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

Carl Peters[1] and Tracy Worzella[2]
[1]BMG LABTECH, Cary, NC [2]Promega, Madison, WI

- **CLARIOstar with ACU maintains cell health and proliferation in untreated cells**
- **Cells can be monitored with duplexed assays**
- **Time- and dose-dependent compounds effects can be discovered directly in the cell culture**

Introduction

Analyses of cell viability and cytotoxicity 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 antiproliferative change occurs. When these assessments are performed with a microplate reader multiple samples and concentrations can be monitored on a single plate.

Two assays from Promega (RealTime-Glo® MT Cell Viability and the CellTox Green Cytotoxicity Assay) were measured over 72 hours. In order to maintain cell health the CLARIOstar was equipped with an Atmospheric Control Unit (ACU). This enables the detection of both time- and dose-dependent effects on cell proliferation and cytotoxicity.

Assay Principle

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. 1).

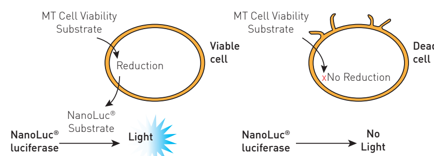

Fig. 1: The RealTime-Glo® MT Cell Viability Assay Principle.

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. 2).

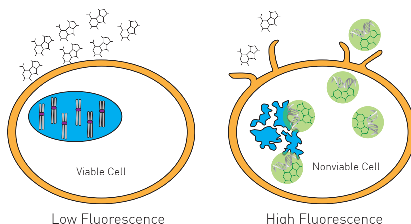

Fig. 2: The CellTox™ Green Cytotoxicity Assay Principle.

Materials & Methods

- 384-well, white, clear bottom microplates from Corning
- RealTime-Glo™ MT Cell Viability Assay from Promega
- CellTox™ Green Cytotoxicity Assay from Promega
- CLARIOstar® microplate reader from BMG LABTECH

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.

Fluorescence instrument settings

Method:	bottom reading
Flashes per well:	50
Optic Settings:	Excitation: F: 482-16/ Emission F: 530-40
Dichroic:	LP 504
No. of cycles:	73
Cycle time:	60 min
Gain + Focus :	adjusted prior to test

Luminescence instrument settings

Measurement interval time:	1.0 s
Optic settings:	No Filter
No. of cycles:	73
Cycle time:	60 min
Gain:	3500

ACU settings

CO_2	5 %
O_2	monitoring
Target temperature	37 °C

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Keywords: **ACU - Atmospheric Control Unit, cell viability, cytotoxicity, multiplexing, NanoLuc luciferase**

Results & 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. 3).

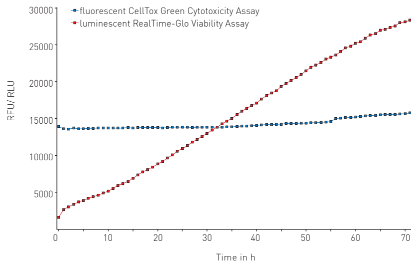

Fig. 3: Multiplexed RealTime-Glo® and CellTox™ Green assay. Average results of 10 replicates shows that cell viability increases and cytotoxicity is unchanged over 72 hours in untreated cells.

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. 4).

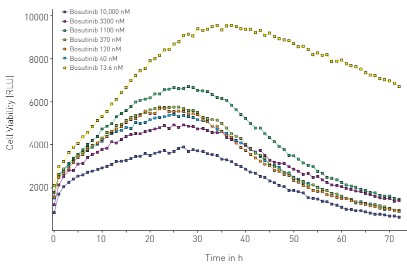

Fig. 4: Effect of varying concentrations of bosutinib on cell viability assesed using RealTime-Glo® MT Cell Viability Assay. Average results of triplicates at the indicated concentrations of bosutinib.

All concentrations of bosutinib also increased cytotoxicity to some degree. Cytotoxicity begins to increase at around 30 hours coincident with decreased viability (Fig. 5).

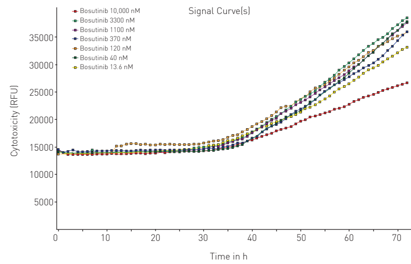

Fig. 5: Effect of varying concentrations of bosutinib on cytotoxicity assessed using CellTox™ Green Cytotoxicity Assay. Average results of triplicates at the indicated concentrations of bosutinib.

Conclusion

The CLARIOstar with ACU keeps cells happy so that long term cell-based assays can be achieved. In this case both cell viability and cytoxicity were monitored over 72 hours. Here we show the hourly assessment of the effect of the tyrosine kinase inhibitor bosutinib. The results show a clear time dependence for cytotoxicity as well as time and dose dependence for cell viability.