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Orion II Microplate Luminometer Simplicity 4

ViaLight™ Cell proliferation and Cytotoxicity BioAssay Kit (Lonza)

The use of ATP detection systems to quantify viable cells with the advantages of increased detection limits, speed and accuracy has been well documented (Crouch et al., 1993). As the level of ATP within a healthy cell is strictly maintained it is assumed that each cell within a population contains a similar amount of ATP. When the cells are in a proliferative state the amount of ATP in the culture can be seen to increase as the cell number increases. Conversely, cells in a cytotoxic environment are unable to maintain their levels of ATP as synthesis becomes compromised and the ATP is consumed by ATPases thus levels are seen to reduce. The level of ATP present within a culture is indicative of the number of viable cells present and ATP assays have been shown to be one of the most predictive general cytotoxicity methods.

The ViaLight™ Assay is based on the bioluminescent measurement of ATP (adenosine triphosphate). This bioluminescent method utilises luciferase, which generates light from ATP and luciferin according to the following reaction:

Reaction

$$\text{ATP} + \text{Luciferin} + O_2 \xrightarrow{\text{Luciferase}} \text{Oxyluciferin} + \text{AMP} + PP_i + CO_2 + \text{Light}$$

The emitted light intensity is linearly related to the ATP concentration and is measured using a luminometer.

The ViaLight™ assay provides a luminescent method for determining the number of viable cells. ATP is released from cells by a simple lysis step and detected by the addition of an ATP monitoring reagent. The resulting light emission (RLU/s) is directly proportional to the number of viable cells in the culture.

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Materials

Luminometer: Orion II Microplate Luminometer
Software: Simplicity 4
Assay : ViaLight™ Cell Proliferation and Cytotoxicity Bio Assay Kit (Lonza)
Microplates: opaque microplates (solid, white, 96 well; Porvair)

Method

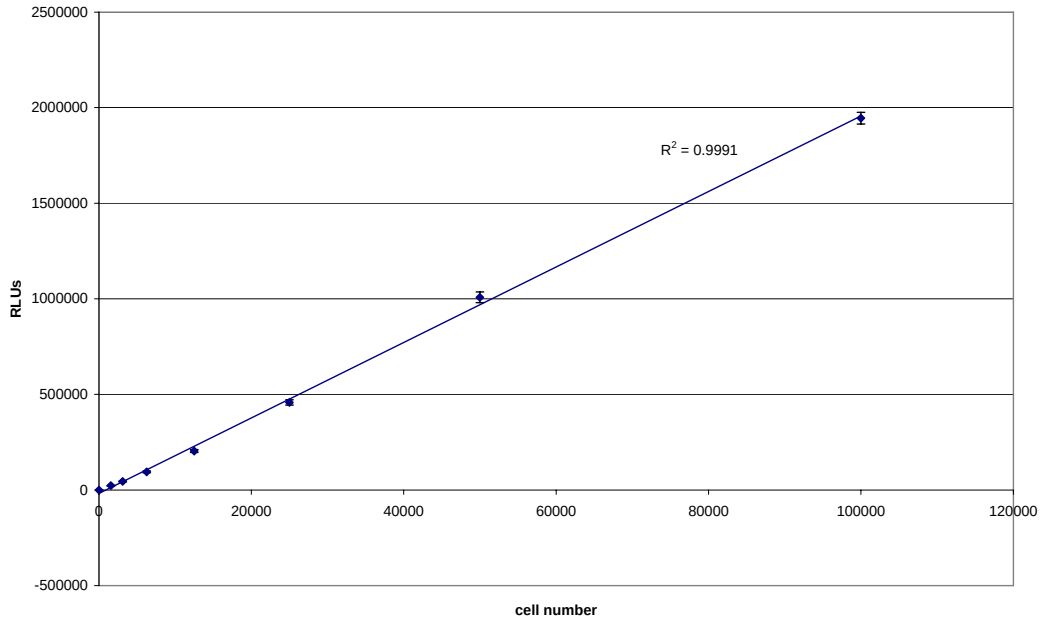
100µl cell culture samples were exposed to drug for a determined time period. These were lysed by the addition of 50µl of Cell Lysis Reagent followed by a 10 minute incubation at room temperature. 100µl of AMR Plus (ATP Monitoring Reagent Plus) was added to each sample and allowed to incubate at room temperature for 2 minutes to allow signal stabilization.
The plate was placed into the Orion II and a 1s integrated read taken.

For detailed assay instructions please visit:

www.lonzabioscience.com/content/Documents/Bioscience/Vialight_plus18880-060502.pdf

Example

The assay as detailed above was conducted using triplicate dilutions of HL60 cells at predetermined cell numbers.



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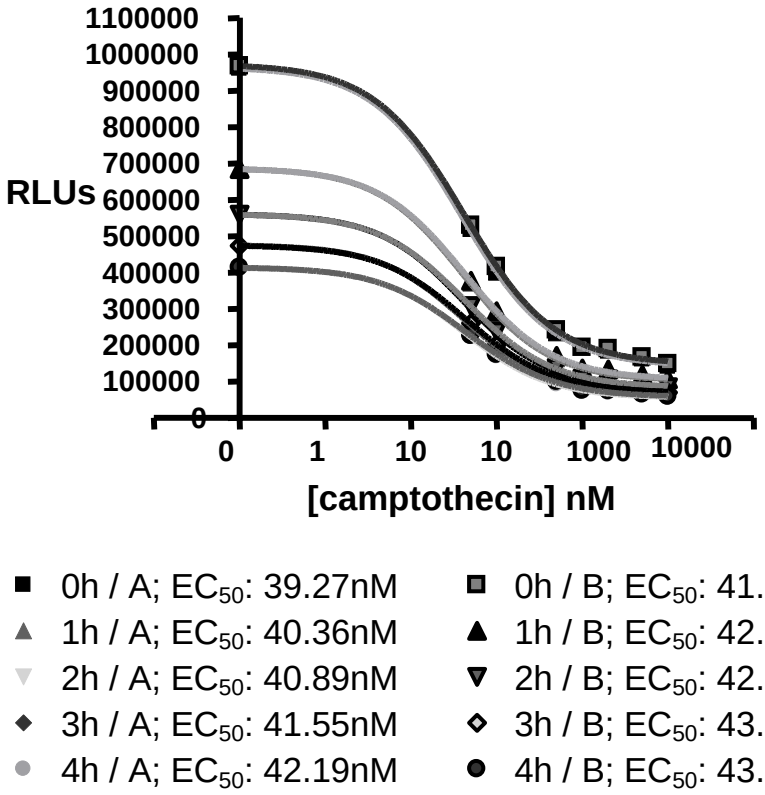

Figure 2: EC_{50} data generated using the Orion II with Lonza ViaLight™ Plus Cell Proliferation and Cytotoxicity BioAssay Kit. HL60 cells were dosed with camptothecin for 24 h and assayed using ViaLight Plus. Cells were read in an Orion II luminometer and the plate repeatedly read every 10 minutes for 4 h. Data shown is the results of two separate data sets (n=8 for each). EC_{50} data was calculated using Graphpad prism.

Result

The Orion II microplate luminometer showed excellent sensitivity and linearity in the detection of a wide range of cell numbers and easily generated EC_{50} data in combination with the Lonza ViaLight™ Cell Proliferation and Cytotoxicity BioAssay Kit.

Acknowledgement
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Figures and text by courtesy of Lonza Nottingham Ltd., UK

Lonza

www.lonza.com