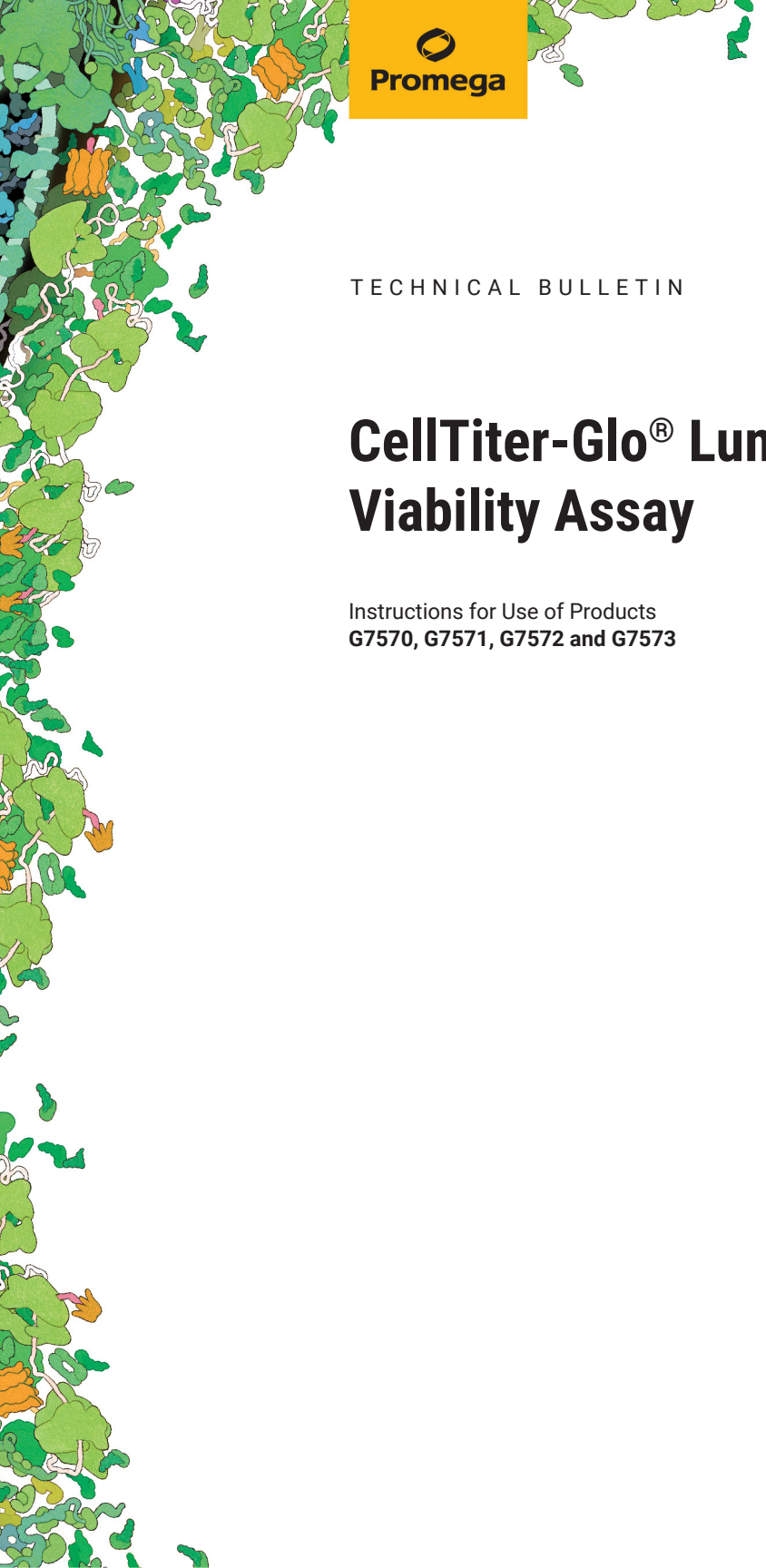

TECHNICAL BULLETIN

CellTiter-Glo® Luminescent Cell Viability Assay

Instructions for Use of Products
G7570, G7571, G7572 and G7573

Revised 1/23
TB288

CellTiter-Glo® Luminescent Cell Viability 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 Bulletin.
E-mail Promega Technical Services if you have questions on use of this system: techserv@promega.com

1. Description

The CellTiter-Glo® Luminescent Cell Viability Assay[a] is a homogeneous method to determine the number of viable cells in culture based on quantitation of the ATP present, which signals the presence of metabolically active cells. The CellTiter-Glo® Assay is designed for use with multiwell plate formats, making it ideal for automated high-throughput screening (HTS) and cell proliferation and cytotoxicity assays. The homogeneous assay procedure (Figure 1) involves adding a single reagent (CellTiter-Glo® Reagent) directly to cells cultured in serum-supplemented medium. Cell washing, removal of medium or multiple pipetting steps are not required.

The homogeneous "add-mix-measure" format results in cell lysis and generation of a luminescent signal proportional to the amount of ATP present (Figure 2). The amount of ATP is directly proportional to the number of cells present in culture in agreement with previous reports (1). The CellTiter-Glo® Assay relies on the properties of a proprietary thermostable luciferase (Ultra-Glo™ Recombinant Luciferase), which generates a stable "glow-type" luminescent signal and improves performance across a wide range of assay conditions. The luciferase reaction for this assay is shown in Figure 3. The half-life of the luminescent signal resulting from this reaction is greater than five hours (Figure 4). This extended half-life eliminates the need for reagent injectors and provides flexibility for continuous or batch-mode processing of multiple plates. The unique homogeneous format reduces pipetting errors that may be introduced during the multiple steps required by other ATP-measurement methods.

1. Description (continued)

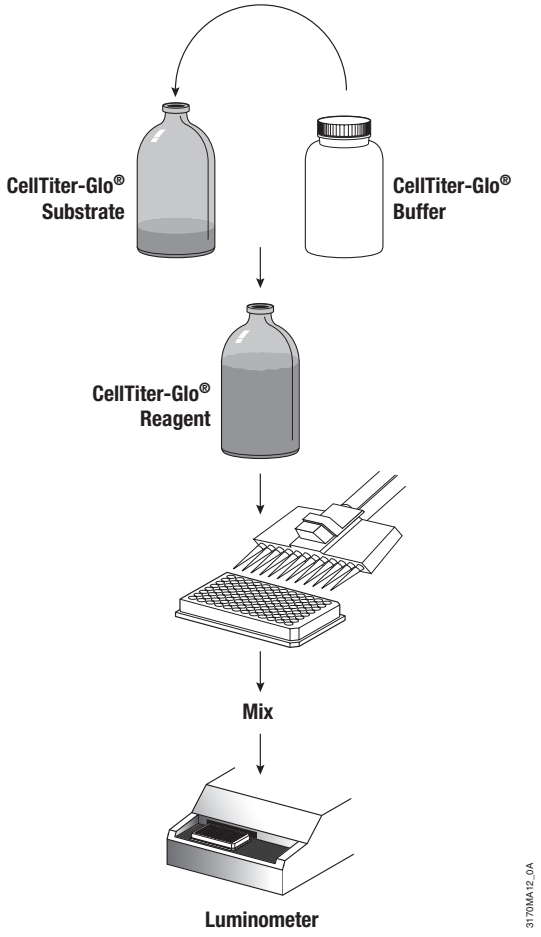

Figure 1. Flow diagram showing preparation and use of CellTiter-Glo® Reagent.

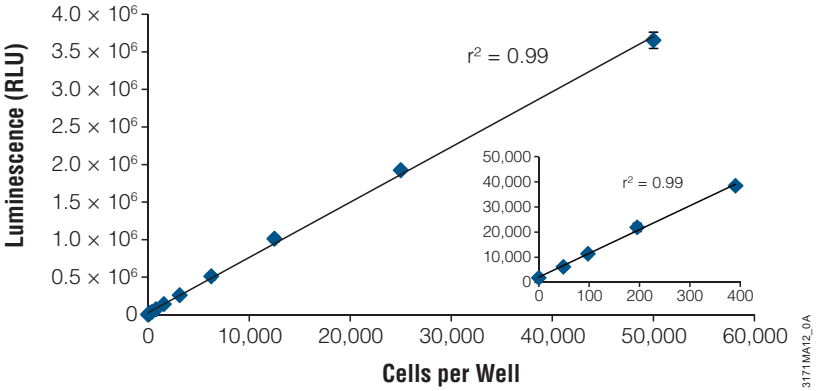

Figure 2. Cell number correlates with luminescent output. A direct relationship exists between luminescence measured with the CellTiter-Glo® Assay and the number of cells in culture over three orders of magnitude. Serial twofold dilutions of HEK293 cells were made in a 96-well plate in DMEM with 10% FBS, and assays were performed as described in Section 3.B. Luminescence was recorded 10 minutes after reagent addition using a GloMax®-Multi+ Detection System. Values represent the mean ± S.D. of four replicates for each cell number. The luminescent signal from 50 HEK293 cells is greater than three times the background signal from serum-supplemented medium without cells. There is a linear relationship (r^2 = 0.99) between the luminescent signal and the number of cells from 0 to 50,000 cells per well.

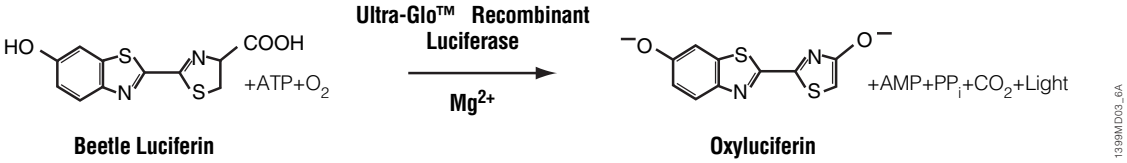

Figure 3. The luciferase reaction. Mono-oxygenation of luciferin is catalyzed by luciferase in the presence of Mg^{2+}, ATP and molecular oxygen.

1. Description (continued)

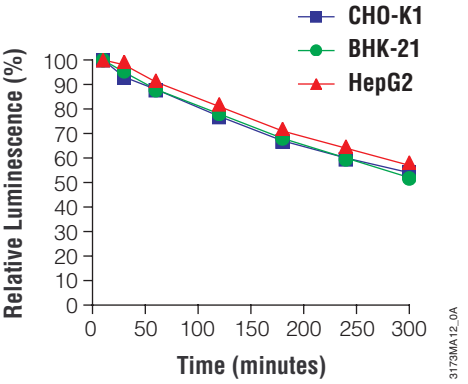

Figure 4. Extended luminescent half-life allows high-throughput batch processing. Signal stability is shown for three common cell lines. HepG2 and BHK-21 cells were grown and assayed in MEM containing 10% FBS, while CHO-K1 cells were grown and assayed in DME/F-12 containing 10% FBS. CHO-K1, BHK-21 and HepG2 cells, at 25,000 cells per well, were added to a 96-well plate. After an equal volume of CellTiter-Glo® Reagent was added, plates were shaken and luminescence monitored over time with the plates held at 22°C. The half-life of luminescent signals for CHO-K1, BHK-21 and HepG2 cells was approximately 5.4, 5.2 and 5.8 hours, respectively.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
CellTiter-Glo® Luminescent Cell Viability Assay	**10ml**	**G7570**

Substrate is sufficient for 100 assays at 100µl/assay in 96-well plates or 400 assays at 25µl/assay in 384-well plates. Includes:

- 1 × 10ml CellTiter-Glo® Buffer
- 1 vial CellTiter-Glo® Substrate (lyophilized)

PRODUCT	SIZE	CAT.#
CellTiter-Glo® Luminescent Cell Viability Assay	**10 × 10ml**	**G7571**

Each vial of substrate is sufficient for 100 assays at 100µl/assay in 96-well plates or 400 assays at 25µl/assay in 384-well plates (1,000 to 4,000 total assays). Includes:

- 10 × 10ml CellTiter-Glo® Buffer
- 10 vials CellTiter-Glo® Substrate (lyophilized)

PRODUCT	SIZE	CAT.#
CellTiter-Glo® Luminescent Cell Viability Assay	**100ml**	**G7572**

Substrate is sufficient for 1,000 assays at 100µl/assay in 96-well plates or 4,000 assays at 25µl/assay in 384-well plates. Includes:

- 1 × 100ml CellTiter-Glo® Buffer
- 1 vial CellTiter-Glo® Substrate (lyophilized)

PRODUCT	SIZE	CAT.#
CellTiter-Glo® Luminescent Cell Viability Assay	**10 × 100ml**	**G7573**

Each vial of substrate is sufficient for 1,000 assays at 100µl/assay in 96-well plates or 4,000 assays at 25µl/assay in 384-well plates (10,000 to 40,000 total assays). Includes:

- 10 × 100ml CellTiter-Glo® Buffer
- 10 vials CellTiter-Glo® Substrate (lyophilized)

Storage Conditions: For long-term storage, store the lyophilized CellTiter-Glo® Substrate and CellTiter-Glo® Buffer at –30°C to –10°C. For frequent use, the CellTiter-Glo® Buffer can be stored at +4°C to +10°C or room temperature for 48 hours without loss of activity. See product label for expiration date information. Reconstituted CellTiter-Glo® Reagent (Buffer plus Substrate) can be stored at room temperature for up to 8 hours with <10% loss of activity, at 4°C for 48 hours with ~5% loss of activity, at 4°C for 4 days with ~20% loss of activity or at –20°C for 21 weeks with ~3% loss of activity. The reagent is stable for up to ten freeze-thaw cycles, with less than 10% loss of activity.

3. Performing the CellTiter-Glo® Assay

Materials to Be Supplied by the User

- opaque-walled multiwell plates adequate for cell culture
- multichannel pipette or automated pipetting station for reagent delivery
- device (plate shaker) for mixing multiwell plates
- luminometer, CCD camera or imaging device capable of reading luminescence in multiwell plates
- **optional:** ATP for use in generating a standard curve (Section 3.C)

3.A. Reagent Preparation

1. Thaw the CellTiter-Glo® Buffer, and equilibrate to room temperature prior to use. For convenience CellTiter-Glo® Buffer may be thawed and stored at room temperature for up to 48 hours prior to use.
2. Equilibrate the lyophilized CellTiter-Glo® Substrate to room temperature prior to use.
3. Transfer the appropriate volume (10ml for Cat.# G7570 and G7571, or 100ml for Cat.# G7572 and G7573) of CellTiter-Glo® Buffer into the amber bottle containing CellTiter-Glo® Substrate to reconstitute the lyophilized enzyme/substrate mixture. This forms CellTiter-Glo® Reagent.
4. Mix by gently vortexing, swirling or inverting the contents to obtain a homogeneous solution. The CellTiter-Glo® Substrate should go into solution easily in less than 1 minute.

3.B. Protocol for the Cell Viability Assay

We recommend that you perform a titration of your particular cells to determine the optimal number and ensure that you are working within the linear range of the CellTiter-Glo® Assay. Figure 2 provides an example of such a titration of HEK293 cells using 0 to 50,000 cells per well in a 96-well format.

1. Prepare opaque-walled multiwell plates with mammalian cells in culture medium, 100µl per well for 96-well plates or 25µl per well for 384-well plates.

   Multiwell plates must be compatible with the luminometer used.

2. Prepare control wells containing medium without cells to obtain a value for background luminescence.
3. Add the test compound to experimental wells, and incubate according to culture protocol.
4. Equilibrate the plate and its contents at room temperature for approximately 30 minutes.
5. Add a volume of CellTiter-Glo® Reagent equal to the volume of cell culture medium present in each well (e.g., add 100µl of reagent to 100µl of medium containing cells for a 96-well plate, or add 25µl of reagent to 25µl of medium containing cells for a 384-well plate).
6. Mix contents for 2 minutes on an orbital shaker to induce cell lysis.
7. Allow the plate to incubate at room temperature for 10 minutes to stabilize luminescent signal.

   Note: Uneven luminescent signal within standard plates can be caused by temperature gradients, uneven seeding of cells or edge effects in multiwell plates.

8. Record luminescence.

   Note: Instrument settings depend on the manufacturer. An integration time of 0.25–1 second per well should serve as a guideline.

3.C. Protocol for Generating an ATP Standard Curve (optional)

It is a good practice to generate a standard curve using the same plate on which samples are assayed. We recommend ATP disodium salt (Cat.# P1132, Sigma Cat.# A7699 or GE Healthcare Cat.# 27-1006). The ATP standard curve should be generated immediately prior to adding the CellTiter-Glo® Reagent because endogenous ATPase enzymes found in sera may reduce ATP levels.

1. Prepare 1µM ATP in culture medium (100µl of 1µM ATP solution contains 10^{-10} moles ATP).
2. Prepare serial tenfold dilutions of ATP in culture medium (1µM to 10nM; 100µl contains 10^{-10} to 10^{-12} moles of ATP).
3. Prepare a multiwell plate with varying concentrations of ATP standard in 100µl medium (25µl for a 384-well plate).
4. Add a volume of CellTiter-Glo® Reagent equal to the volume of ATP standard present in each well.
5. Mix contents for 2 minutes on an orbital shaker.
6. Allow the plate to incubate at room temperature for 10 minutes to stabilize the luminescent signal.
7. Record luminescence.

4. Appendix

4.A. Overview of the CellTiter-Glo® Assay

The assay system uses the properties of a proprietary thermostable luciferase to enable reaction conditions that generate a stable "glow-type" luminescent signal while simultaneously inhibiting endogenous enzymes released during cell lysis (e.g., ATPases). Release of ATPases will interfere with accurate ATP measurement. Historically, firefly luciferase purified from *Photinus pyralis* (LucPpy) has been used in reagents for ATP assays (1,2–5). However, it has only moderate stability in vitro and is sensitive to its chemical environment, including factors such as pH and detergents, limiting its usefulness for developing a robust homogeneous ATP assay. We have successfully developed a stable form of luciferase based on the gene from another firefly, *Photuris pennsylvanica* (LucPpe2), using an approach to select characteristics that improve performance in ATP assays. The unique characteristics of this mutant (LucPpe2^{m}) enabled design of a homogeneous single-reagent-addition approach to perform ATP assays with cultured cells. Properties of the CellTiter-Glo® Reagent overcome the problems caused by factors, such as ATPases, that interfere with ATP measurement in cell extracts. The reagent is physically robust and provides a sensitive and stable luminescent output.

4.A. Overview of the CellTiter-Glo® Assay (continued)

Sensitivity and Linearity: The ATP-based detection of cells is more sensitive than other methods (6–8). In experiments performed by Promega scientists, the luminescent signal from 50 HEK293 cells is greater than three standard deviations above the background signal from serum-supplemented medium without cells. There is a linear relationship (r^2 = 0.99) between the luminescent signal and the number of cells from 0 to 50,000 cells per well in the 96-well format. The luminescence values in Figure 2 were recorded after 10 minutes of incubation at room temperature to stabilize the luminescent signal as described in Section 3.B. Incubation of the same 96-well plate used in the experiment shown in Figure 2 for 360 minutes at room temperature had little effect on the relationship between luminescent signal and number of cells (r^2 = 0.99).

Speed: The homogeneous procedure to measure ATP using the CellTiter-Glo® Assay is quicker than other ATP assay methods that require multiple steps to extract ATP and measure luminescence. The CellTiter-Glo® Assay also is faster than other commonly used methods to measure the number of viable cells (such as MTT, alamarBlue® or Calcein-AM) that require prolonged incubation steps to enable the cells' metabolic machinery to convert indicator molecules into a detectable signal.

4.B. Additional Considerations

Temperature: The intensity and decay rate of the luminescent signal from the CellTiter-Glo® Assay depends on the luciferase reaction rate. Environmental factors that affect the luciferase reaction rate will change the intensity and stability of the luminescent signal. Temperature is one factor that affects the rate of this enzymatic assay and thus the light output. For consistent results, equilibrate assay plates to a constant temperature before performing the assay. Transferring eukaryotic cells from 37°C to room temperature has little effect on ATP content (3). We have demonstrated that removing cultured cells from a 37°C incubator and allowing them to equilibrate to 22°C for 1–2 hours had little effect on ATP content. For batch-mode processing of multiple assay plates, take precautions to ensure complete temperature equilibration. Plates removed from a 37°C incubator and placed in tall stacks at room temperature will require longer equilibration than plates arranged in a single layer. Insufficient equilibration may result in a temperature gradient effect between wells in the center and at the edge of the plates. The temperature gradient pattern also may depend on the position of the plate in the stack.

Chemicals: The chemical environment of the luciferase reaction affects the enzymatic rate and thus luminescence intensity. Differences in luminescence intensity have been observed using different types of culture media and sera. The presence of phenol red in culture medium should have little impact on luminescence output. Assaying 0.1µM ATP in RPMI medium without phenol red resulted in ~5% increase in luminescence output (in relative light units [RLU]) compared to assays in RPMI containing the standard concentration of phenol red, whereas assays in RPMI medium containing twice the normal concentration of phenol red showed a ~2% decrease in luminescence.

Solvents for the various test compounds may interfere with the luciferase reaction and thus the light output from the assay. Interference with the luciferase reaction can be detected by assaying a parallel set of control wells containing medium without cells. Dimethylsulfoxide (DMSO), commonly used as a vehicle to solubilize organic chemicals, has been tested at final concentrations of up to 2% in the assay and only minimally affects light output.

Plate Recommendations: We recommend using standard opaque-walled multiwell plates suitable for luminescence measurements. Opaque-walled plates with clear bottoms to allow microscopic visualization of cells also may be used; however, these plates will have diminished signal intensity and greater cross talk between wells. Opaque white tape may be used to decrease luminescence loss and cross talk.

Cellular ATP Content: Different cell types have different amounts of ATP, and values reported for the ATP level in cells vary considerably (1,2,9–11). Factors that affect the ATP content of cells may affect the relationship between cell number and luminescence. Anchorage-dependent cells that undergo contact inhibition at high densities may show a change in ATP content per cell at high densities, resulting in a nonlinear relationship between cell number and luminescence. Factors that affect the cytoplasmic volume or physiology of cells also will affect ATP content. For example, oxygen depletion is one factor known to cause a rapid decrease in ATP (1).

Mixing: Optimal assay performance is achieved when the CellTiter-Glo® Reagent is mixed completely with the cultured cells. Suspension cell lines (e.g., Jurkat cells) generally require less mixing to achieve lysis and extract ATP than adherent cells (e.g., L929 cells). Tests were done to evaluate the effect of shaking the plate after adding the CellTiter-Glo® Reagent. Suspension cells cultured in multiwell plates showed only minor differences in light output whether or not the plates were shaken after adding the CellTiter-Glo® Reagent. Adherent cells are more difficult to lyse and show a substantial difference between shaken and nonshaken plates.

Several additional parameters related to reagent mixing include the force of delivery of CellTiter-Glo® Reagent, sample volume and dimensions of the well. All of these factors may affect assay performance. The degree of reagent mixing required may be affected by the method used to add the CellTiter-Glo® Reagent to the assay plates. Automated pipetting devices using a greater or lesser force of fluid delivery may affect the degree of subsequent mixing required. Complete reagent mixing in 96-well plates should be achieved using orbital plate shaking devices built into many luminometers and the recommended 2-minute shaking time. Special electromagnetic shaking devices that use a radius smaller than the well diameter may be required to efficiently mix contents of 384-well plates. The depth of medium and geometry of the multiwell plates may have an effect on mixing efficiency. We recommend that you take these factors into consideration when performing the assay and empirically determine whether a mixing step is necessary for the individual application.

Luminometers

For highly sensitive luminometric assays, the luminometer model and settings greatly affect the quality of data obtained. Luminometers from different manufacturers will vary in sensitivities and dynamic ranges. We recommend the GloMax® products because these instruments do not require gain adjustments to achieve optimal sensitivity and dynamic range. Additionally, GloMax® instruments are preloaded with Promega protocols for ease of use.

If you are not using a GloMax® luminometer, consult the operating manual for your luminometer to determine the optimal settings. The limits should be verified on each instrument before analysis of experimental samples. The assay should be linear in some portion of the detection range of the instrument used. For an individual luminometer there may be different gain settings. We recommend that you optimize the gain settings.

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4.C. References

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5. Gerhardt, R.T. *et al.* (1991) Characterization of in vitro chemosensitivity of perioperative human ovarian malignancies by adenosine triphosphate chemosensitivity assay. *Am. J. Obstet. Gynecol.* **165**, 245–55.
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8. Maehara, Y. *et al.* (1987) The ATP assay is more sensitive than the succinate dehydrogenase inhibition test for predicting cell viability. *Eur. J. Cancer Clin. Oncol.* **23**, 273–6.
9. Stanley, P.E. (1986) Extraction of adenosine triphosphate from microbial and somatic cells. *Methods Enzymol.* **133**, 14–22.
10. Beckers, B. *et al.* (1986) Application of intracellular ATP determination in lymphocytes for HLA-typing. *J. Biolumin. Chemilumin.* **1**, 47–51.
11. Andreotti, P.E. *et al.* (1995) Chemosensitivity testing of human tumors using a microplate adenosine triphosphate luminescence assay: Clinical correlation for cisplatin resistance of ovarian carcinoma. *Cancer Res.* **55**, 5276–82.

4.D. Related Products

Viability Assays

Product	Size	Cat.#
RealTime-Glo™ MT Cell Viability Assay	100 reactions	G9711
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.#
LDH-Glo™ Cytotoxicity Assay	10ml	J2380
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.

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4.D. Related Products (continued)

Oxidative Stress and Metabolism Assays

Product	Size	Cat.#
Cholesterol/Cholesterol Ester-Glo	5ml	J3190
Glycerol-Glo	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	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

5. Summary of Changes

The following changes were made to the 1/23 revision of this document:

1. Legal disclaimers and Related Products were updated.
2. Advantages section and related references removed. Advantages are available at: **www.promega.com**
3. Cover image and font were updated.

[a]U.S. Pat. Nos. 7,741,067, 8,361,739 and 8,603,767 and other patents and patents pending.