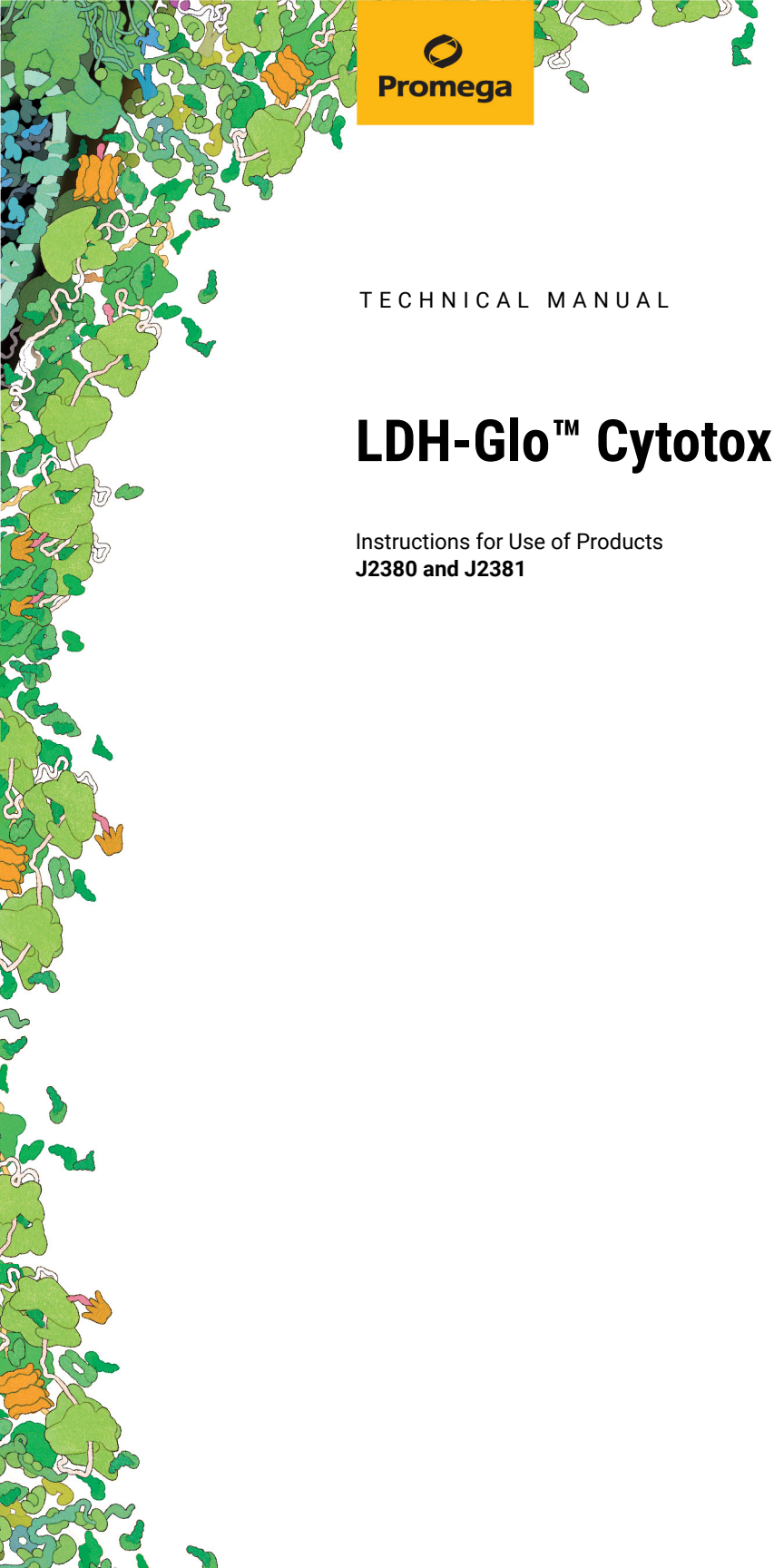


TECHNICAL MANUAL

LDH-Glo™ Cytotoxicity Assay

Instructions for Use of Products
J2380 and J2381

Revised 7/23
TM548

Promega

LDH-Glo™ Cytotoxicity Assay

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

Lactate dehydrogenase (LDH) is a soluble stable cytosolic enzyme present in many cell types that is rapidly released into the cell culture medium upon disruption of the plasma membrane. LDH is a widely used marker in cytotoxicity studies.

The LDH-Glo™ Cytotoxicity Assay[a, b] provides a simple bioluminescent method for quantifying LDH release. The bright luminescent signal provides the sensitivity to determine cytotoxicity in samples low in cell number such as 3D microtissue spheroids, microfluidic cell culture chips, primary cells and stem cells.

In the LDH-Glo™ Assay protocol, LDH Detection Reagent (containing Lactate, NAD^+, Reductase, Reductase Substrate and Ultra-Glo™ rLuciferase) is added to a sample of diluted cell culture media. If the sample contains LDH, the enzyme-coupled reactions shown in Figure 1 start and progress simultaneously. The luminescent signal generated is proportional to the amount of LDH in the sample. The signal increases until all reductase substrate is consumed and the reaction signal is no longer in the linear range of the assay.

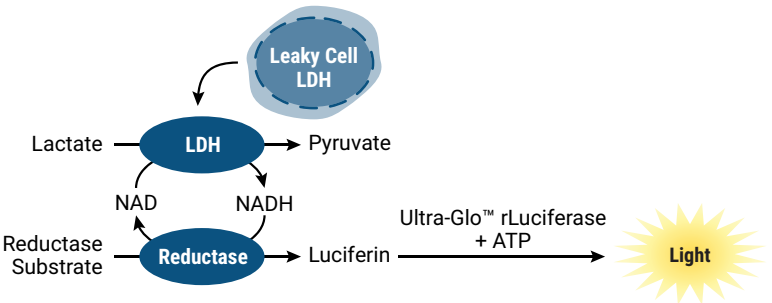


Figure 1. LDH-Glo™ Cytotoxicity Assay principle. Lactate Dehydrogenase (LDH) catalyzes the oxidation of lactate with concomitant reduction of NAD^+ to NADH. Reductase uses NADH and reductase substrate to generate luciferin, which is converted to a bioluminescent signal by Ultra-Glo™ rLuciferase. The light signal generated is proportional to the amount of LDH present.

Protocol Overview

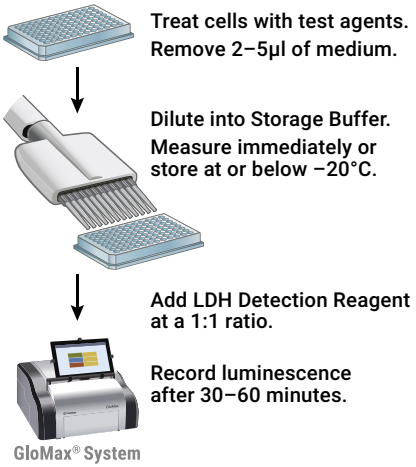


Figure 2. LDH-Glo™ Cytotoxicity Assay protocol. To measure LDH released from non-viable cells, a small amount of culture medium (2–5µl) is removed and diluted into LDH Storage Buffer (Section 3.B). The dilution factor used (25–100 fold) varies depending on the amount of cells and the presence of serum in the medium (see Table 1, Section 3.C). LDH activity is measured by adding an equal volume of LDH Detection Reagent to the diluted sample.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
LDH-Glo™ Cytotoxicity Assay	**10ml**	**J2380**

Contains sufficient reagents to perform 200 reactions in 96-well plates (50µl of sample + 50µl of prepared LDH Detection Reagent). Includes:

- 10ml LDH Detection Enzyme Mix
- 55µl Reductase Substrate
- 1 vial Lactate Dehydrogenase

PRODUCT	SIZE	CAT.#
LDH-Glo™ Cytotoxicity Assay	**50ml**	**J2381**

Contains sufficient reagents to perform 1,000 reactions in 96-well plates (50µl of sample + 50µl of prepared LDH Detection Reagent). Includes:

- 50ml LDH Detection Enzyme Mix
- 275µl Reductase Substrate
- 1 vial Lactate Dehydrogenase

Storage Conditions: Store complete kits below –65°C. Alternatively, store the Reductase Substrate below –65°C protected from light, and store all other components at –30°C to –10°C. Do not freeze-thaw the kit components more than three times.

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

3. Before You Begin

Please read the entire protocol (Sections 3 and 4) to become familiar with the assay procedure before beginning your experiments.

3.A. Materials to Be Supplied By the User

- cells and cell culture medium
- LDH Storage Buffer (Section 3.B; 200mM Tris-HCl (pH 7.3), 10% Glycerol, 1% BSA)
- 96- or 384-well, opaque-walled assay plates (with clear or opaque bottom) compatible with a standard plate reader
- single- and multi-channel pipettors and tips
- single-channel and 12-channel reagent reservoirs
- plate-reading luminometer (e.g., GloMax® Discover System, Cat.# GM3000)
- **optional:** Triton® X-100
- **optional:** menadione (e.g., Sigma Cat.# M5625)

3.B. Reagent Preparation

LDH Detection Reagent

1. Thaw the LDH Detection Enzyme Mix and Reductase Substrate at room temperature. Once thawed, equilibrate the LDH Detection Enzyme Mix to room temperature. Reductase Substrate should be placed on ice. Mix thawed components to ensure homogeneous solutions prior to use.
2. **Immediately before use**, prepare LDH Detection Reagent by combining the LDH Detection Enzyme Mix and Reductase Substrate as shown in the table below. The volumes given are for a 96-well plate format using 50µl of sample and 50µl of LDH Detection Reagent per well. Prepare the amount of reagent needed for your experiment, bearing in mind that some volume may be lost during pipetting. Typically, 5ml of detection reagent is sufficient for a full 96-well plate.

Component	Per Reaction	Per 96-Well Plate
LDH Detection Enzyme Mix	50µl	5ml
Reductase Substrate	0.25µl	25µl

3. Mix the LDH Detection Reagent by gently inverting five times.

   Note: Store unused LDH Detection Enzyme Mix below –65°C or at –30°C to –10°C. Store unused Reductase Substrate below –65°C protected from light. Do not store prepared LDH Detection Reagent.

3. Before You Begin (continued)

LDH Storage Buffer

Prepare LDH Storage Buffer from stock solutions to a final concentration of 200mM Tris-HCl (pH 7.3), 10% Glycerol, 1% BSA. Store at 4°C. LDH Storage Buffer is used for diluting and freezing samples. Samples frozen in medium or PBS will have significantly decreased LDH activity (see Section 5.B).

3.C. Assay Linearity and LDH Dilution Factor Recommendations

To perform the LDH-Glo™ Assay, an aliquot of cell culture medium is diluted into LDH Storage Buffer and transferred to the assay plate. The dilution factor required depends on the amount of LDH in the sample, the presence or absence of serum in the medium and reaction time, and is optimized to fit within the linear range of the assay (Figure 3).

Lower dilutions will increase sensitivity while higher dilutions are required to extend linearity when working with high cell numbers or in the presence of serum (contains significant amount of LDH). General recommendations for sample dilution are given in Table 1.

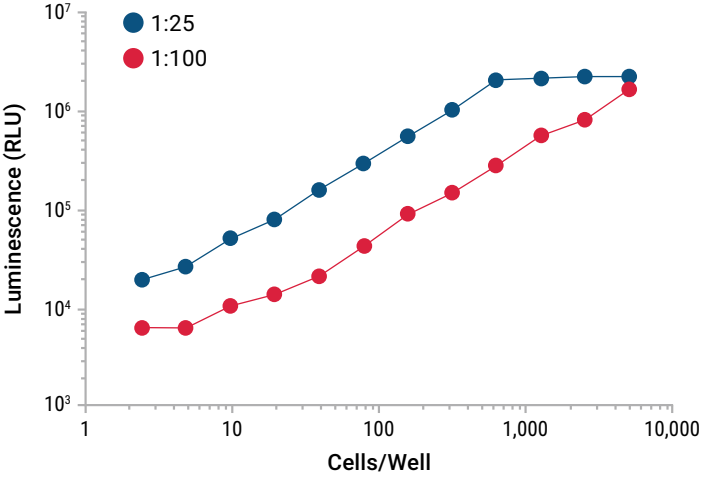


Figure 3. Linearity of LDH-Glo™ Assay depends on dilution factor. A549 cells lysed in F12 medium without serum were diluted 1:25 or 1:100 in LDH Storage Buffer and incubated with an equal volume of LDH Detection Reagent for 60 minutes at room temperature before recording luminescence. Cells/Well on x axis indicates the amount of lysed cells before dilution. The number of cells used in the LDH-Glo™ Assay was 25- and 100-fold less. RLU = Relative Luminescence Units.

Table 1. Dilution Recommendations for LDH Assay Set Up.

Media Composition	Cells/100µl	Fold Dilution
Without Serum	< 1,000	5X
	1,000–10,000	25X
	10,000–50,000	100X
With 10% Serum	1,000–25,000	100X
	25,000–50,000	300X

3.D. Temperature and Reagent Compatibility

The intensity and stability of the luminescent signal is temperature sensitive. For consistent results, equilibrate the reagents and samples to room temperature before use.

Avoid the presence of DTT and other reducing agents in the samples to be tested. Reducing agents will react with the Reductase Substrate and increase background.

3.E. Assay Incubation Time

Record luminescence 30–60 minutes after adding LDH Detection Reagent. We do not recommend taking readings before 30 minutes to ensure that the different coupled enzyme reactions reach equilibrium. Incubation times longer than 60 minutes will increase assay sensitivity, but it is important to confirm that the assay results are still in the linear range and that the luminescence signal continues to increase.

3.F. Plates and Equipment

Most standard plate readers designed for measuring luminescence are suitable for this assay. Some instruments do not require gain adjustment, while others may require optimization of the gain settings to achieve sensitivity and dynamic range. An integration time of 0.5–1 second per well should serve as guidance. For exact instrument settings, consult your instrument manual.

Use opaque, white multiwell plates that are compatible with your luminometer (e.g., Corning Costar® #3917 96-well or Costar® #3570 384-well plates). Luminescence signal is diminished in black plates and increased well-to-well cross-talk is observed in clear plates. The RLU values shown in the figures of this technical manual vary depending on the plates and luminometers used to generate the data. Although relative luminescence output will vary with different instruments, this variation should not affect assay performance.

4. Protocol

This protocol uses 50µl of sample and 50µl of LDH Detection Reagent in a 96-well plate. The assay can be adapted to other volumes provided that a 1:1 ratio of LDH Detection Reagent volume to sample volume is maintained (e.g., 12.5µl of sample and 12.5µl LDH Detection Reagent in a 384-well format).

Please read the entire protocol to become familiar with the assay procedure before beginning your experiments.

4.A. Recommended Controls

Perform the recommended controls on every assay plate, processing the controls in the same way as experimental samples.

- **No-Cell Control:** Set up triplicate wells without cells to serve as a negative control to determine culture medium background.
- **Vehicle-Only Cells Control:** Set up triplicate wells with untreated cells to serve as a vehicle control. Add the same solvent used for test compounds to the vehicle control wells.
- **Maximum LDH Release Control (Optional):** Set up triplicate wells to determine the Maximum LDH Release. Add 2µl of 10% Triton® X-100 per 100µl to Vehicle-Only Cells for 10–15 minutes or longer before collecting the samples for LDH detection. **Note:** This control is only required if calculating percent cytotoxicity.

4.B. Example Cytotoxicity Assay Protocol

The LDH-Glo™ Cytotoxicity Assay can be used to determine cell death following treatment with a cytotoxic drug or compound. Measurements can be taken at a single time point or at multiple time points. Figure 4 shows an example of measuring cytotoxicity by repeat sampling from individual wells. Other example data showing use of the LDH-Glo™ Assay to measure time-dependent cytotoxicity in spheroid cultures, and cell-specific cytotoxicity in ADCC Assays is provided in the Appendix (Figures 8 and 9).

1. Set up a 96-well assay plate containing cells in culture medium. Include wells without cells as negative control to determine background luminescence from LDH in the medium.
2. Add test compounds and vehicle-only controls to the appropriate wells (see Section 4.A). Return treated assay plates to the cell culture incubator for the duration of the treatment time.
3. Collect samples of the culture medium at the desired experimental time points by removing 2–5µl into 48–95µl LDH Storage Buffer (Section 3.B). Mix well by pipetting up and down 2–3 times (without touching or disturbing cells) to ensure the sample is homogenous.

   Note: Removing up to 10% of the starting medium from a well (e.g., removing 2.5µl at four time points from a starting volume of 100µl) should not affect the remaining cells. Refer to Figure 4 for an example. The effect on specific experimental systems should be determined empirically.

   Optional: If a Maximum LDH Release Control is required, add 2µl of 10% Triton® X-100 (per 100µl original volume) to the vehicle-only wells, mix and incubate for at least 10–15 minutes before sample removal.
4. After collecting and diluting all samples, proceed to Step 5 or store at or below –20°C for future assay.

5. On the day of the assay, thaw frozen samples and further dilute in LDH Storage Buffer (if needed) to fit the linear range of the assay. General recommendation for sample dilution are given in Table 1.

Ensure that samples are equilibrated to room temperature before proceeding to Step 6.

6. Transfer 50µl of diluted sample into a 96-well opaque-walled, non-transparent assay plate (with clear or opaque bottom).

7. Add 50µl of LDH Detection Reagent prepared as described in Section 3.B to each well.

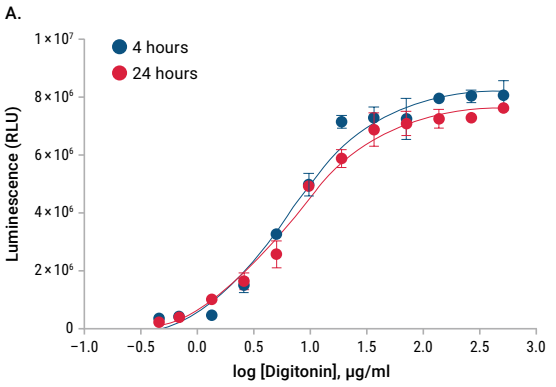
8. Incubate for 60 minutes at room temperature.

Note: Luminescence can be recorded 30–60 minutes after adding LDH Detection Reagent. We do not recommend taking readings before 30 minutes to ensure the different coupled enzyme reactions reach equilibrium. Incubation times longer than 60 minutes will increase assay sensitivity, but it is important to confirm that the assay is still in the linear range and that the luminescent signal continues to increase.

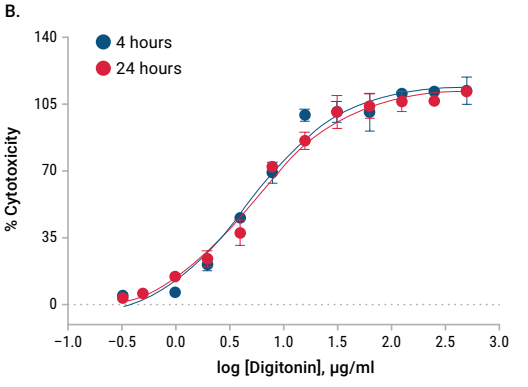
9. Record luminescence (See Section 3.F).

10. Calculate Percent cytotoxicity if needed:

$$\text{Percent Cytotoxicity} = 100 \times \frac{(\text{Experimental LDH Release} - \text{Medium Background})}{(\text{Maximum LDH Release Control} - \text{Medium Background})}$$



	4 hours	24 hours
EC50	5.11	4.697



	4 hours	24 hours
EC50	5.138	4.672

Figure 4. Measuring cytotoxicity by repeat sampling from the same well. 20,000 A549 cells in 100µl F12 medium without serum were treated with increasing concentrations of digitonin. Samples (5µl) were collected from the same wells at 4 and 24 hours and frozen in LDH Storage Buffer at a 1:20 dilution. Samples were thawed, further diluted twofold, and 50µl of the diluted samples combined with 50µl LDH Detection Reagent. Luminescence was recorded after 60 minutes incubation at room temperature. **Panel A.** RLU values plotted against digitonin concentration. **Panel B.** Percent cytotoxicity was calculated using the formula above and plotted against digitonin concentration. RLU = Relative Luminescence Units.

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4.C. LDH Positive Control

An LDH positive control (purified Lactate Dehydrogenase from rabbit muscle) is included in the LDH-Glo™ Assay. A positive control can be used to verify performance of other system components, to establish the linear range of the assay or as a normalization factor for comparing data between multiple plates or experiments. An example LDH titration curve is shown in Figure 5.

1. Reconstitute Lactate Dehydrogenase with 275µl of LDH Storage Buffer to make a 1,000 U/ml LDH Standard. Gently mix to dissolve and place on ice. Prepare aliquots to avoid multiple freeze-thaw cycles and store below –20°C.
2. Dilute the 1,000U/ml LDH Standard to 3.2U/ml by adding 10µl LDH Standard to 3.115ml LDH Storage Buffer
3. Using a 12-channel reagent reservoir, further dilute the 3.2U/ml LDH to 32mU/ml as described in the table below, then perform serial dilutions.

Dilution #	Volume LDH (µl)	Volume LDH Storage Buffer (µl)	mU/ml
1	10µl of 3.2U/ml (Section 4.C, Step 2)	990	32
2	500µl from Dilution #1	500	16
3	500µl from Dilution #2	500	8
4	500µl from Dilution #3	500	4
5	500µl from Dilution #4	500	2
6	500µl from Dilution #5	500	1
7	500µl from Dilution #6	500	0.5
8	0	500	0

4. Transfer 50µl of each LDH Standard dilution (dilutions #1–8) to a 96-well assay plate in triplicate. The remainder of the plate can be used for samples.

   Note: If desired, the highest and lowest dilutions only (#1 and #8) can be used for the positive control standard.
5. Prepare LDH Detection Reagent as described in Section 3.B and transfer to a reagent reservoir.
6. Using a multi-channel pipette, add 50µl prepared LDH Detection Reagent to each well of a 96-well assay plate containing LDH standards.
7. Incubate for 60 minutes at room temperature.

8. Record luminescence.

Notes:

a. To determine if sample relative luminescence unit (RLU) values are within the linear range of the assay, first plot the RLU values for the LDH Positive Control standard curve as shown in Figure 5. If sample RLU values fall within the linear range of the LDH Positive Control standard curve, the sample values are within the linear range of the assay. If the sample values fall outside of the linear range of the LDH Positive Control standard curve, the sample dilution scheme should be adjusted. As RLU values are dependent on assay conditions, sample RLU values should only be compared to LDH Positive Control standard curve RLU values generated during the same assay.

b. Experimental samples and LDH Positive Control standard curve samples should be prepared in the same Storage Buffer and run on the same plate to verify that RLU values of experimental samples are within the linear range of the assay.

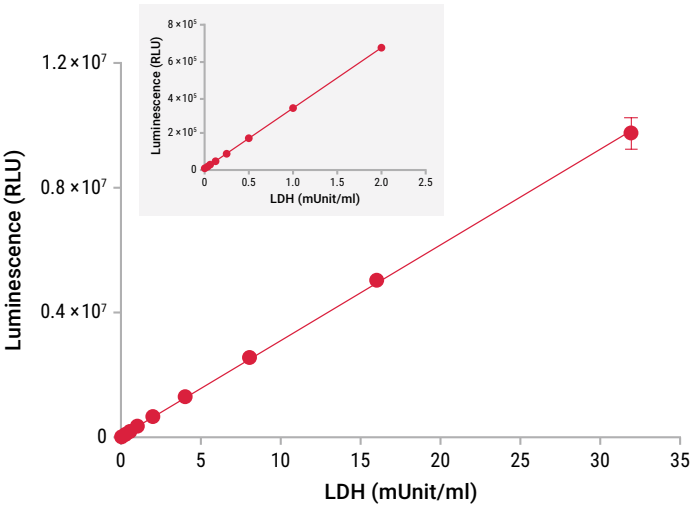


Figure 5. Linear range of LDH positive control standard curve. An equal volume of LDH positive control diluted in LDH Storage Buffer was combined with LDH Detection Reagent and incubated for 1 hour at room temperature. Luminescence was recorded using a GloMax® instrument. Values represent the mean ± SD of three replicate samples. RLU = Relative Luminescence Units.

5. General Considerations

5.A. Cell Culture Media and Serum

The LDH-Glo™ Cytotoxicity Assay is compatible with different media formulations. The assay sensitivity allows dilution of samples in culture media 5–300-fold, minimizing any potential effect of media composition on assay chemistry.

Animal serum used to supplement tissue culture medium may contain significant amounts of LDH, which can increase background luminescence and decrease sensitivity. The presence of serum in the medium does not affect the ability to use the LDH-Glo™ Cytotoxicity Assay with small sample volumes or to measure changes over the time using frozen and diluted samples.

Using reduced serum concentrations or serum-free medium can reduce or eliminate background luminescence. Background luminescence can be corrected by including a negative control to allow determination of luminescence from serum-supplemented culture medium in the absence of cells. The luminescence value determined from this control is used to normalize the luminescence values obtained from other samples. It is important to handle negative controls in the same way as experimental samples. For example, if the samples are diluted 100-fold in LDH Storage Buffer before assay, the negative control samples should also be diluted 100-fold in the same buffer.

5.B. LDH Stability in Media and Upon Freezing

To evaluate the stability of LDH released from cells after freezing, we compared the titration curves of lysed cells before and after freezing. As shown in Figure 6, Panel A, no significant decrease in LDH activity was observed when lysed cells were frozen in LDH Storage Buffer. However, LDH activity decreased by up to 85% if cell lysates were diluted and frozen in PBS supplemented with 1% BSA (Figure 6, Panel B).

The stability of LDH released from dead cells in culure media at 37°C was examined by measuring LDH activity at different time points after cell lysis. Our data indicates no significant loss of LDH activity over time (to at least 72 hours; data not shown).

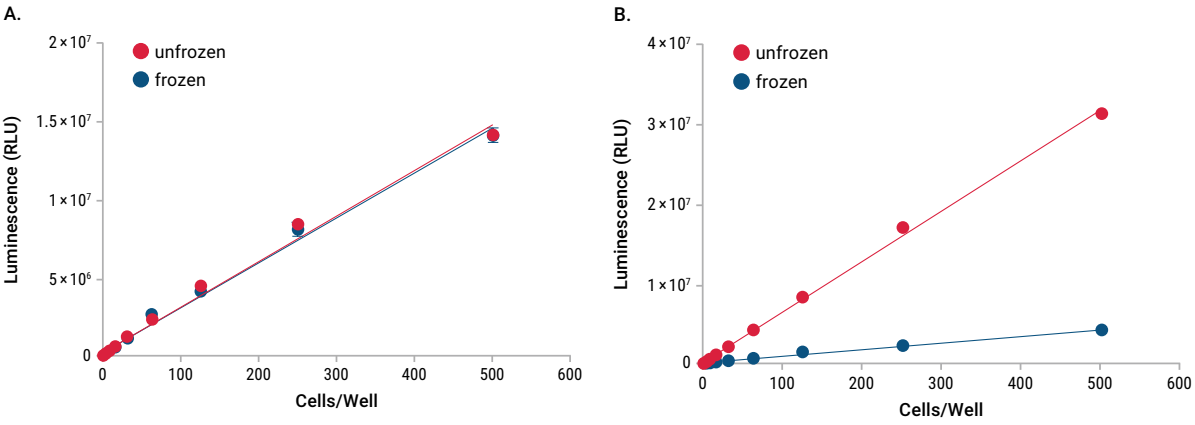


Figure 6. Stability of LDH upon freezing. A549 cells at a starting concentration of 0.2 × 10^6 cells/ml were lysed and diluted 1:20 in medium to 10,000 cells/ml. Then, cells were further diluted twofold in LDH Storage Buffer (**Panel A**) or PBS + 1% BSA (**Panel B**). LDH activity was measured by combining 50µl diluted sample + 50 µl prepared LDH Detection Reagent, and recording luminescence after a 60-minute room-temperature incubation. Samples were assayed immediately (unfrozen) or after one freeze-thaw cycle (frozen). RLU = Relative Luminescence Units.

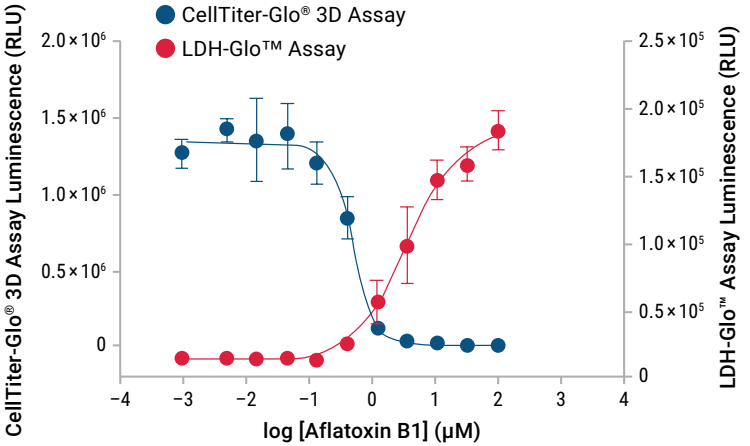
5.C. Use of Stop Solution to Achieve Steady Luminescent Signal

If a stable light signal is desired (e.g., when multiple plates need to be processed at the same time), the increase in signal after adding the LDH Detection Reagent can be stopped at any time by adding a reductase inhibitor, such as menadione. Addition of menadione stops the continued generation of luciferin and allows the plates to be read at a later time. Luminescent signal remains stable for 1–2 hours. Menadione (e.g., Sigma Cat.# M5625) can be prepared as a 40mM DMSO stock solution and added to the samples to a final concentration of 0.25mM. Refer to Section 3 for information on assay timing, and optimal assay sensitivity and linearity.

5.D. Multiplexing with Viability and Other Assays

The LDH-Glo™ Assay can be multiplexed with cell viability assays, including the Promega RealTime-Glo™ MT, CellTiter-Glo® and CellTiter-Fluor™ Assays. After the media sample is removed for measurement with the LDH-Glo™ Cytotoxicity assay, the remainder of the sample can be used for RealTime-Glo™, CellTiter-Fluor™ or CellTiter-Glo™ viability measurements following the protocols provided with these assays. Multiplexing assays may be advantageous for confirming changes in cell health, or identifying cytostatic versus cytotoxic effects of treatment.

The nondestructive nature of the LDH-Glo™ Assay also allows use with other endpoint assays for orthogonal measures of cell health, including metabolism. For example, remaining cells and media can be used to determine caspase activity (Caspase-Glo® 3/7 Assay) or changes in metabolite levels (Glucose-Glo™ or Glutamate-Glo™ Assays).



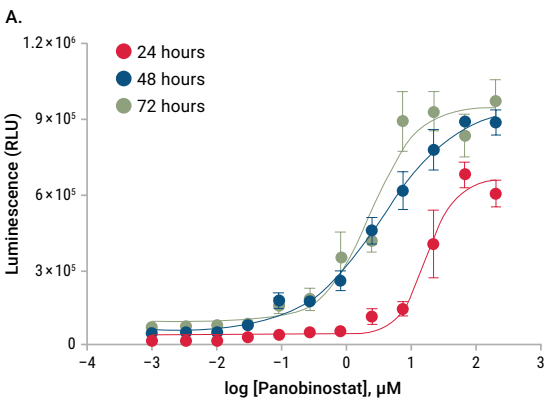
	LDH Assay	CellTiter-Glo® 3D Assay
EC_{50}	3.52	0.4999

Figure 7. Multiplexing the LDH-Glo™ Cytotoxicity Assay and the CellTiter-Glo® 3D Cell Viability Assay. Human liver microtissues were treated with aflatoxin B1 for 48 hours. Samples (10µl) were collected in PBS at a 1:2.5 dilution, then further diluted tenfold. Change in toxicity during treatment was determined using the LDH-Glo™ Cytotoxicity Assay by combining 15µl diluted samples with 15µl LDH Detection Reagent and recording luminescence after a 60-minute, room-temperature incubation. After samples were removed for LDH determination, an equal volume of CellTiter-Glo® 3D Reagent was added to the remaining microtissue sample to assess viability. RLU = Relative Luminescence Units.

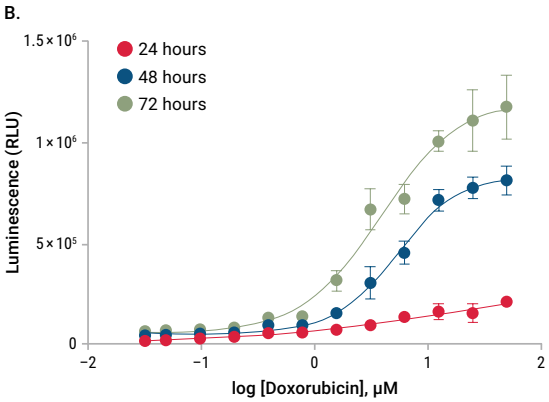
6. Appendix

6.A. Measuring Time-Dependent Cytotoxicity in Spheroid Cultures

The LDH-Glo™ Cytotoxicity Assay is well-suited for measuring LDH released from membrane-damaged cells using small numbers of cells. Here we show an example of using the assay for measuring drug-induced toxicity in HCT116 spheroids formed in Corning® 384-well Ultra-Low Attachment Microplates. Time-dependent toxicity measurements were performed by repeatedly sampling media from the same wells. Samples were stored frozen as described in Section 4.B until the LDH-Glo™ Assay was performed.



	24 hours	48 hours	72 hours
EC50	17.16	3.544	2.311

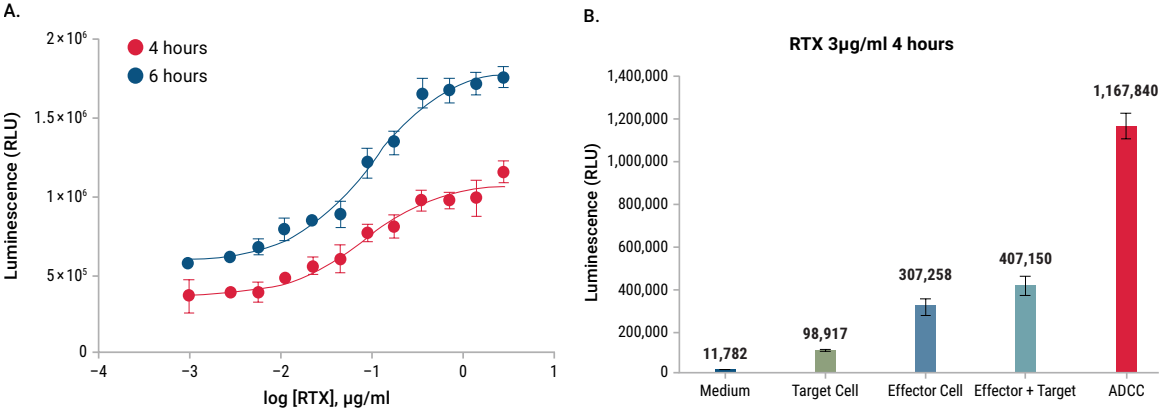


	24 hours	48 hours	72 hours
EC50	17.09	5.474	3.844

Figure 8. Time-dependent cytotoxicity from the same sample well. HCT116 spheroids were formed using 2,500 cells/well in Corning 384-well Ultra-Low Attachment plates and treated with panobinostat (**Panel A**) or doxorubicin (**Panel B**). Samples (2.5µl) were collected from the same wells at the indicated time points and frozen in LDH Storage Buffer at a 1:10 dilution. Samples were thawed and further diluted 2.5-fold before measuring LDH levels using the LDH-Glo™ Cytotoxicity Assay. RLU = Relative Luminescence Units.

6.B. Detecting Cell-Specific Cytotoxicity in ADCC Assays

The LDH-Glo™ Cytotoxicity Assay can be used to measure LDH activity released from membrane-damaged target cells into cell culture medium in antibody-dependent-cell mediated cytotoxicity (ADCC) studies. This ADCC example demonstrates that the LDH-Glo™ Assay can be used to detect rituximab monoclonal antibody-mediated killing of target cells. EC_{50} values are consistent with those reported in the literature for rituximab (1).



	4 hours	6 hours
EC50	0.08958	0.09978

Figure 9. ADCC example. Cells were plated at a 20:1 Effector (PBMC):Target (Daudi) ratio and treated with varying concentrations of rituximab (RTX) in triplicate for 4 or 6 hours. Medium samples (2.5µl) from the same wells were removed and diluted into LDH Storage Buffer (47.5µl) and frozen. Twenty-five microliters of thawed samples were added to an equal volume of LDH Detection Reagent and luminescence was recorded after 60 minutes incubation at room temperature. **Panel A.** Concentration of rituximab versus RLU was plotted and data was fit to 4PL curve to calculate EC_{50}. **Panel B.** RLU values from control wells (performed on the same plate as the rituximab treatment) indicate that there is minimal luminescent signal from target or effector cells alone, and that addition of rituximab is required for cell killing. ADCC = Effector cells + Target cells + RTX. RLU = Relative Luminescence Units.

6.C. Assessing Cell Number and Cell Proliferation

Lactate dehydrogenase is an abundant cytosolic protein present in the cytoplasm of many cell types. LDH activity directly correlates with the number of cells in a well and can be used to quantitate changes in cell number during proliferation, or as a normalization factor for determining the total number of cells in a well. The number of cells present will be directly proportional to the luminescence values, which represent LDH activity. In addition, by measuring the amount of LDH released into the medium and comparing to the changes in total LDH, drugs affecting cell proliferation can be identified and characterized. An example is provided in Figure 10. Upon treating cells with metformin, no significant release of LDH into the medium was detected (blue bars). The total amount of LDH increased with time in control samples, but not metformin-treated samples. These data indicate that metformin inhibits cell proliferation but does not lyse the cells.

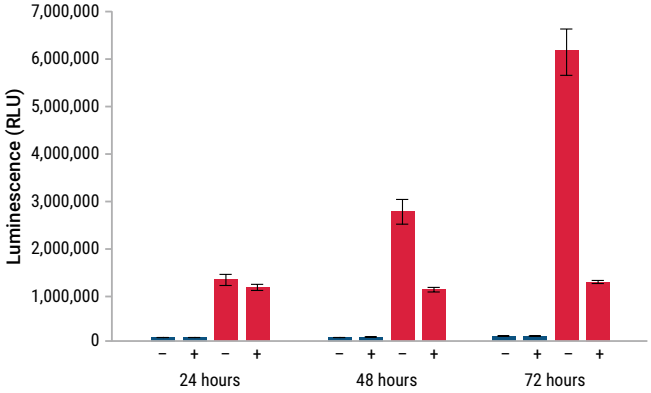


Figure 10. Detecting cell proliferation. Two thousand five hundred (2,500) A549 cells were plated in a 96-well plate and incubated in the absence (–) or presence (+) of 10mM metformin for 24, 48 or 72 hours. To measure LDH release (blue bars) into medium from cells at each time point, 5µl samples were removed, diluted into LDH Storage Buffer (95µl) and frozen. To measure total LDH release (red bars), samples were incubated for 10 minutes with Triton® X-100, collected and frozen the same way. All samples were thawed, further diluted 1:5 in LDH Storage Buffer, an equal volume (50µl) of LDH Detection Reagent was added and luminescence was recorded after 60 minutes incubation at room temperature. RLU = Relative Luminescence Units.

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6.D. Reference

1. Tada, M. *et al.* (2014) Development of a cell-based assay measuring the activation of FcRIIa for the characterization of therapeutic monoclonal antibodies. *PLoS ONE* **94**, e95787.

6.E. Related Products

Viability Assays

Product	Size	Cat.#
RealTime-Glo™ MT Cell Viability Assay	100 reactions	G9711
CellTiter-Glo® Luminescent Cell Viability Assay	10ml	G7570
CellTiter-Glo® 2.0 Assay (luminescent)	10ml	G9241
CellTiter-Glo® 3D Assay	100ml	G9681
CellTiter-Glo® One Solution Assay	100ml	G8461
CellTiter-Fluor™ Cell Viability Assay (fluorescent)	10ml	G6080

Not for Medical Diagnostic Use. Additional kit formats are available.

Cytotoxicity Assays

Product	Size	Cat.#
CellTox™ Green Cytotoxicity Assay	10ml	G8741
CellTox™ Green Express Cytotoxicity Assay	200µl	G8731
CytoTox-Fluor™ Cytotoxicity Assay (fluorescent)	10ml	G9260
CytoTox-Glo™ Cytotoxicity Assay (luminescent)	10ml	G9290
MultiTox-Fluor Multiplex Cytotoxicity Assay (fluorescent; dual assay)	10ml	G9200
MultiTox-Glo Multiplex Cytotoxicity Assay (luminescent and fluorescent; dual assay)	10ml	G9270

Not for Medical Diagnostic Use. Additional kit formats are available.

Apoptosis Products

Product	Size	Cat.#
RealTime-Glo™ Annexin V Apoptosis and Necrosis Assay	100 assays	JA1011
Caspase-Glo® 3/7 Assay	2.5ml	G8090
Caspase-Glo® 3/7 3D Assay	10ml	G8981
Caspase-Glo® 8 Assay	2.5ml	G8200
Caspase-Glo® 9 Assay	2.5ml	G8210
Apo-ONE® Homogeneous Caspase-3/7 Assay	1ml	G7792

Not for Medical Diagnostic Use. Additional kit formats are available.

Oxidative Stress and Metabolism Assays

Product	Size	Cat.#
Cholesterol/Cholesterol Ester-Glo™ Assay	5ml	J3190
Glycerol-Glo™ Assay	5ml	J3150
Glucose Uptake-Glo™ Assay	5ml	J1341
Glucose-Glo™ Assay	5ml	J6021
Glutamate-Glo™ Assay	5ml	J7021
Glutamine/Glutamate-Glo™ Assay	5ml	J8021
GSH-Glo™ Glutathione Assay	10ml	V6911
GSH/GSSG-Glo™ Assay	10ml	V6611
Lactate-Glo™ Assay	5ml	J5021
Mitochondrial ToxGlo™ Assay	10ml	G8000
NAD/NADH-Glo™ Assay	10ml	G9071
NADP/NADPH-Glo™ Assay	10ml	G9081
ROS-Glo™ H_2O_2 Assay	10ml	G8820
Triglyceride-Glo™ Assay	5ml	J3160

Not for Medical Diagnostic Use. Additional kit formats are available.

Luminometers

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

7. Summary of Changes

The following changes were made to the 7/23 revision of this document:

1. Updated patent statements and Section 6.E.
2. Changed font and cover image.
3. Made minor text edits.

Promega

[a]Patents Pending.

[b]U.S. Pat. Nos. 9,273,343 and 9,951,372, European Pat. No. 2751089, Japanese Pat. No. 6067019 and other patents pending.

Products may be covered by pending or issued patents or may have certain limitations. Please visit our Web site for more information.

All prices and specifications are subject to change without prior notice.

Product claims are subject to change. Please contact Promega Technical Services or access the Promega online catalog for the most up-to-date information on Promega products