viability Assays

Product	Size	Cat.#
CellTiter-Glo® Luminescent Cell Viability Assay	10ml	G7570
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.

Promega

8.G. Related Products (continued)

Energy Metabolism and Oxidative Stress Assays

Product	Size	Cat.#
NAD/NADH-Glo™ Assay	10ml	G9071
NADP/NADPH-Glo™ Assay	10ml	G9081
ROS-Glo™ H2O2 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; 10,648,971; and other patents and patents pending.

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