The new Atmospheric Control Unit (ACU) for the CLARIOstar® provides versatility in long term cell-based assays from Promega and Platypus

BMG LABTECH
The Microplate Reader Company

Carl Peters[1], Tracy Worzella[2], Jennifer Fronczak[3]
[1]BMG LABTECH, Cary, NC [2]Promega, Madison, WI [3]Platypus Technologies, Madison, WI

CLARIOstar with ACU

Analyses of cell health and cell migration are examples of cell based assays that derive great benefit from the ability to detect changes to these parameters in real time. Real-time detection allows identification of an exact moment in time when a cytotoxic or anti-proliferative change occurs. Furthermore, assessing cell migration in real time allows the determination of migration rate.

CLARIOstar ACU settings:
In order to maintain cell health CLARIOstar temperature control was used and the ACU was set as follows:

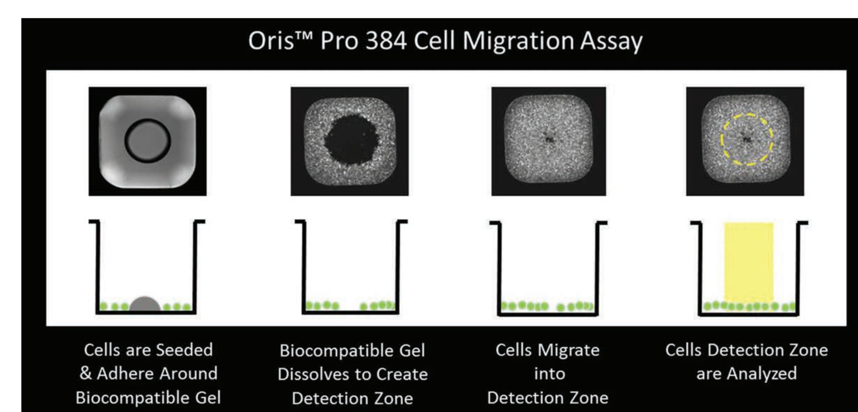
CO_2 %	5
O_2	monitoring
Target temperature	37 °C

Platypus: Oris™ Pro 384 Cell Migration Assay

Introduction

The Oris™ Pro 384 Cell Migration Assay from Platypus Technologies employs their biocompatible gel based approach in an assay suitable for high throughput. A cell free zone is created in the center of the well so that the amount of migration is based on the amount of cells in this zone.

The ACU maintained an environment suitable for the health of fluorescently labeled cells which allowed cell migration to be assessed in two ways. First, by taking fluorescent readings in the center well an increase in signal will indicate an increase in cells migrating into the cell free zone. Second, the CLARIOstar can perform high resolution well scanning. This enables a heat map to be generated which depicts the extent of cell migration. These images can be collected throughout the migration process.



Materials and Methods

MDA-MB-231 cells were stained with Cell Tracker Deep Red from Life Technologies and plated in Oris™ Pro 384-well, Tissue Culture Treated plates. The CLARIOstar was equipped with fixed filters (Ex: 590-50, Dichroic: LP639, Em: 675-50) and the microplate read from the bottom in both well scanning and orbital averaging mode (scan diameter=1 mm). These two assay protocols could be read sequentially using BMG's Script mode. The ACU maintained cells at the appropriate 37° C and 5% CO_2 for the duration of the 23 hour experiment.

Results and Discussion

A portion of the 384 well to depict the representative heat map data that can be generated using the CLARIOstar in well scanning mode (Fig.2):

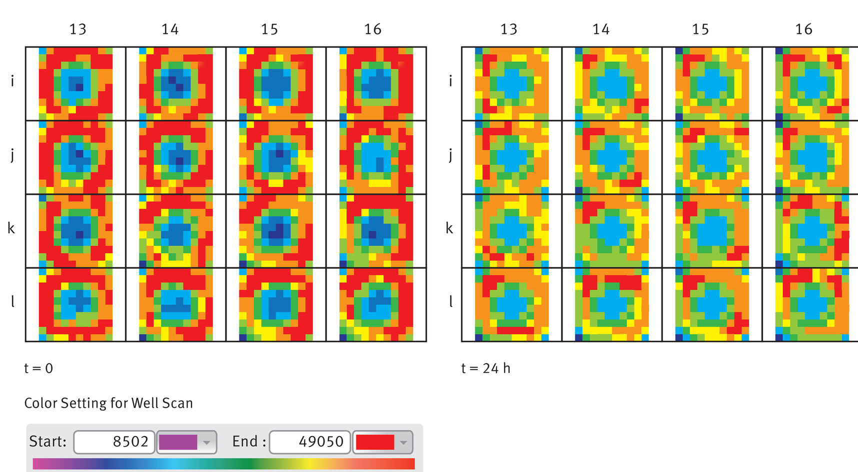


Fig. 2: Representative well scan data of MDA-MB-231 cells is presented. At t = 0 the heat map generated from a well scan shows high cell density outside of the cell free zone. At t = 24 hours the heat map shows a decreased cell density outside of the cell free zone while the cell free zone exhibits increased fluorescent signal indicating of cell migration.

Using orbital averaging allows the detection of changes in fluorescent signal within the cell free zone. The ACU enables changes in this signal, and therefore cell migration, to be quantified over time.

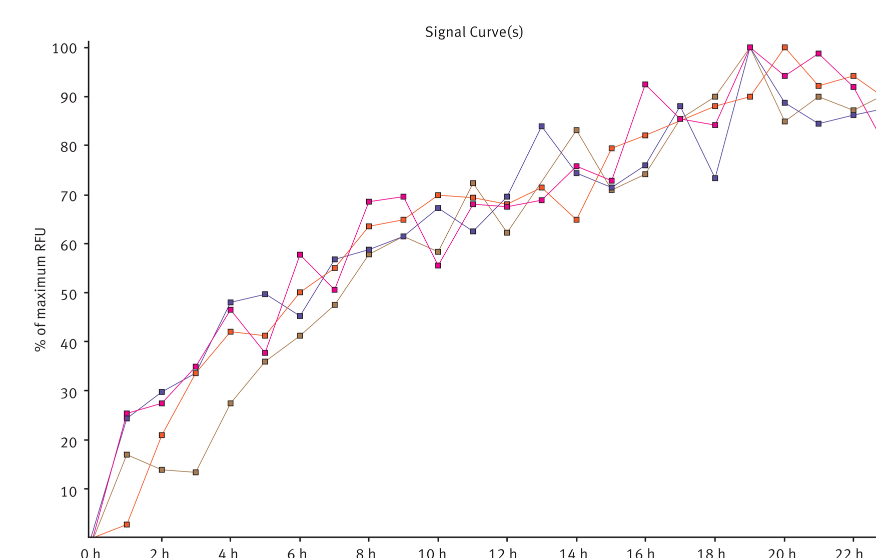


Fig. 3: Monitoring the change in fluorescence intensity in the cell free zone correlates with cell migration. Results from replicate wells (n= 4) for MDA-MB-231. The positive controls for migration indicate that reproducible changes in relative signal intensity and thus cell migration are observed over time.

Promega: Multiplexing Cell Viability Assay and Cytotoxicity Assay

Introduction

The RealTime-Glo® MT Cell Viability Assay is a bioluminescent assay that relies on the metabolic (MT) reducing potential of cells. NanoLuc® luciferase and cell-permeant pro-NanoLuc® substrate are added to cells in culture. Viable cells reduce the substrate which then diffuses into the medium where it is rapidly used by NanoLuc® enzyme to produce a luminescent signal proportional to viable cell number (Fig. 4)

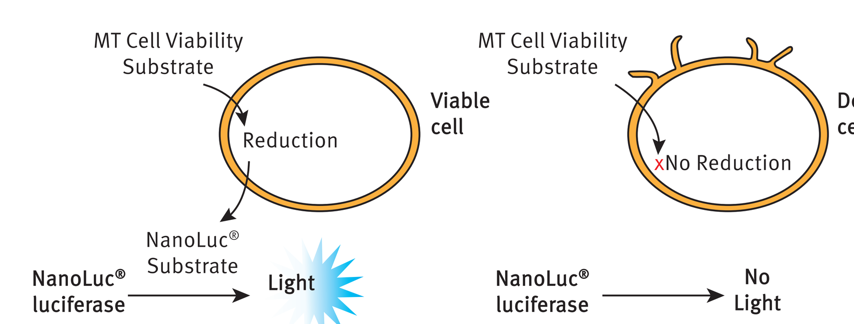


Fig. 4: The RealTime-Glo® MT Cell Viability Assay Principle.

The non-activity based CellTox™ Green Cytotoxicity Assay is comprised of a cell membrane impermeant dye that is excluded from viable cells. When the cell membrane becomes compromised, the dye enters the cell where it binds to DNA and becomes fluorescent. Fluorescent signal is proportional to the number of dead cells in culture (Fig. 5).

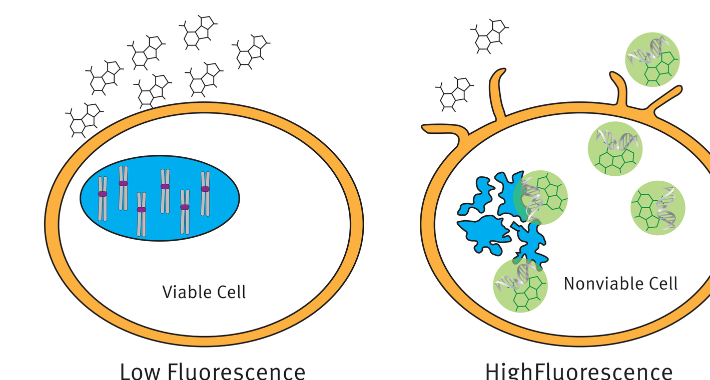


Fig. 5: The CellTox™ Green Cytotoxicity Assay Principle.

Materials and Methods

K562 cells were plated in 384 well microplates and treated with a panel of test compounds with known effects on proliferation and cytotoxicity. The CLARIOstar with ACU was used to both incubate cells and quantify luminescence and fluorescent signal changes every hour for 72 hours.

Results and Discussion

The CLARIOstar with ACU was able to fully sustain the normal proliferation and health of untreated cells for the entire 72 hour time course (Fig. 6)

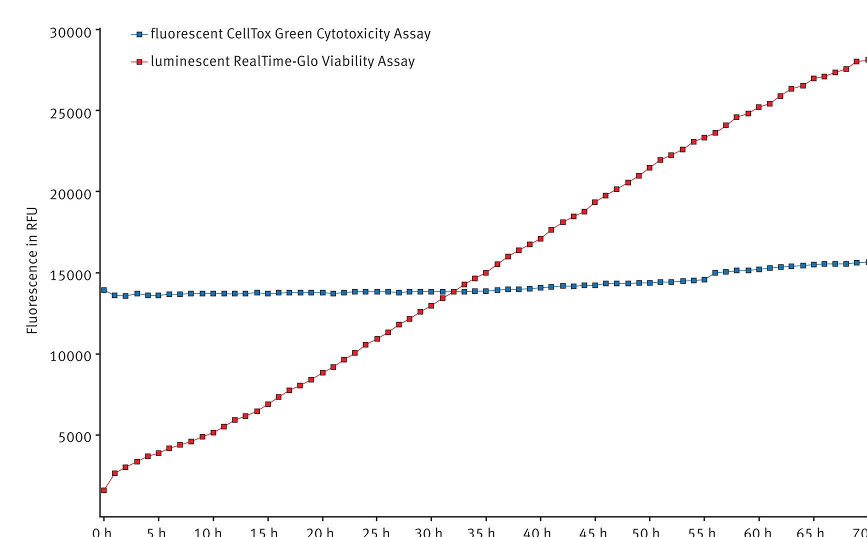


Fig. 6: Multiplexed RealTime-Glo® and CellTox™ Green assay. Average results of 10 replicates shows that cell viability increases and cytotoxicity is unchanged over 72 hours in untreated cells.

Cells treated with varying concentrations of the tyrosine kinase inhibitor bosutinib, initially exhibit proliferation although higher concentrations suppressed proliferation. For all but the lowest concentration, a change appears to occur around 25 hours and cell viability begins to decrease (Fig. 7).

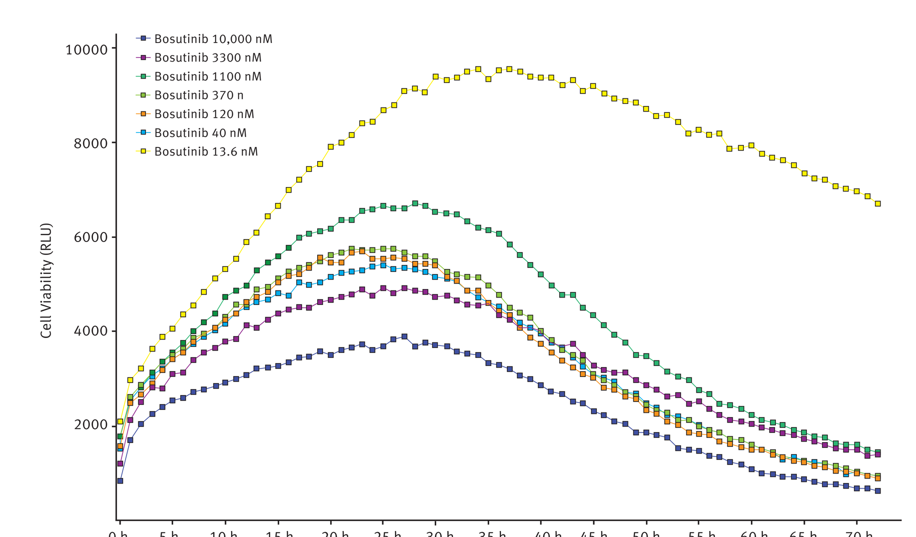


Fig. 7: Effect of varying concentrations of bosutinib on cell viability assessed using RealTime-Glo® MT Cell Viability Assay. Average results of triplicates at the indicated concentrations of bosutinib.

All concentrations of bosutinib also increased cytotoxicity to some degree. Cytotoxicity begins to increase at around 30 hours coincident with decreased viability (Fig. 8).

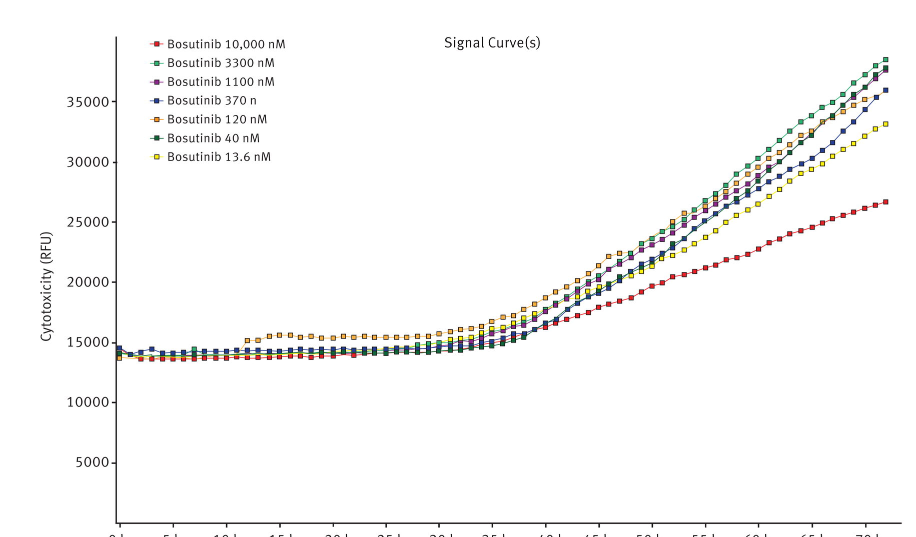


Fig. 8: Effect of varying concentrations of bosutinib on cytotoxicity assessed using CellTox™ Green Cytotoxicity Assay. Average results of triplicates at the indicated concentrations of bosutinib.

Visit

Booth #929