Promega

Application Note

A GloMax® 96 Microplate Luminometer Method for

CellTiter-Glo® Luminescent Cell Viability Assay

1. INTRODUCTION

The GloMax® 96 Microplate Luminometer in combination with the CellTiter-Glo® Luminescent Cell Viability Assay provides a convenient, rapid, and sensitive procedure for determining the number of viable cells in a culture based on quantitation of ATP, which signals the presence of metabolically active cells[1].

The CellTiter-Glo® Luminescent Cell Viability Assay kit uses luciferase as the detection enzyme because of the absence of endogenous luciferase activity in mammalian cells. The UltraGlo™ luciferase used in the CellTiter-Glo® Assay generates a stable, glow-type signal that has a half-life of greater than four hours. This extended signal allows batch-mode processing of multiple plates. Luciferase enzyme requires ATP 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.

The CellTiter-Glo® Luminescent Cell Viability Assay maximizes the sensitivity of the assay reagent and provides a long-lasting luminescent signal. The cell viability protocol is an efficient "add-mix-measure" method. The CellTiter-Glo® Reagent is compatible with commonly used culture media for mammalian cells (RPMI 1640, MEMα, DMEM and Ham's F12) and tolerates phenol red and organic solvents.

The extended dynamic range of the GloMax® 96 Microplate Luminometer allows the user to easily measure very dim and very bright samples on the same plate using the CellTiter-Glo® Reagent. A pre-installed template on the GloMax® 96 facilitates quick assay set-up. The GloMax® 96 detects as little as 1.5×10^{-15} moles ATP using CellTiter-Glo® Substrate. Measurements are linear from 760 fg to 5.1 ng ATP or more than 4 orders of magnitude (Figure 1).

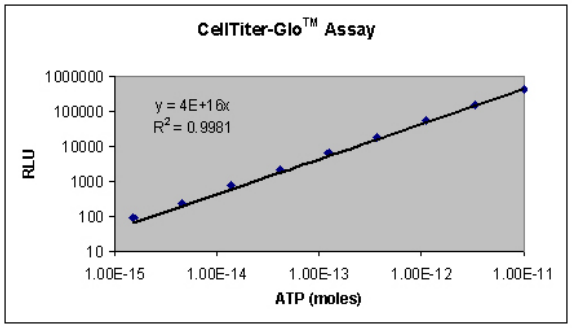

Figure 1. CellTiter-Glo® Assay on the GloMax® 96 Microplate Luminometer using ATP standard diluted in HEPES buffer.

2. MATERIALS REQUIRED

- GloMax® 96 Microplate Luminometer
- 96-well plates, white (E&K Scientific EK-25075)
- CellTiter-Glo® Luminescent Cell Viability Assay (Cat # G7570, G7571, G7572, G7573)
- p200 pipette and pipette tips

3. PROTOCOL

3.1 Reagent Preparation

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 to one bottle of CellTiter-Glo® Substrate. Mix by inversion until the substrate is thoroughly dissolved. Use reconstituted reagent on the same day it is prepared or store at 4°C for one to two days with 5—20% loss of activity.

Note: The temperature of 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.

3.2 Instrument Setup

3.2.1 Double-click on the GloMax® 96 icon to start the software.

3.2.2 Click on "Run Promega Protocol" from the "Welcome to Veritas" dialog box.

3.2.3 Select "CellTiterGlo" from the list of Promega protocols.

3.2.4 Enter your information into the "Experiment", "Operator", "Plate No.", and "Notes" fields in the "Main Dialog Box".

3.2.5 Click on "Options" from the "Main Dialog Box" to select the wells to be read and modify the number of runs. Once modified, click the "Apply Changes" button to return to the "Main Dialog Box".

3.3 Sample Analysis

3.3.1 Add 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.

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

3.3.3 Add an equal volume (100 µL) of CellTiter-Glo® Reagent. Mix gently for 2 minutes on an orbital shaker. Incubate at room temperature for 10 minutes to allow luminescent signal to stabilize.

3.3.4 Insert plate into the GloMax® 96 Microplate Luminometer and click on "Start" to begin assay. RLU values measured by the GloMax® 96 Microplate Luminometer will appear in the Excel spreadsheet after each row of the selected wells have been read. If you encounter an error message, refer to the troubleshooting guide for more information.

3.3.5 Once the measurements are complete, you can access Excel to analyze your data.

3.3.6 Remove your plate after measurement.

4. 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.

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 concentration of DTT is less than 1%. However, we recommend the use of gloves, lab coats and eye protection when working with these or any chemical reagents. Promega assumes no liability for damage resulting from handling or contact with these products.

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