Setting up luminometric cell viability assays with the Thermo Scientific multimode microplate reader and Reagent Dispenser

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Goal

This application note describes how to perform a cell viability assay using the Thermo Scientific Varioskan LUX multimode microplate reader and the Thermo Scientific Multidrop Combi reagent dispenser. Cell viability was measured using the Promega CellTiter-Glo Luminescent Cell Viability Assay. The procedure and performance of the system and the assay were followed after their optimisation.

Introduction

Cell viability is an important parameter measured in a variety of research areas including drug screening. The CellTiter-Glo Luminescent Cell Viability Assay measures the viability of cells based upon the quantification of cellular ATP levels. The assay relies upon the generation of a 'glow-type' luminescent signal which is proportional to the amount of ATP, and thus the number of cells that are present (***Figure 1***).

Cells are plated into microtiter plates and treated with compounds whose effect on cell viability is to be determined. Subsequent to the incubation of cells in the presence of the compounds, the CellTiter-Glo Luminescent Cell Viability Assay reagent is added and the luminescent signal monitored.

An early understanding of compound-mediated cell toxicity is important in pre-clinical drug discovery. If this is overlooked, it can have a profound effect as to whether the compound will fail due to its safety when investigated in clinical trials.[1]

This application note describes how the CellTiter-Glo Luminescent Cell Viability Assay was used to screen the ENZO SCREEN-WELL FDA approved drug library V2 (ENZO compound library) against HEK293 cells.

Materials and methods

- Varioskan LUX multimode microplate reader, Thermo Fisher Scientific
- 384-well white microtiter plate, Greiner
- HEK293 cells, DSMZ, Braunschweig, Germany
- CellTiter-Glo Luminescent Cell Viability Assay kit, Promega
- Multidrop Combi reagent dispenser, Thermo Fisher Scientific
- ECHO acoustic dispenser, Labcyte

The assay was performed according to CellTiter-Glo Luminescent Cell Viability Assay protocol.[2] The ENZO compound library was screened at 10µM in duplicate microtiter plates.

The measurement protocol was set up with the Thermo Scientific SkanIt software, a PC software for controlling the Varioskan LUX multimode microplate reader. The default parameters for luminescent assays were used for all measurements (***Figure 2***).

Users can adjust the measurement speed for specific throughput requirements. In this case, measurement time was set to 200milliseconds. A shorter measurement time naturally has an effect on the assay performance, but in high throughput screens this may be a valuable feature. It is also possible to lengthen the measurement times to improve precision. Results were converted to inhibition percentages using the 'Normalisation' step, and later classified according to their viability values, using the 'Classification' step.

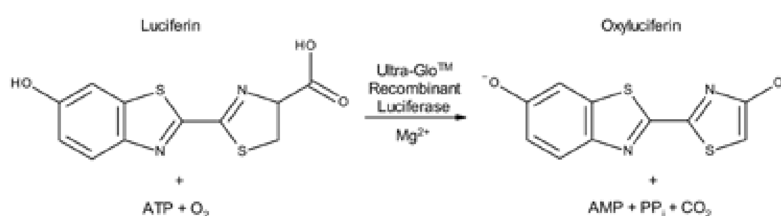

Figure 1: Principle of the CellTiter-Glo Luminescent Cell Viability Assay

Figure 2: Luminescence protocol set-up with SkanIt software

1. Miniaturisation of the CellTiter-Glo Luminescent Cell Viability Assay

The assay was miniaturised into 384-well microtiter plates. Briefly, a solution of HEK293 cells was prepared after sub-culturing the cells. Cells were grown in cell culture media with 10% inactivated FBS, 100 IU/mL penicillin G and 100µg/mL streptomycin, and kept in a humidified incubator at 37°C and 5% CO_2. Cells were dispensed into 384-well microtiter plates (500 cells/well in a volume of 20µL) using the Multidrop Combi reagent dispenser and the standard tube dispensing cassette.

After overnight incubation of the microtiter plates in a humidified incubator (48hr at 37°C), 10µL/well of the CellTiter-Glo Luminescent Cell Viability Assay reagent was added. All additions were performed using the Multidrop Combi with default speed set to HIGH. After a 10-minute incubation at room temperature, the luminescence signal was measured using the Varioskan LUX multimode microplate reader.

2. Screening of the ENZO compound library against the HEK293 cell-line

The miniaturised assay described above was used to screen the ENZO compound library at 10µM. Before adding cells to each well of the microtiter plates, compounds were added using the Labcyte ECHO (0.1µL/well of a 1mM stock in 100% DMSO v/v). The last column of each microtiter plate was filled with cell culture media alone since this was used as the negative control. The positive control was cells without any treatment.

Results

The layout of the ENZO compound library (***Figure 3***) contained appropriate negative and positive controls and the raw luminescence signal for each compound was normalised using these.

Z' is a dimensionless parameter, which describes the quality of a screening assay.[3] The value ranges from 0-1 and assays with values above 0.5 can be considered very good. In this case, the Z' for each of the screened microtiter plates was >0.8, indicating excellent performance of this cell-based assay even after its miniaturisation. Calculations of the Z' values can be directly made using the SkanIt software using the four involved variables and the 'Custom-Formula' tool.

The screening of the ENZO compound library yielded excellent correlation, and the data for one 384-well microtiter plate is shown (***Figure 4***). As expected, most of these compounds are approved drugs and did not significantly reduce the viability of HEK293 cells. In fact, viabilities were over 75% for most compounds.

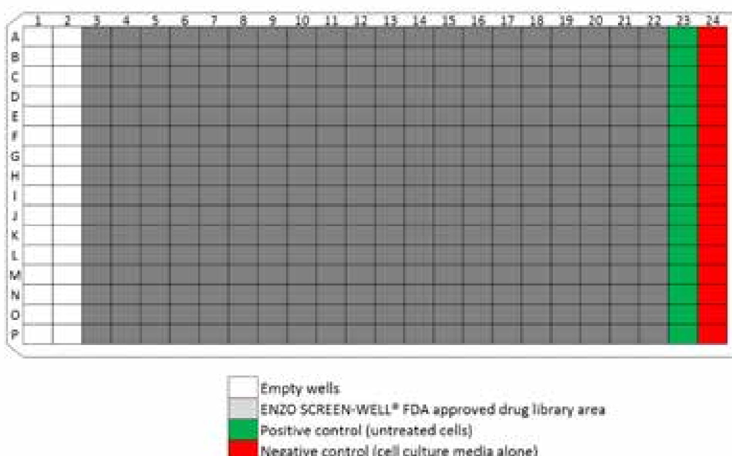

Figure 3: Layout of the ENZO SCREEN-WELL FDA approved drug library, positive and negative controls in the 384-well microtiter plates for the screening of against the HEK293 cell-linesoftware

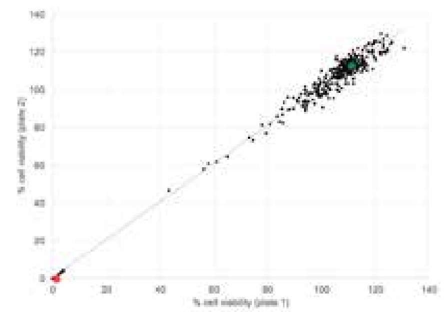

Figure 4: Correlation of the ENZO SCREEN-WELL FDA approved drug library screen against HEK293 cells (r^2 = 0.99). Positive control region = ● Negative control region = ●

Conclusions

The Varioskan LUX multimode microplate reader and the Multidrop Combi reagent dispenser provide both easy-to-use and high-performance tools for luminescence assays. The Varioskan LUX contains optimised parameters for all of the measurement technologies, but also provides the opportunity to adjust the measurement parameters for assay set-up and optimisation. Coupled with the in-built calculations of SkanIt software, this makes the system a very flexible tool from research to high-throughput screening. The Multidrop Combi assures a fast and gentle way for cell dispensing, resulting in cells remaining intact and viable. ■

References

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