High-throughput Screening with the CellTiter-Glo™ Luminescent Cell Viability Assay

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The CellTiter-Glo™ Assay is a homogeneous, scalable, assay for measuring cell viability and cytotoxicity. The versatility of the assay makes it well suited for high-throughput applications. The assay produces a luminescent signal that is proportional to the number of cells present regardless of culture volume. Results can be achieved in as little as 10 minutes, while the extended glow luminescence offers flexibility in plate processing. To meet the needs of high-throughput users, the CellTiter-Glo™ Assay can be scaled to work in 384 and 1536 well formats with little or no loss in sensitivity.

Introduction

The CellTiter-Glo™ Luminescent Cell Viability Assay[a] is designed to measure cell viability and cytotoxicity in high-throughput applications. With the increased availability of equipment designed to accommodate 384 well plates, many laboratories have scaled their screening methods to this smaller format. As a result of this miniaturization, cell viability assays need to be sensitive, fast and adaptable. Promega's recently introduced CellTiter-Glo™ Luminescent Cell Viability Assay meets these criteria.

The assay determines the number of viable cells based on the measurement of ATP using luciferase. A single addition of reagent (CellTiter-Glo™ Reagent) directly to cells in culture eliminates the need for multiple additions, extractions or washes. The luminescent signal generated is directly proportional to the number of viable cells present and is a "glow" type signal with an extended half-life typically greater than 5 hours, depending on cell type, medium and serum used. This long half-life eliminates the need for luminometers with reagent injectors and allows flexibility in plate processing. After addition and mixing of the CellTiter-Glo™ Reagent, results are obtained in just 10 minutes. This is advantageous over metabolism-based cell viability assays that rely on cells to metabolize substrates such as calcein-AM or tetrazoliums. The CellTiter-Glo™ Assay eliminates the need for extended incubations and can give a better indication of cell viability following drug treatments, particularly in situations where cells are treated for short periods of time.

Simple to prepare

The CellTiter-Glo™ Reagent is prepared by reconstituting the lyophilized CellTiter-Glo™ Substrate with the CellTiter-Glo™ Buffer. The final volume of CellTiter-Glo™ Reagent used is dependent on the final volume of cells and test compounds. A volume of CellTiter-Glo™ Reagent equal to the volume of the cells is added to the culture wells. The plates are agitated briefly, and the luminescence detected. As shown in Figure 1, luminescence correlates with cell number. The CellTiter-Glo™ Assay is able to detect 4 Jurkat cells/well ($p<0.001$) and has an r^2 value of 0.99 up to 4,000 cells/well in a 384 well assay format.

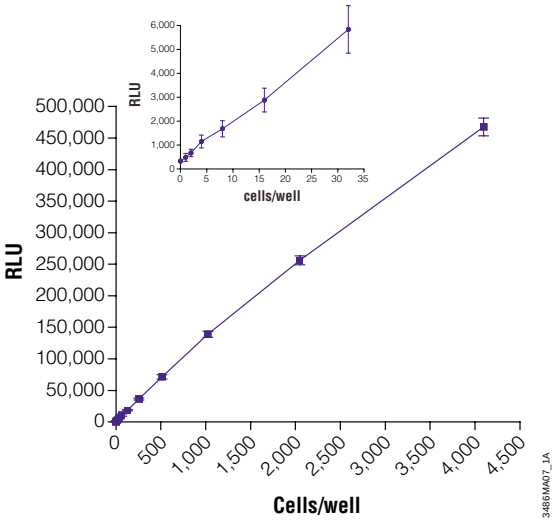

Figure 1. Cell number correlates with luminescent output. There is a direct relationship between luminescence and cell number as measured with the CellTiter-Glo™ Assay. Serial two-fold dilutions of Jurkat cells in RPMI 1640 + 10% FBS were made in a 384 well solid white plate as 25µl/well samples. An equal volume of CellTiter-Glo™ Reagent was added, mixed and the luminescence was recorded at 10 minutes using a Wallac® Victor 1420 MultiLabel counter. Values represent the mean and standard deviation of 8 replicates of each cell number (r^2=0.99 between 0–4000 cells per well). A Student's t-test indicates that the luminescence from 4 cells is signficant over background ($p < 0.001$).

Scalable and sensitive

The CellTiter-Glo™ Assay produces a luminescence signal that is proportional to the number of cells per sample, rather than their effective "concentration". Therefore, culture volumes have little effect on assay results. Similarly, distribution of cells throughout the sample has minimal effect. Reproducible luminescence intensity is achieved as long as a 1:1 volume of CellTiter-Glo™ Reagent to sample volume is maintained. This allows for greater flexibility of cell culture conditions and experimental design and enables scaling for 384 and 1536 well formats. To illustrate this, Figure 2 shows the light output obtained using equivalent quantities of cells in different sample

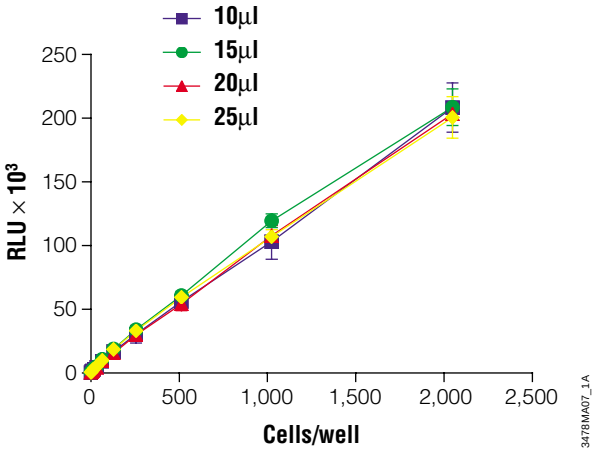

Figure 2. Luminescence correlates with cell number regardless of sample volume. Serial two-fold dilutions of Jurkat cells in RPMI 1640 + 10% FBS were made in 384 well solid white plates such that an equivalent quantity of cells was contained in various sample volumes (10, 15, 20 and 25µl/well). Equal volumes of CellTiter-Glo™ Reagent were added, the plate shaken, and luminescence was recorded after 10 minutes using a Wallac® Victor 1420 MultiLabel counter. Values represent the mean +/– S.D. of 4 replicates. For a given quantity of cells, light output was nearly equivalent regardless of sample volume.

volumes, ranging from 10–25µl. The results obtained are essentially equivalent and independent of sample volume.

The detection of such few cells by itself is somewhat inconsequential; however, a sensitive assay will ultimately allow the researcher to use small quantities of cells for drug screening assays. Two examples of cytotoxicity assays quantitated using CellTiter-Glo™ are shown in Figures 3 and 4. Figure 3 shows a TNFα cytotoxicity assay and compares the detection methods of the CellTiter-Glo™ Assay and the CellTiter 96® AQ_{ueous} One Solution Cell Proliferation Assay. The second assay is a colorimetric cell viability assay using the tetrazolium MTS (Owen's reagent), which is bioreduced by cells into a colored formazan product that is soluble in tissue culture medium. Although each assay measures a different indicator of metabolism, both means of cell viability quantitation give a similar ED_{50} value (~8pg/ml). The CCD camera image aligned above the graph was obtained from the same plate used for the luminometer.

Figure 4 shows a tamoxifen toxicity assay on HepG2 cells in a 384 well format using two different quantities of cells, 200 and 1,000 cells per well. Viability was determined using the CellTiter-Glo™ Assay and results indicate a similar ED_{50} value for both cell numbers assayed. The sensitivity of CellTiter-Glo™ assay allows testing using a small number of cells in a 384 well format.

Conclusion

The CellTiter-Glo™ Assay possesses many qualities that make it a robust assay, ideal for a variety of screening situations. We have validated the assay with a variety of cell types including Jurkat, HepG2, BHK-21, CHO-K1 and SH-SY5Y (human neuroblastoma) cells. It is relatively insensitive to the presence of phenol red (approximately a 5% decrease in light output with RPMI 1640). Common drug vehicles such as DMSO (10% increase at 0.5%),

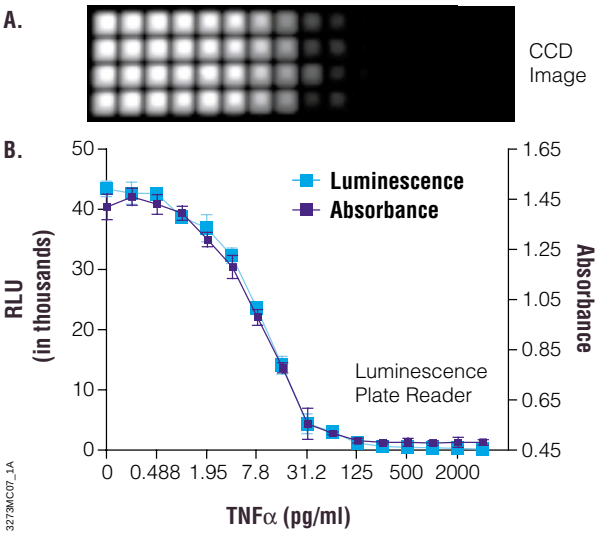

Figure 3. TNFα cytotoxicity of L929 cells as measured with the CellTiter-Glo™ Assay and the CellTiter 96® AQ_{ueous} One Solution Assay in 384 well plates. We plated L929 cells (1,000 cells/well) in a solid white and a clear bottom 384 well plate and allowed to attach and grow for 24 hours. We added varying concentrations of TNFα (n = 4) in the presence of actinomycin D (1µg/ml final) and incubated for 20 hours. Cytotoxicity was determined by adding an equal volume (30µl/well) of CellTiter-Glo™ Reagent, shaking the plate, and recording the luminescence 10 minutes later using a Berthold® Orion® plate luminometer. Alternatively, 6µl per well of CellTiter 96® AQ_{ueous} One Solution Reagent was added to wells of the clear bottom plate. This plate was incubated for 2 hours at 37°C prior to determining absorbance at 490nm on a Wallac® Victor 1420 MultiLabel counter. **Panel A.** Luminescence visualized with an Alpha Innotech Multi-image Light Cabinet CCD camera (30 second exposure at medium sensitivity). Each column of replicates corresponds to the mean data point on the graph as determined by the luminometer. **Panel B.** The ED_{50} values determined by both assays were approximately equivalent (8pg/ml).

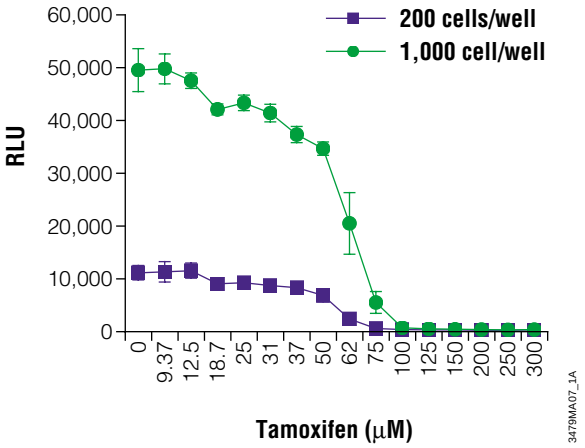

Figure 4. Tamoxifen cytotoxicity with HepG2 cells in 384 well format using the CellTiter-Glo™ Assay. We plated HepG2 cells (200 and 1,000 cells/well in 15µl volume) grown in MEM + 10% FBS in a solid white 384 well plate and allowed to attach and grow overnight. The following day, we added varying concentrations of tamoxifen (n = 4) in medium and incubated for 3 hours at 37°C. Cytoxicity was determined by adding an equal volume (30µl/well) of CellTiter-Glo™ Reagent, shaking the plate, and determining luminescence using a Wallac® Victor 1420 MultiLabel counter. The ED_{50} values obtained were similar for both cell concentrations used, and demonstrate the ability to perform toxicity testing on approximately 200 cells/well in a 384 well format.

Detailed Protocol

***CellTiter-Glo™ Luminescent Cell Viability Assay Technical Bulletin* (#TB288), Promega Corporation.**

(www.promega.com/tbs/tb288/tb288.pdf)

acetonitrile (2% increase at 1%) and ethanol (no change up to 5%) have little effect on light output as well. The reconstituted CellTiter-Glo™ Reagent is stable, with only a 12% decrease in activity after 18 hours at ambient temperature and no loss in activity following 5 weeks of storage at –20°C. The Reagent withstands the effects of freezing and thawing (less than 5% change following 10 cycles).

CellTiter-Glo™ Luminescent Cell Viability Assay is a homogeneous, single addition, extended-glow luminescent assay for the determination of cell viability. The assay is sensitive, fast and easy to use. Here, we have shown its usefulness for drug screening and quantitation in a 384 well format.

Ordering Information

Product	Size	Cat.#
CellTiter-Glo™ Luminescent Cell Viability Assay[a]	10ml	G7570
	10 × 10ml	G7571
	100ml	G7572
	10 × 100ml	G7573
CellTiter 96® AQ_{ueous} One Solution Cell Proliferation Assay*	200 assays	G3582
	5,000 assays	G3581
	1,000 assays	G3580

*For Laboratory Use.
[a] Patent Pending.
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