CellTiter-Glo™ Luminescent Cell Viability Assay: A Sensitive and Rapid Method for Determining Cell Viability

by Rita Hannah, Ph.D., Michael Beck, B.S., Richard Moravec, B.S., and Terry Riss, Ph.D.
Promega Corporation

Promega's new CellTiter-Glo™ Luminescent Cell Viability Assay is a highly sensitive method for assaying cell proliferation and cytotoxicity. The assay uses the luciferase reaction to measure ATP, a global indicator of cellular metabolism. While providing a powerful tool for the life science researcher, the "add, mix, measure" format of the assay also satisfies the need for a simple and rapid assay for high-throughput screening.

Introduction

The CellTiter-Glo™ Luminescent Cell Viability Assay[a] uses ATP, a required co-factor of the luciferase reaction, as an indicator of metabolically active cells (1–3). The enzyme luciferase acts on luciferin in the presence of Mg^{2+} and ATP to produce oxyluciferin and to release energy in the form of luminescence (4). Since the luciferase reaction requires ATP, the luminescence produced is proportional to the amount of ATP present, an indicator of cellular metabolic activity. The assay has several advantages over other methods for determining cell viability.

Simply add, mix and measure

The assay is simple to use and requires only a single addition of reagent directly to cells in culture medium. The procedure does not require additional handling steps, such as removal of medium or washing of cells, making CellTiter-Glo™ Luminescent Cell Viability Assay easy and convenient to use.

To perform the assay, prepare CellTiter-Glo™ Reagent by reconstituting the lyophilized CellTiter-Glo™ Substrate in CellTiter-Glo™ Buffer. Add the Reagent in a volume equivalent to the amount of medium in which the cells are plated. Mix for 2 minutes, and after a 10-minute incubation, detect the emitted luminescence using a plate reading luminometer. Alternatively, a CCD camera may be used to record the luminescence.

One feature of this assay is that the reaction occurs at pH 6.0. Upon addition of the Reagent to medium containing phenol red, the reaction mixture changes in color from pink to yellow. This is advantageous when pipetting into a multiwell plate, since observation of a color change indicates addition of CellTiter-Glo™ Reagent.

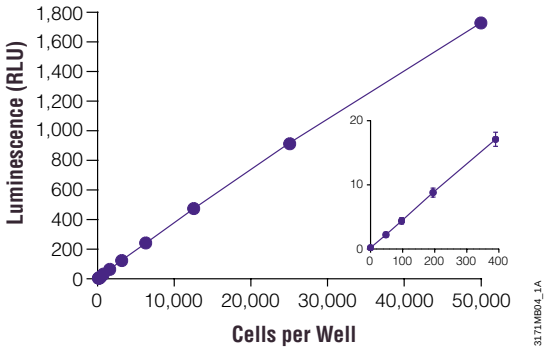

Figure 1. Cell number correlates with luminescent output. We made serial two-fold dilutions of Jurkat cells in a 96 well plate in RPMI 1640 + 10% FBS according to the protocol in Technical Bulletin #TB288. Luminescence was recorded at 10 minutes using a Dynex MLX Microtiter® plate luminometer. Values of the insert graph represent the mean ± S.D. of 4 replicates for each cell number. 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 assays performed in a 96 well format.

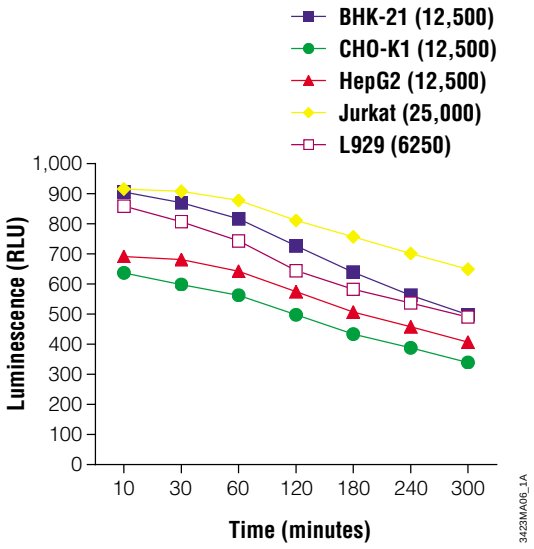

Figure 2. Extended luminescent half-life allows for high-throughput batch processing. The stability of the signal is shown for five different cell lines. We cultured and assayed HepG2 and BHK-21 in MEM containing 10% FBS. CHO-K1 cells were grown and assayed in DME/F-12 containing 10% FBS. Jurkat cells were grown in RPMI 1640 + 10% FBS, and L929 cells were cultured in DME/F-12 + 10% HS. The number of cells per well for each cell line is indicated in the figure legend. We added a volume of CellTiter-Glo™ Reagent equivalent to the volume of cell suspension in each well and monitored luminescence over time at 22°C. (HS, horse serum; FBS, fetal bovine serum).

Sensitivity and linearity up to 3 logs

The CellTiter-Glo™ Luminescent Cell Viability Assay is sensitive and linear up to 3 logarithms (Figure 1). The signal equivalent to 50 Jurkat cells is greater than two standard deviations above the background signal from serum-supplemented medium without cells. The r^2 value of 0.99 demonstrates the linear nature of the assay between 0 and 50,000 cells. For some cell lines, detection of as few as 4 cells is possible (5). This extreme sensitivity extends the linear range of the assay to 4 logs for some cell lines. The linearity of the assay is similar for both adherent and suspension cell lines tested at Promega.

The sensitivity and reproducibility of the CellTiter-Glo™ Assay are reflected in its excellent Z-factor value. Z-factor is a dimensionless measure of high-throughput assay quality that is based on the dynamic range of the assay and the data variability. Assays with Z-factors approaching 1.0 are considered excellent (6). The CellTiter-Glo™ Luminescent Cell Viability Assay has a Z-factor value of 0.96.

Long half-life: Generally greater than 5 hours

The CellTiter-Glo™ Luminescent Cell Viability Assay generates a "glow-type" luminescent signal that has a half-life generally of 5 hours depending on cell type, medium and serum used (Figure 2). Historically, reagents for ATP assays used firefly luciferase purified from *Photinus pyralis* (7). However, *P. pyralis* luciferase has only moderate stability in vitro and is sensitive to environmental factors such as pH and detergents. These characteristics limit the potential for developing a robust, homogeneous ATP assay. However, by selecting for characteristics that improve performance in various environmental conditions, Promega has developed a more stable form of firefly luciferase derived from *Photuris pennsylvanica.* This modified luciferase with its improved stability is the basis for robustness of the CellTiter-Glo™ Assay.

Protection from endogenous ATPases

During cell death, levels of ATP drop rapidly as metabolism shuts down and ATP is degraded by endogenous ATPases. The CellTiter-Glo™ Reagent contains inhibitors that block the activity of these ATPases upon cell lysis. Figure 3 illustrates the protection from ATPases conferred by the presence of inhibitors in CellTiter-Glo™ Buffer.

Minimal effect of phenol red and other additives on assay performance

Phenol red is used as a pH indicator in many culture media, but its presence adversely affects some luciferase assays. To assess the effect of phenol red on the CellTiter-Glo™ Assay, we spiked a sample of RPMI 1640 (without phenol red) + 10% FBS with 1µM ATP and added varying concentrations of phenol red [1X or normal working concentration (5.3g/l), 0.5X, and 2X]. The phenol red had minimal effect on the assay, with less than 10% loss in activity and no effect on half-life (data not shown).

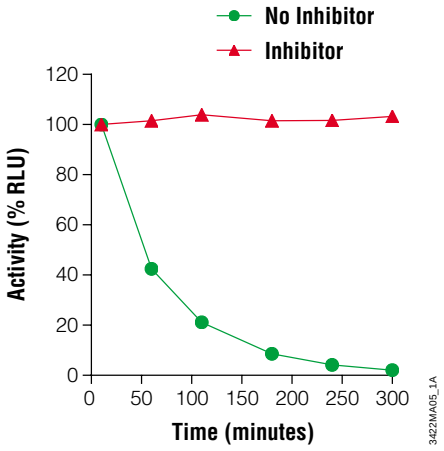

Figure 3. Inhibition of ATPase activity by the CellTiter™-Glo Reagent. We used cell lysate obtained through 3–4 freeze-thaws of 1.5×10^5 L929 cells/ml of DME/F-12 (1:1) contianing 10% horse serum. We prepared two pools of cell lysate that were incubated at 22°C. To one pool, we added an equal volume of 50mM HEPES (pH 7.5; no inhibitor), and to the other we added an equal volume of CellTiter-Glo™ Buffer (inhibitor). We removed 100µl aliquots at ten 60-minute time intervals and added 20µl 5X CellTiter-Glo™ Substrate (luciferin/luciferase mixture in buffer), mixed, and read luminescent output. Quadruplicate samples were taken at each time point.

Table 1. Effect of Solvent on Luciferase Reaction: Percent RLU Compared to DPBS-Only Control.

	Concentration				
Solvent	0.5%	1.0%	2.0%	4.0%	5.0%
DMSO	110	114	119	124	N.D.
DMF	104	98	106	96	N.D.
Acetonitrile	99	102	97	ND	N.D.
Methylpyridone	110	89	112	77	N.D.
Ethanol	N.D.	100	N.D.	N.D.	100

N.D., no data available.

For these experiments, we prepared a 96 well plate containing 5×10^4 cells/well in 50µl RPMI + 10%FBS. To this, we added 50µl of medium containing a 4X concentration of the solvent tested in each assay. We added 100µl CellTiter-Glo™ Reagent, mixed and read luminescence.

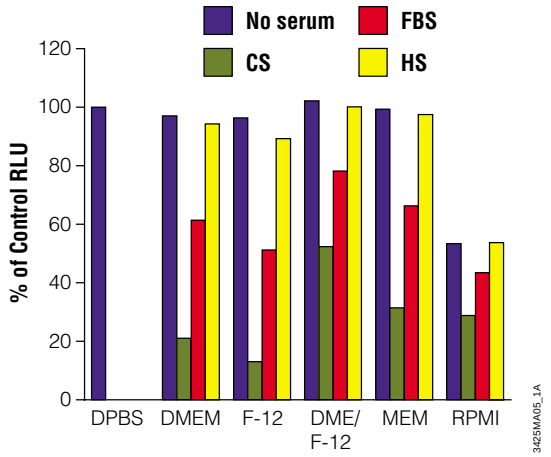

Figure 4. Effect of serum addition to media on luciferase reaction. We prepared a 96 well plate with 50µl RPMI 1640 + 2µM ATP. To each medium tested, we added an additional 50µl of the medium containing 20% of the different sera and mixed. We next added 100µl of CellTiter-Glo™ Reagent, mixed and recorded luminescence. Each experiment was performed in quadruplicate. Experimental wells were compared to a well containing 1µM ATP in 100µl DPBS. (CS, calf serum; HS, horse serum; FBS, fetal bovine serum).

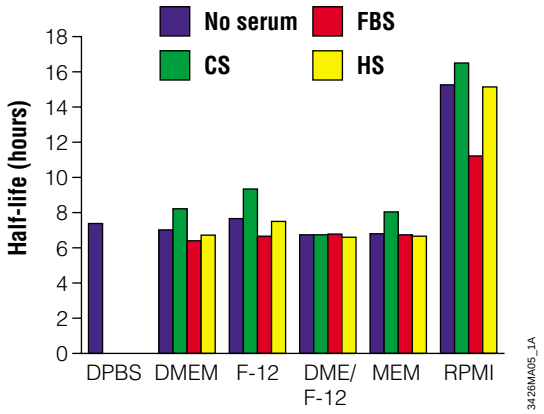

Figure 5. Effect of serum addition to media on reaction half-life. We prepared plates as described for Figure 4 and collected data over 60-minute intervals for five hours. From the data obtained, we calculated reaction half-life. (CS, calf serum; HS, horse serum; FBS, fetal bovine serum).

Solvents used to dissolve treatment agents can potentially affect the outcome of luciferase assays. Table 1 contains data that describe the effect of a variety of solvents on the CellTiter-Glo™ Assay. Most solvents have little or no effect on assay results.

Chemicals and serum supplements in culture media can also affect luciferase assays. Of the media tested with the CellTiter-Glo™ Assay, adverse effects on the assay performance have been observed with some lots of RPMI 1640. Tests of other media result in assay activity similar to that observed for control samples prepared in DPBS. The three sera tested affected the luciferase reaction differently. Calf serum showed the most inhibition and horse serum the least (Figure 4). However, in our experience, adding sera to most media does not change the half-life of the reaction, with the exception of RPMI (Figure 5).

Conclusion

The CellTiter-Glo™ Luminescent Cell Viability Assay is a homogeneous, extended-glow cell viability assay based on ATP detection. It is a rapid procedure that does not involve lengthy incubation time for development of the reaction. The assay is excellent for those interested in moving away from radioactive assays such as [^{3}H]-thymidine incorporation. The CellTiter-Glo™ Assay offers flexibility in measurement techniques and is ideal for high-throughput formats.

Ordering Information

Product	Size	Cat.#
CellTiter-Glo™ Luminescent Cell Viability Assay[a]	10ml	G7570
	10 × 10ml	G7571
	100ml	G7572
	10 × 100ml	G7573

[a]Patent Pending.
CellTiter-Glo is a trademark of Promega Corporation.
Microtiter is a registered trademark of Dynex Technologies, Inc.

References

1. Crouch, S.P.M. *et al.* (1993) *J. Immunol. Meth.* **160**, 81–88.
2. Kabakov, A.E. and Gabai, V.L. (1996) In *Heat shock proteins and cytoprotection: ATP deprived mammalian cells.* R.G. Landes Company, Austin, TX.
3. DeLuca, M. *et al.* (1979) *Anal. Biochem.* **95**, 194–198.
4. Conti, E. *et al.* (1996) *Structure* **4**, 287–298.
5. Moravec, R. (2001) *Cell Notes* **2**, 14–16.
6. Zhang, J-H., Chung, T.D.Y. and Oldenburg, K.F. (1999) *J. Biomol. Screen.* **4**, 67–73.
7. *CellTiter-Glo™ Luminescent Cell Viability Assay Technical Bulletin* #TB288, Promega Corporation.
(www.promega.com/tbs/tb288/tb288.html)