

A Rapid and Sensitive “Mix-and-Measure” Luminescent Cell Viability Assay



Promega

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Abstract

We have developed a simple and highly sensitive homogeneous assay (CellTiter-Glo™ Assay) for quantifying viable cells in multi-well plates. The assay is based on detection of adenosine triphosphate (ATP), which is an indicator of viability present in all cells. The homogeneous “add-mix-measure” protocol is performed by adding a single reagent directly to cells cultured in serum-containing medium, mixing, and recording luminescence with a luminometer or CCD imaging device. The luminescence is proportional to ATP concentration and correlates to the number of viable cells over 3-4 logs depending on the type of multi-well plates and cells used. Assay sensitivity is sufficient to detect 4 cells/well in a 384 well format. The bioluminescent “glow” signal can be read within 10 minutes of reagent addition and has a half-life of ~5 hours, allowing flexibility for batch or continuous automated processing. We will demonstrate the usefulness of the CellTiter-Glo™ Assay for high-throughput cytotoxicity screening.

Homogeneous Method CellTiter-Glo™ Assay Protocol



- Combine Substrate and Buffer to prepare Reagent
- Add directly to cells in culture medium (1:1 Ratio)
- Mix
- Record Luminescence

Sensitivity CellTiter-Glo™ Assay can Detect 4 Cells/Well

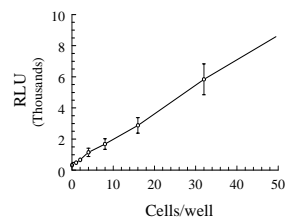


Figure 2. CellTiter-Glo™ Assay results from serial dilution of Jurkat cells in 384 well plate. Luminescent signal from 4 cells is greater than 2 standard deviations above signal from zero cells/well.

Linearity

ATP Content is Proportional to Number of Viable Cells

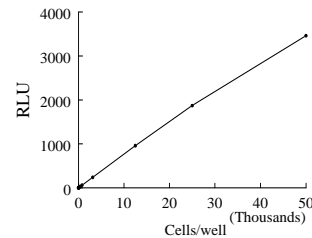


Figure 3. The CellTiter-Glo™ Assay produces a 4 log linear ($r^2=0.99$) response between luminescent signal and the number of Jurkat cells. Serial dilution of 50,000 – 5 cells/well were done across the rows of a 96 well plate. Values are mean \pm SD of 8 wells. SD bars are plotted; but are smaller than data points.

Half-Life

Luminescent Signal Lasts Greater Than 5 Hours

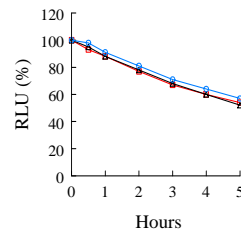


Figure 4. The half-life of luminescent signal produced by CellTiter-Glo™ Reagent is greater than 5 hours for the cell lines shown. The long half-life of luminescent signal provides the flexibility of performing “continuous” or “batch mode” handling of assay plates for HTS.

Mixing of Cells and Reagent Effect of Shaking Plates After Reagent Addition

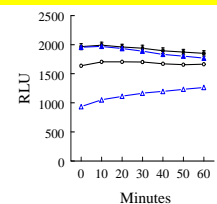


Figure 5. Plots of the RLU values from Jurkat cells in suspension are parallel for shaken and non-shaken plates (NS) whereas values from shaken and non-shaken plates with attached L929 fibroblasts are non-parallel. The non-shaken plate shows increasing RLU over the first hour suggesting slower lysis of attached cells.

ATP as Indicator of Cell Viability

ATP Levels Decrease Rapidly in Non-Viable Cells

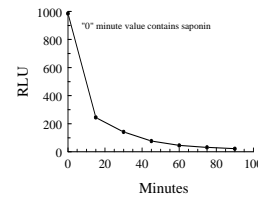


Figure 6. CHO-K1 cells (15,000/well) were plated into a 96 well plate in DME/F-12 + 10% FBS and allowed to attach overnight at 37°C, 5% CO₂. Wells (n=4) were treated with staggered additions of 0.2% saponin to lyse cells and incubated at 37°C to allow ATPase activity to occur. CellTiter-Glo™ Reagent then was added to measure ATP in all samples simultaneously. The results show that under normal culture conditions, ATP levels rapidly drop upon cell lysis.

Correlation with Accepted Methods

Comparison of ATP and MTS Tetrazolium Assays

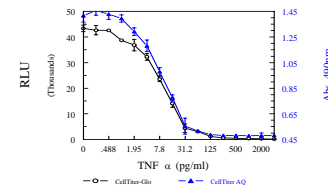


Figure 7. Similar IC₅₀ values (8pg/ml) were obtained for TNF α cytotoxicity using determination of ATP and MTS tetrazolium assays. L929 cells (1,000 cells/well in parallel 384-well plates) were treated with TNF α to induce cytotoxicity. Cytotoxicity was then determined using the standard CellTiter-Glo™ Assay (ATP) or CellTiter 96® Aqueous One Solution (MTS) Assay protocols.

Reagent Stability

Reconstituted Reagent is Stable During -20°C Storage

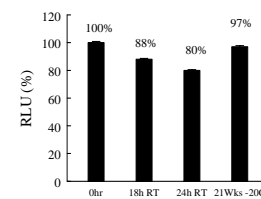


Figure 8. CellTiter-Glo™ Reagent was prepared by reconstituting the lyophilized CellTiter-Glo™ Substrate with the CellTiter-Glo™ Buffer. The samples of Reagent were prepared and stored at room temperature for 24 hours or at -20°C for 21 Weeks. Samples were used to assay for 1 μ M ATP and compared to freshly reconstituted Reagent (0hr Control = 100% RLU).

CCD Camera Imaging

Data Recorded with CCD Camera & Luminometer

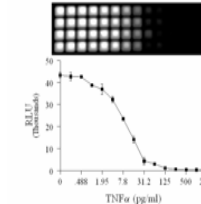


Figure 9. CellTiter-Glo™ Assay data can be recorded with a luminometer or a CCD camera. The results illustrate data recorded from the same 384 well plate using a Berthold Orion plate luminometer and an Alpha Innotech Multi-image Light Cabinet (CCD) camera (30 sec exposure, medium sensitivity).

1536 Well Format

1000 Cells/well was Determined to be Optimum

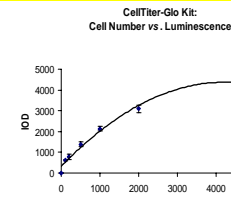
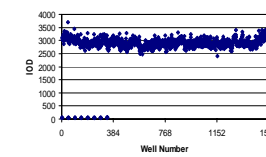


Figure 10. 293-EBNA cells were dispensed into wells of a Corning 1536-well test plate (1 μ l/well; 8 wells per concentration) using a BlueBird™ automated reagent dispenser. After 24 h, 1 μ l CellTiter-Glo™ Reagent was added, and luminescence recorded using a ViewLux™. 1000 cells/well (equivalent to 10⁶ cells/ml) were determined to be optimum for this 1536 well format. Data provided by Karen Wilson and Robert Swanson, Pharmacopia, Inc.

1536 Well Validation Assay Data

All Values are Acceptable for uHTS Screening Applications



Neg. Ctrl. Avg.	41.4	Assay Well. Avg.	2714.4	Pos. Ctrl. Avg.	2645.9
Neg. Ctrl. Std. Dev.	3.8	Assay Well. Std. Dev.	247.9	Pos. Ctrl. Std. Dev.	140.2
Neg. Ctrl. CV (%)	9.1	Assay Well CV (%)	9.1	Z Factor	0.84

Figure 11. Scattergram of validation assay plate using CellTiter-Glo™ Assay. Integrated Optical Density units (IOD) are plotted along the y-axis. The signal was consistent across the entire plate. The CV, Z factor, and signal to background were within acceptable ranges for screening. Data provided by Karen Wilson and Robert Swanson, Pharmacopia, Inc.

Use of 200 vs 1000 HepG2 cells/well

Low Cell Numbers Reduce Costs & Yield Similar IC₅₀ Values

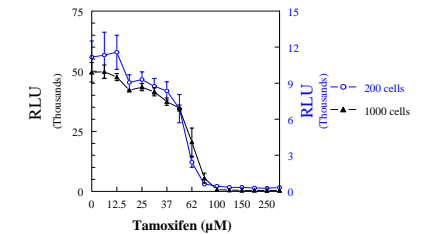


Figure 12. CellTiter-Glo™ Assay of 200 and 1000 HepG2 cells/well in 384 well plate resulted in the same tamoxifen IC₅₀ value. Assay sensitivity allows the use of low cell numbers, thus more assays can be run in situations where cells are limiting such as primary human hepatocytes for *in vitro* cytotoxicity testing.

Summary

CellTiter-Glo™ Assay Characteristics

- Homogeneous: “Add-Mix-Measure” format to measure ATP as an indicator of cell viability
- Sensitivity capable of detecting 4 cells/well
- Luminescent signal directly proportional to the number of viable cells
- Provides a glow type luminescent signal with a half-life of >5 hours
- Utilizes the properties of a stable form of luciferase proprietary to Promega to enable reaction conditions that inhibit endogenous ATPases
- Results of ATP measurement using CellTiter-Glo™ Assay correlate with widely accepted MTS tetrazolium methods
- Reconstituted Reagent is stable upon storage making it convenient for HTS applications
- Assay chemistry has little interference from solvents used to deliver test compounds (not shown)
- Luminescent output is bright enough to be detected by CCD camera
- Assay is compatible with 1536 well format
- Assay sensitivity allows most efficient use of limited cell types such as primary human hepatocytes for *in vitro* cytotoxicity testing
- For additional technical information, see <http://www.promega.com/tbs/tb288/tb288.html>