APPLICATION NOTE

Measure cancer cell viability using a homogeneous, stable luminescence assay

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Benefits

- **Highly sensitive luminescence detection down to 20 cells per well**
- **Easy correlation of cell number and ATP content**
- **Convenient single-reagent workflow**

Introduction

Luminescent cell viability assays offer sensitivity and an easy workflow for monitoring the effects of various experimental conditions. These use the firefly luciferase reaction as a way to determine the relative numbers of living cells under different treatments or experimental conditions. Metabolically active cells produce ATP, which is required by the luciferase reaction. When ATP is the limiting component in the reaction, the amount of light produced is proportional to and serves as the readout for the number of viable cells (Figure 1).

Here, we validated performance of Promega's CellTiter-Glo 2.0 Cell Viability Assay[1] on the SpectraMax® iD5 Multi-Mode Microplate Reader. Both ATP and viable cells were used to generate standard curves demonstrating the sensitivity and linearity of the assay.

To confirm the utility of the CellTiter-Glo 2.0 assay in cytotoxicity assessment, MCF7, an estrogen receptor (ER)-positive breast cancer cell line, was treated with the selective estrogen receptor modulator (SERM) tamoxifen to induce cell death.

Materials

- CellTiter-Glo 2.0 Assay (Promega cat. #G5241)
- 96-well solid white, tissue culture-treated microplate (Corning cat. #3917)
- MCF7 cells (Breast cancer cell line, ATTC cat. #HTB-22)
- Tamoxifen (Tocris cat. #6342)
- SpectraMax iD5 Multi-Mode Microplate Reader (Molecular Devices cat. #iD5-STD)

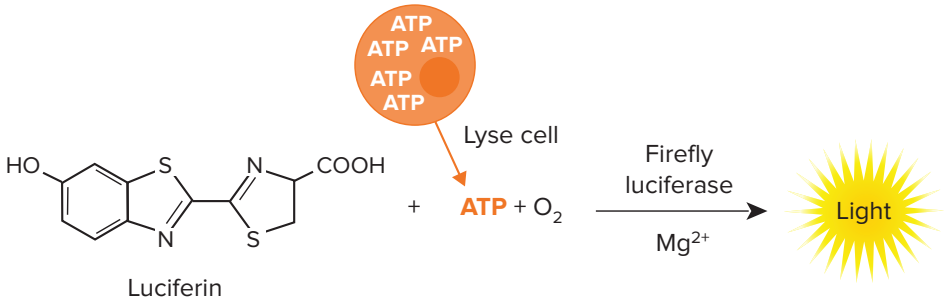

Figure 1. Luciferase reaction used to measure cell viability. Cells are lysed and added to the luciferase reaction, where they provide the ATP needed to produce light. The more live cells are present, the more light is produced.

Methods

ATP standard curve

A 1:10 serial dilution of ATP was prepared in medium. 100-µL samples ranging from 1 nM to 10 µM were added to triplicate wells of a solid white 96-well microplate. Controls containing medium only were included to measure background luminescence. 100 µL of CellTiter-Glo 2.0 reagent was added to each well, and the microplate was placed on a plate shaker for two minutes, then incubated at room temperature for 10 minutes. Luminescence was then detected on the SpectraMax iD5 reader using the settings shown in Table 1.

Correlating cell number with luminescent output

MCF7 cells were seeded from 20 to 20,000 cells per well in a solid, white, 96-well microplate. Background control wells contained medium only. Cells and controls were run in duplicate at 100 µL per well. Cells were allowed to adhere to the wells for three hours at 37°C, 5% CO_2. The microplate was equilibrated to room temperature for 30 minutes, and 100 µL of CellTiter-Glo 2.0 reagent was added to each well. The plate was placed on a plate shaker for two minutes to induce lysis and allowed to incubate at room temperature for 10 minutes before luminescence was measured on the SpectraMax iD5 reader.

Cell viability assay

MCF7 cells were seeded at 15,000 cells per well in a solid, white, 96-well, tissue culture-treated microplate and allowed to attach overnight at 37°C, 5% CO_2. Control wells containing medium without cells were included to determine background luminescence. The next day, a 1:2 serial dilution of tamoxifen ranging from 0.2 µM to 100 µM was added to the cells. Cells were incubated for 48 hours. The microplate was equilibrated to room temperature for 30 minutes prior to the addition of 100 µL of CellTiter-Glo 2.0 per well. The plate was shaken for two minutes to induce cell lysis and incubated at room temperature for 10 minutes to stabilize the luminescent signal before reading. The ATP content of cells can be calculated using the ATP standard curve in a preconfigured CellTiter-Glo protocol in SoftMax® Pro Software.

Parameter	Setting
Read mode	Luminescence
Read type	Endpoint
Wavelengths	All Wavelengths
Plate type	96 well Costar
PMT and optics	Integration time: 1000 ms Read height: 3.54 mm

Table 1. Instrument settings for luminescence readings on the SpectraMax iD5 reader. The All Wavelengths setting (no wavelength selection) was chosen to maximize the luminescence detected by the reader. Read height was optimized using the Read Height Adjustment setting in SoftMax Pro Software.

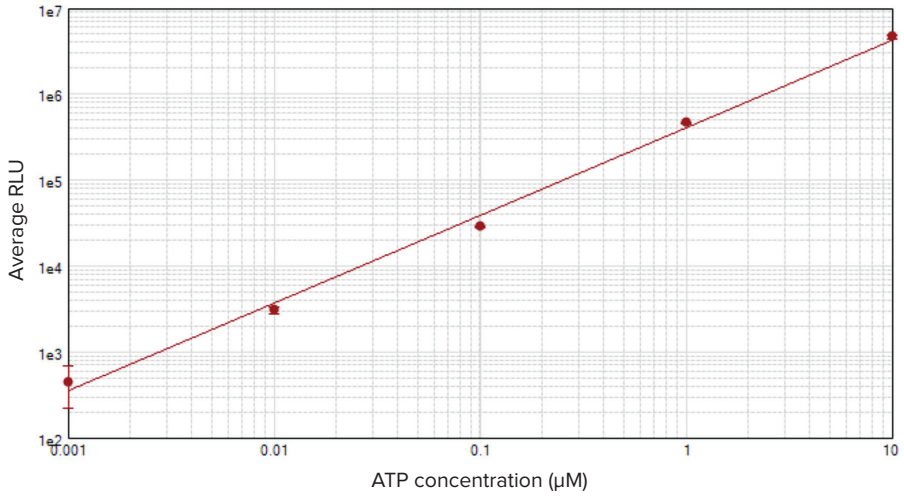

Figure 2. ATP standard curve. Average luminescence vs. ATP concentration was plotted using the log-log curve fit in SoftMax Pro Software (r^2 = 0.996).

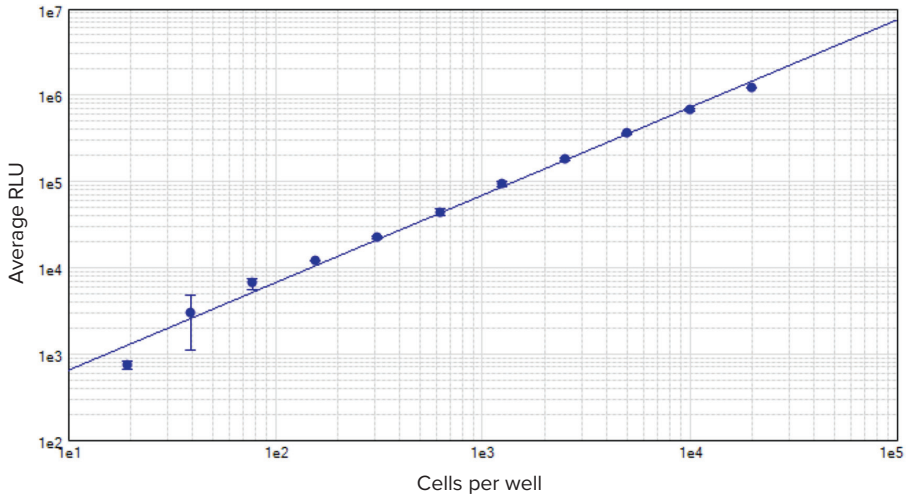

Figure 3. MCF7 cell dilution series in 96-well format. Cell concentrations as low as 20 cells per well could be detected using the CellTiter-Glo 2.0 assay (r^2 = 0.993).

Results

The CellTiter-Glo 2.0 assay was used to generate an ATP standard curve, which can be used to determine the amount of ATP present in a cell sample. When detected using the SpectraMax iD5 reader, this curve was linear over concentrations of ATP spanning four orders of magnitude (Figure 2).

A standard curve consisting of MCF7 cells seeded from 20 to 20,000 per well in a 96-well plate was linear across the entire range with r^2 = 0.993 (Figure 3), validating the assay's usefulness over a wide range of viable cell numbers. MCF7 cells treated with tamoxifen for 48 hours were assayed for cytotoxicity, and the resulting concentration response curve was plotted using a 4-parameter curve fit in SoftMax Pro Software (Figure 4). From the curve, an IC_{50} value of 19.7 µM was automatically calculated by the software. This result is consistent with other published results in MCF7[2].

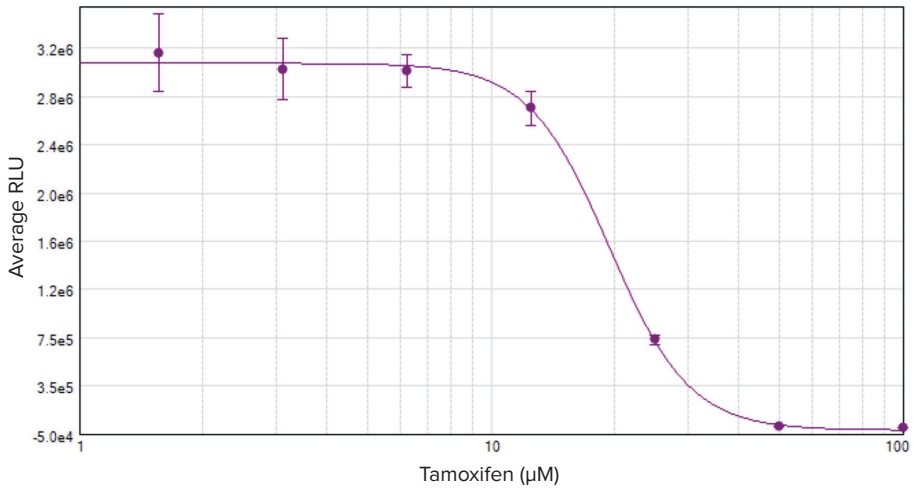

Figure 4. MCF7 cells treated with tamoxifen. Results were plotted using a 4-parameter curve fit (IC_{50} = 19.70 µM).

Conclusion

Screening of cell viability in response to experimental treatment is most easily done using an assay that is homogeneous and stable. The CellTiter-Glo 2.0 assay consists of a single reagent that is added directly to cells cultured in a microplate and is stable at a variety of storage conditions. The signal half-life is greater than three hours, making the assay amenable to a variety of screening and other workflows.

Used together with the SpectraMax iD5 reader, which features an ultra-cooled PMT for improved luminescence signal to noise, this assay enables linear detection of a wide range of cell numbers, down to 20 cells per well. The SpectraMax iD5 reader also offers the capability of multiplexing the CellTiter-Glo assay with other assays requiring additional detection modes.

References

1. Technical Manual: CellTiter-Glo 2.0 Assay. Promega Corporation.
2. Li AP, Bode C, and Sakai Y. A novel *in vitro* system, the integrated discrete multiple organ cell culture (IdMOC) system, for the evaluation of human drug toxicity: comparative cytotoxicity of tamoxifen towards normal human cells from five major organs and MCF-7 adenocarcinoma breast cancer cells. *Chemico-Biological Interactions* 150 (2004) 129–136.

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