TECHNICAL BULLETIN

CellTiter-Glo® One Solution Assay

Instructions for Use of Products
G8461 and G8462

Revised 2/16
TB370

Promega

CellTiter-Glo® One Solution Assay

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1. Description

The CellTiter-Glo® One Solution Assay[a–c] provides a homogeneous method to determine the number of viable cells in culture based on quantitation of ATP present, which indicates the presence of metabolically active cells. This frozen, ready-to-use reagent is based on the original CellTiter-Glo® Luminescent Cell Viability Assay chemistry and eliminates the need to combine buffer with lyophilized substrate when preparing reagent. The CellTiter-Glo® One Solution 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 addition of a single reagent (CellTiter-Glo® One Solution) directly to cells cultured in serum-supplemented medium. Cell washing, removal of medium and multiple pipetting steps are not required.

The "add-mix-measure" format results in cell lysis and generation of a luminescent signal that is proportional to the amount of ATP present (Figure 2). The amount of ATP is directly proportional to the number of cells present in culture (1). The CellTiter-Glo® One Solution 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 resulting luminescent signal is greater than 3 hours. This extended half-life eliminates the need for reagent injectors and provides flexibility for continuous or batch-mode processing of multiple plates.

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1. Description (continued)

The single-reagent-addition format reduces pipeting errors that may be introduced during the multiple steps required by other ATP-measurement methods.

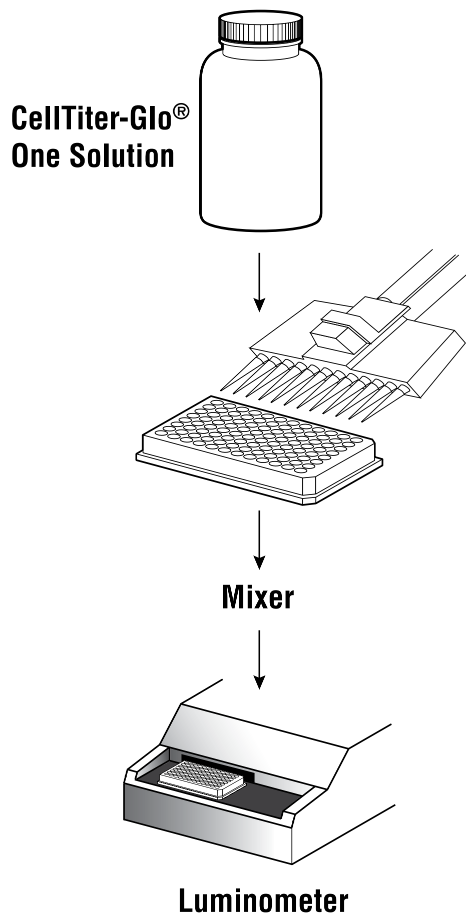

Figure 1. Flow diagram showing preparation and use of the CellTiter-Glo® One Solution.

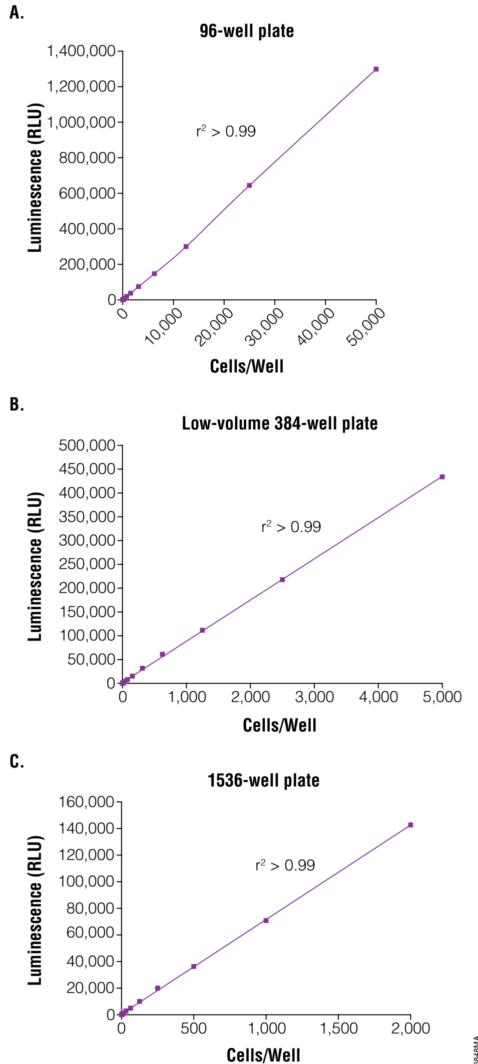

Figure 2. Cell number correlates with luminescent output. A direct relationship exists between luminescence measured with the CellTiter-Glo® One Solution Assay and the number of cells in culture over three orders of magnitude. Serial twofold dilutions of Jurkat cells were made in RPMI 1640 with 10% FBS and plated in three formats using automated dispensing equipment. Volumes plated were 100µl of cells per well in a 96-well plate (**Panel A**), 10µl of cells per well in low-volume 384-well plate (**Panel B**) and 4µl of cells per well in a 1536-well plate (**Panel C**). An equivalent volume of CellTiter-Glo® One Solution was dispensed into each well. Luminescence was recorded 10 minutes after addition of CellTiter-Glo® One Solution. There is a linear relationship ($r^2 > 0.99$) between luminescent signal and cell number in each plate format.

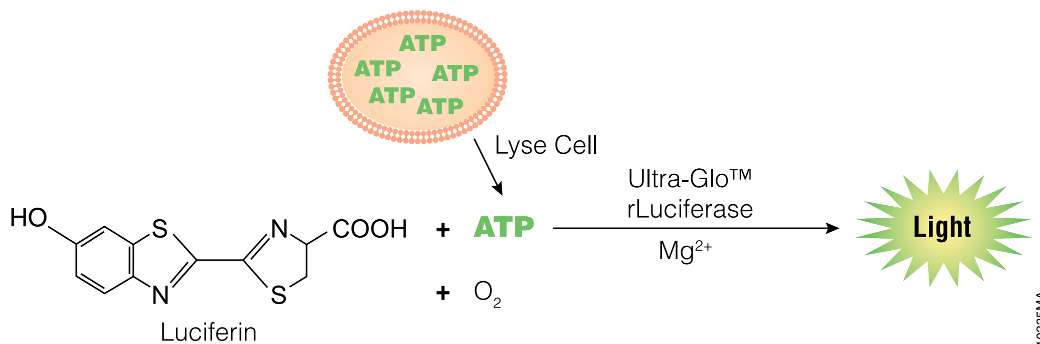

Figure 3. The luciferase reaction. Mono-oxygenation of luciferin is catalyzed by luciferase in the presence of Mg^{2+}, ATP and molecular oxygen.

System Advantages

- **Convenient:** No reagent preparation is required; simply thaw and "add-mix-measure". Volumes are convenient for HTS applications.
- **Homogeneous:** The "add-mix-measure" format reduces the number of plate-handling steps.
- **Fast:** Data can be recorded 10 minutes after adding reagent.
- **Sensitive:** The assay measures cell numbers below the detection limits of standard colorimetric and fluorometric assays.
- **Flexible:** The assay can be used with various multiwell formats (96-well, regular or low-volume 384-well and 1536-well plates). Data can be recorded by luminometer, CCD camera or other imaging device capable of reading luminescence in multiwell plates.
- **Robust:** Luminescent signal is stable, with a half-life >3 hours, depending on cell type and culture medium used.
- **Able to Multiplex:** The assay can be used with other nonlytic assays from Promega (2,3).

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
CellTiter-Glo® One Solution Assay	**100ml**	**G8461**

For in vitro Research Use Only. CellTiter-Glo® One Solution is sufficient for 1,000 assays at 100µl/assay in 96-well plates or 4,000 assays at 25µl/assay in 384-well plates.

PRODUCT	SIZE	CAT.#
CellTiter-Glo® One Solution Assay	**500ml**	**G8462**

For in vitro Research Use Only. CellTiter-Glo® One Solution is sufficient for 5,000 assays at 100µl/assay in 96-well plates or 20,000 assays at 25µl/assay in 384-well plates.

Storage Conditions: Store the CellTiter-Glo® One Solution Assay below –10°C. The CellTiter-Glo® One Solution Assay can be stored at 4°C for 48 hours or at 22°C for 10 hours with ~10–12% loss of activity. CellTiter-Glo® One Solution can withstand two additional freeze-thaw cycles after the first thaw, with approximately 10% loss of activity with each freeze-thaw cycle. We do not recommend dispensing the CellTiter-Glo® One Solution into aliquots due to the risk of ATP contamination.

3. Performing the CellTiter-Glo® One Solution Assay

Materials to Be Supplied by the User

- 22°C water bath
- opaque-walled multiwell plates adequate for cell culture
- multichannel pipette or automated pipetting station
- 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 in Section 3.C (Cat.# P1132, Sigma Cat.# A7699 or GE Healthcare Cat.# 27-1006)

3.A. Reagent Thawing and Preparation

1. Thaw the CellTiter-Glo® One Solution at 4°C overnight.

Do not thaw reagent by transferring the frozen bottle directly to a 37°C water bath because the bottle may break.

2. Equilibrate the thawed CellTiter-Glo® One Solution to room temperature by placing the reagent in a 22°C water bath prior to use.

   Note: In a 22°C water bath, 100ml of the thawed reagent requires approximately 30 minutes to equilibrate and 500ml requires approximately 90 minutes.

3. Mix gently by inverting the contents to obtain a homogeneous solution.

   Note: Use caution when removing the seal of the CellTiter-Glo® One Solution bottle to avoid ATP contamination.

3.B. Protocol for the Cell Viability Assay

Prepare and equilibrate the CellTiter-Glo® One Solution as described in Section 3.A prior to performing the assay.

1. Prepare opaque-walled multiwell plates with mammalian cells in culture medium. Volumes and cell number should be optimized for experimental conditions.

Multiwell plates must be compatible with the luminometer used.

2. Prepare control wells containing medium without cells to determine background luminescence.
3. Add test compound to experimental wells, and incubate according to your culture protocol.
4. Equilibrate plate and its contents at room temperature for approximately 30 minutes.
5. Add a volume of CellTiter-Glo® One Solution equal to the volume of cell culture medium present in each well (e.g., for a 96-well plate, add 100µl of CellTiter-Glo® One Solution to 100µl of medium containing cells. For a 384-well plate, add 25µl of CellTiter-Glo® One Solution to 25µl of medium containing cells).
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.
8. Record luminescence.

Notes:

1. Instrument settings depend on the manufacturer. Use an integration time of 0.25–1 second per well as a guideline.
2. Uneven luminescent signal within plates can be caused by temperature gradients, uneven seeding of cells or edge effects in multiwell plates.

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. The ATP standard curve should be generated immediately prior to adding the CellTiter-Glo® One Solution because endogenous ATPase enzymes found in serum may reduce ATP levels. We recommend ATP disodium salt (Cat.# P1132, Sigma Cat.# A7699 or GE Healthcare Cat.# 27-1006).

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, respectively).
3. Prepare a multiwell plate with varying concentrations of ATP standard in 100µl of medium (25µl for a 384-well plate).
4. Add a volume of CellTiter-Glo® One Solution 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® One Solution Assay

The CellTiter-Glo® One Solution Assay takes advantage of the properties of a proprietary thermostable luciferase, Ultra-Glo™ Recombinant 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 (1,4–7). However, LucPpy 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. Promega has successfully developed a stable form of luciferase based on the gene from another firefly, *Photuris pennsylvanica* (LucPpe2) using an approach to select characteristics that improved performance in ATP assays. The unique characteristics of this mutant (LucPpe2m), Ultra-Glo™ Recombinant Luciferase, enabled design of a homogeneous single-reagent-addition approach for performing ATP assays on cultured cells. Properties of the CellTiter-Glo® One Solution overcome problems caused by factors such as ATPases that interfere with the measurement of ATP in cell extracts. The reagent is physically robust and provides a sensitive and stable luminescent output.

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

Sensitivity and Linearity: The ATP-based detection of cells is more sensitive than other methods (8–10). There is a linear relationship between luminescent signal and cell number in different plate formats. The luminescence values shown 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 samples for 3 hours at room temperature had little effect on the relationship between luminescent signal and cell number.

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

4.B. Additional Considerations

Temperature: The intensity and rate of decay of the luminescent signal from the CellTiter-Glo® One Solution Assay depends on the luciferase reaction rate. Environmental factors that affect the luciferase reaction rate will change the intensity of light output 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 (5). 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 a longer equilibration time than plates arranged in a single layer. Insufficient equilibration may result in a temperature gradient between wells in the center and on 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 effect on luminescence output. Solvents for the test compounds may interfere with the luciferase reaction and thus light output. To test for luciferase inhibition, assemble two reactions, one with equal volumes of reconstituted CellTiter-Glo® One Solution and 1µM ATP, and a second reaction with equal volumes of CellTiter-Glo® One Solution and 1µM ATP plus the test compound. Incubate reactions for 10 minutes at 22–25°C, then measure luminescence. A decrease in luminescence in the presence of test compound indicates luciferase inhibition.

Plate Recommendations: We recommend using standard opaque-walled multiwell plates suitable for luminescence measurements. Opaque-walled plates with clear bottoms, which allow microscopic visualization of cells, also may be used; however, assays in 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 a particular cell type vary considerably (1,4,11–13). 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 cytoplasmic volume or cell physiology also will have an effect on ATP content. For example, oxygen depletion is one factor known to cause a rapid decrease in ATP (1).

Mixing: Optimum assay performance is achieved when the CellTiter-Glo® One Solution is completely mixed with the cultured cells. Suspension cell lines (e.g., Jurkat cells) generally require less mixing to achieve lysis and extraction of ATP than adherent cells (e.g., L929 cells).

Several additional parameters related to reagent mixing include: the force of delivery of CellTiter-Glo® One Solution, 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® One Solution to the assay plates. Automated pipetting devices that use 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 using a radius smaller than the well diameter may be required to efficiently mix the 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 consider these factors when performing the assay and determine whether a mixing step is necessary for your application.

ATP Contamination: Strict aseptic technique is essential to prevent ATP contamination of the CellTiter-Glo® One Solution. Wear gloves and avoid contact with potentially contaminated surfaces and equipment. Clean gloves, lab surfaces and equipment with a 10% bleach solution, then pat dry with lab wipes (e.g., KimWipes® tissues). Use individually wrapped or designated ATP-free pipettes and pipette tips whenever possible, and avoid inserting pipettes or pipette tips into the CellTiter-Glo® One Solution bottle multiple times. Discard any unused, dispensed reagent; do not return it to the original bottle.

4.C. References

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4.D. Related Products

Cell Proliferation Products

Product	Size	Cat.#
CellTiter-Glo® Luminescent Cell Viability Assay (Luminescent)	10ml	G7570
	10 × 10ml	G7571
	100ml	G7572
	10 × 100ml	G7573
CellTiter-Fluor™ Cell Viability Assay (Fluorescent)	10ml	G6080
	5 × 10ml	G6081
	2 × 50ml	G6082
CellTiter-Blue® Cell Viability Assay (resazurin; fluorescent)	20ml	G8080
	100ml	G8081
	10 × 100ml	G8082
CellTiter 96® AQ_{ueous} One Solution Cell Proliferation Assay (MTS; colorimetric)	200 assays	G3582
	1,000 assays	G3580
	5,000 assays	G3581

Cytotoxicity Assays

Product	Size	Cat.#
MultiTox-Fluor Multiplex Cytotoxicity Assay (Fluorescent; dual assay)	10ml	G9200
	5 × 10ml	G9201
	2 × 50ml	G9202
MultiTox-Glo Multiplex Cytotoxicity Assay (Luminescent and fluorescent; dual assay)	10ml	G9270
	5 × 10ml	G9271
	2 × 50ml	G9272
CytoTox-Fluor™ Cytotoxicity Assay (Fluorescent)	10ml	G9260
	5 × 10ml	G9261
	2 × 50ml	G9262
CytoTox-Glo™ Cytotoxicity Assay (Luminescent)	10ml	G9290
	5 × 10ml	G9291
	2 × 50ml	G9292

4.D. Related Products (continued)

Cytotoxicity Assays (continued)

Product	Size	Cat.#
CytoTox-ONE™ Homogeneous Membrane Integrity Assay	200–800 assays	G7890
(LDH, fluorometric)	1,000–4,000 assays	G7891
CytoTox-ONE™ Homogeneous Membrane Integrity Assay, HTP	1,000–4,000 assays	G7892

Apoptosis Products

Product	Size	Cat.#
ApoTox-Glo™ Triplex Assay	10ml	G6320
	5 × 10ml	G6321
ApoLive-Glo™ Multiplex Assay	10ml	G6410
	5 × 10ml	G6411
Caspase-Glo® 2 Assay	10ml	G0940
Caspase-Glo® 3/7 Assay	2.5ml	G8090
	10ml	G8091
	100ml	G8092
	10 × 10ml	G8093
Caspase-Glo® 6 Assay	10ml	G0970
Caspase-Glo® 8 Assay	2.5ml	G8200
	10ml	G8201
	100ml	G8202
Caspase-Glo® 9 Assay	2.5ml	G8210
	10ml	G8211
	100ml	G8212
Apo-ONE® Homogeneous Caspase-3/7 Assay	1ml	G7792
	10ml	G7790
	100ml	G7791
Caspase Inhibitor Z-VAD-FMK, 20mM	50µl	G7231
	125µl	G7232

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Oxidative Stress and Metabolism Assays

Product	Size	Cat.#
GSH-Glo™ Glutathione Assay	10ml	V6911
	50ml	V6912
GSH/GSSG-Glo™ Assay	10ml	V6611
	50ml	V6612
Mitochondrial ToxGlo™ Assay	10ml	G8000
	100ml	G8001

Luminometers

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

5. Summary of Changes

The following changes were made to the 2/16 revision of this document:

1. The patent information was updated to remove expired statements.
2. The document design was updated.
3. Related products were updated.

[a]U.S. Pat. Nos. 6,602,677, 7,241,584, 8,030,017 and 8,822,170, European Pat. No. 1131441, Japanese Pat. Nos. 4537573 and 4520084 and other patents pending.

[b]U.S. Pat. Nos. 7,741,067, 8,361,739 and 8,603,767, Japanese Pat. No. 4485470 and other patents pending.

[c]U.S. Pat. Nos 7,083,911, 7,452,663 and 7,732,128, European Pat. No. 1383914 and Japanese Pat. Nos. 4125600 and 4275715.

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