A GloMax® Multi Microplate Fluorometer Method for

CellTiter-Glo® Luminescent Cell Viability Assay

INTRODUCTION

The GloMax® Multi Microplate Luminometer in combination with the CellTiter-Glo® Luminescent Cell Viability Assay Kit provides a convenient, rapid, and sensitive procedure for determining the number of viable cells in a culture. Presence of metabolically active cells[1] is signaled by ATP quantitation.

The CellTiter-Glo® Luminescent Cell Viability Assay Kit uses luciferase as the detection enzyme because mammalian cells lack endogenous luciferase activity. The UltraGlow Luciferase used in the CellTiter-Glo® Luminescent Cell Viability Assay Kit generates a stable, glow-type signal with a half-life greater than four hours. This extended signal allows for batch-mode processing of multiple plates. Luciferase enzyme requires ATP in order to generate light. Metabolically active cells produce ATP as energy for respiration and other vital processes. After an equal volume of CellTiter-Glo® Reagent is added to the cell culture, luminescence is measured. Light signal is proportional to the amount of ATP present which correlates with the number of viable cells present.

The extended dynamic range of the GloMax® Multi Microplate Luminometer allows the user to easily measure various sample signal intensities on the same plate using the CellTiter-Glo® Reagent. The GloMax® Multi Microplate Luminometer detects as little as 1.5×10^{-15} moles ATP using CellTiter-Glo® Substrate. Measurements are linear for more than four orders of magnitude (**Figure 1**).

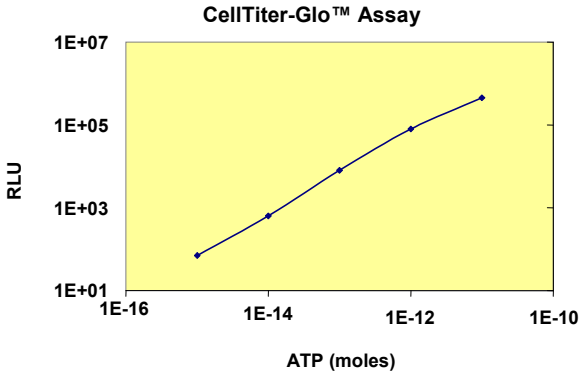

Figure 1. CellTiter-Glo® Assay on the GloMax® Multi Microplate Luminometer using ATP standards diluted in HEPES buffer.

MATERIALS REQUIRED

- GloMax® Multi Microplate Multimode Reader
- GloMax® Multi Microplate Luminesence Module
- 96-well plates, white (E&K Scientific, Cat.# 25075)
- CellTiter-Glo® Luminescent Cell Viability Assay Kit (Cat.#s G7570, G7571, G7572, G7573)
- p200 pipette and pipette tips

CAUTION: The lyophilized CellTiter-Glo® Substrate contains dithiothreitol (DTT) and is therefore classified as hazardous. The reconstituted reagent is not known to present any hazards as the DTT concentration is less than 1%. However, we recommend the use of gloves, lab coats, and eye protection when working with these or any chemical reagents

EXPERIMENTAL PROTOCOL

Reagent Preparation Recommendation

- **CellTiter-Glo® Substrate:** Use as supplied. Store at -20°C.

- **CellTiter-Glo® Buffer:** Use as supplied. Store at -20°C. Buffer may be thawed and stored at room temperature for 48 hours without loss of activity.

- **CellTiter-Glo® Reagent:** Transfer the contents of one bottle of CellTiter-Glo® Buffer into one bottle of CellTiter-Glo® Substrate. Mix by inversion until the substrate is thoroughly dissolved. Use the reconstituted Reagent or store at 4°C for one to two days with 5 - 20% loss of activity.

  Note: The CellTiter-Glo® Reagent should be held constant at room temperature while quantifying luminescence since luciferase activity is temperature dependent. Reagent stored frozen after reconstitution must be thawed below 25°C to ensure Reagent performance. Mix well after thawing. The simplest method for thawing is placing the Reagent in a water bath at room temperature.

Instrument Set Up

1. Go to Select Protocol from the Home screen and follow the protocol wizard to select the preset CellTiter-Glo protocol. Enter the following: Luminescence; at the Preset tab, select CellTiter-Glo; Finish.

2. The Instrument Control screen shows all the reading parameters: integration and that all the plate wells are selected to be read. If desired, change the delay and integration time settings and save the changed protocol under a different protocol name in the User protocol folder.

3. Select wells on the Plate Map according to how samples are loaded into the plate.

4. Refer to the on-screen Help topics, Quick Start Guide, or Operating Manual for detailed instructions.

Sample Analysis

1. Add a compound to be tested to the white 96-well plate containing 100-µL cell cultures. Wells without cells (culture media only) can be used as controls for background. Cultures without compounds should be used as experimental controls. Incubate according to culture protocols.

2. Equilibrate plate and contents to room temperature for approximately 30 minutes.

3. Add an equal volume (100 µL) of CellTiter-Glo® Reagent. Mix gently for two minutes on an orbital shaker. Incubate at room temperature for ten minutes to stabilize the luminescent signal.

4. Open the instrument door by using the Door icon on the touch-screen. Place the plate with A1 well at the top right corner of the microplate sample tray. Close the door by using the Door icon.

5. Touch the Start icon on the touch-screen to begin reading.

6. RLU values measured by the GloMax® Multi Microplate Luminometer will appear on the Results screen of the touch-screen display immediately after each well is measured.

7. Once the measurements are complete, data can be transferred to an external computer for further data analysis in Excel by using the provided USB flash drive.

8. Remove the plate after measurement completion.

RESULTS

Sensitivity: $1.5x10^{-15}$ moles ATP using CellTiter-Glo® Substrate

Dynamic Range: Four orders of magnitude of ATP concentration using CellTiter-Glo® Substrate

CONCLUSION

The GloMax® Multi Microplate Luminometer offers superior sensitivity and dynamic range for luminescence detection, such as the luminescence-based CellTiter-Glo® Assay. The GloMax® Multi Microplate Luminometer achieves its superior performance with a combination of unique detection and optical designs, premium components such as the photomultiplier tube (PMT), low-noise circuitry, and proprietary dual-masking system.

The modular approach of the GloMax® Multi Microplate Luminometer allows for instrument capability expansion as needs in the lab change. Fluorescence and/or absorbance detection modules as well as other accessories can be added after the initial purchase.

The superior performance, ease of use, and utmost flexibility of the GloMax® Multi Microplate make it an ideal microplate reader for today's life science laboratory.

REFERENCES

1. Crouch, S.P.M. *et al.* (1993) The use of ATP bioluminescence as a measure of cell proliferation and cytotoxicity. *J. Immunol. Meth.* **160**, 81.

CONTACT INFORMATION

Toll-Free: (800) 356-9526
Fax: (800) 356-1970

www.promega.com

Email: custserv@promega.com

Mailing Address:

Promega Corporation
2800 Woods Hollow Rd.
Madison, WI 53711 USA