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New and improved cellular health evaluation of 2D and 3D cellular models using microplate reader assays

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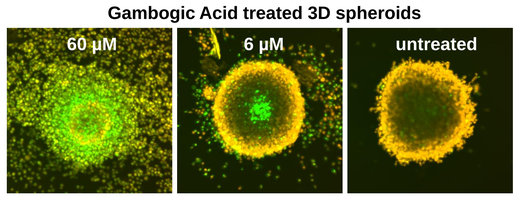


ABSTRACT

High throughput screening (HTS) is an effective method for identifying putative active compounds for therapeutics. Assays that evaluate changes in cellular functions are essential for characterizing test compounds and their potential role as pharmaceuticals.

Researchers in drug discovery and cancer research rely on reproducible biological assays to guide medicinal chemistry programs. A current goal in the cancer research field is to explore and expand the use of microplate readers and assay systems into the realm of 3D cellular models for drug discovery in anticancer research, specifically aimed at modelling solid tumors to screen compounds and confirm their activity. In this study, we screened multiple therapeutic drugs using different HTS assays to establish drug dose response curves and to understand the diversity in cell health assays. Despite the lack of guidelines and enabling technologies to study 3D cellular models, we establish the different impact therapeutics have on 2D versus 3D cellular models using existing cellular assays.

INTRODUCTION

The ability to use diverse cell health assays allows researchers to monitor different cell health read-outs and target specific cellular responses. The differences in potency and drug incubation time between 2D and 3D models indicate that drug effectiveness is dependent on cellular microenvironment. The ability to use existing microplate tools that are established for 2D cell models in 3D cell models provides a time and cost efficient basis for understanding therapeutic effects on 3D cellular models, allowing for rapid therapeutic characterization in drug discovery with minimal investment.

MATERIALS AND METHODS

When comparing existing cell health assays, we evaluated the differences between PrestoBlue™ HS and PrestoBlue. The PrestoBlue HS assay has a larger dynamic range, minimal background signal, and allows the removal of hotspots (false-positives). The PrestoBlue HS assay has the lowest detection threshold among the resazurin-based fluorescent assays available for detection of cell viability. With this advantage, the limit of quantification can be studied. Additionally, as an improved reduction potential assay, PrestoBlue HS can be used to interrogate the differences between 2D and 3D cellular models.

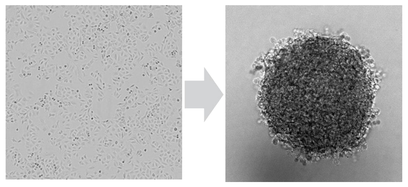
We demonstrate that existing microplate assays, such as PrestoBlue High Sensitivity (HS), CyQUANT® XTT, and CyQUANT® Direct, can be used to quantify functional differences between 2D and 3D cellular models. Using all three cell health assays, we found that a longer incubation time with drug is required to have an effect on the health of 3D models compared to 2D models. These results suggest that drug treatment is less potent in 3D models than 2D models, as demonstrated by a shift in the apparent IC50 values. Additionally, the dense compact structure of 3D models is preserved at low to no concentrations of drug, indicating that higher concentrations of drug are required to kill cells grown in 3D culture compared to 2D monolayers.

- High-Content Screening Platforms provide special and temporal resolution.
- High-Content Screening Platforms are equipped with wide field or confocal optics to allow for ease of multiplexing.
- Assays for microplate readers allow for rapid quantification of cell health measurements and enzyme activity.

RESULTS

Spheroids in Cancer Drug Discovery

Relevant pharmacology & better tumor models

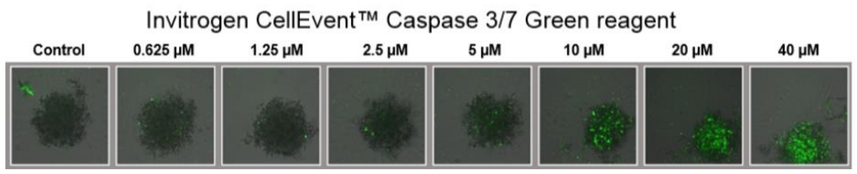


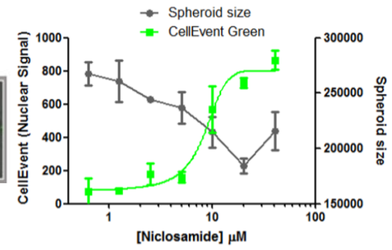
Working in 3D involves formation of spheroids. Spheroids are aggregates that can either be grown in suspension, encapsulated, or grown on the top of a 3D matrix.

	2D Monolayer	3D spheroids
Cellular Activity	Cell to Cell	Cell to ECM
Cellular Interactions	Cellular Adhesion, Proliferation, & modified genes	Proliferative Ring & Apoptotic core
Micro-environment	Immune Response therapy	Gradients to Oxygen, metabolites & nutrients

3D High Content Screening Platform: CellInsight™ CX7

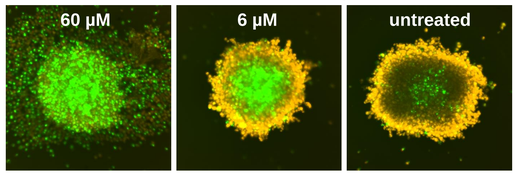
Assessing Spheroid Health using High Content Analysis

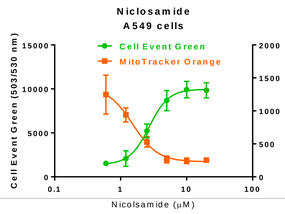




- CellEvent™ Caspase 3/7 detection reagent measures cleaved caspase 3, hall mark of apoptosis cells
- The spheroid size measurements is a phenotypic readout to measure drug effects.

Niclosamide treated 3D spheroids

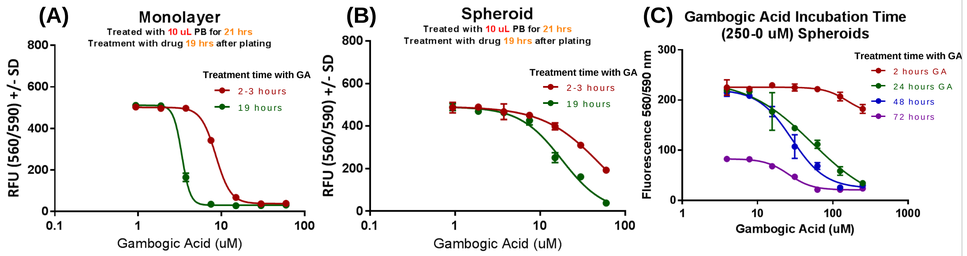




- MitoTracker™ detection reagent measures mitochondria health in live cells. The reagent is dependent upon membrane integrity.
- Mitochondria health is inversely proportional to the apoptotic pathway.

Measuring Cellular Health on microplate reader Varioskan™ LUX versus High Content Analysis System CX7

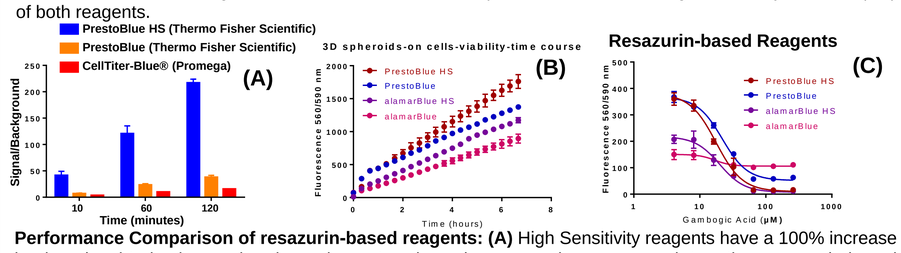
Measuring Cellular Health on Monolayer (2D) and Spheroid (3D) Cellular Models



Drug Potency on Monolayer (2D) and Spheroid (3D) Cellular Models: (A) As incubation time increases with gambogic Acid, the drug becomes more potent to 2D monolayer cells. Gambogic Acid is less potent to 3D spheroids than to 2D monolayer cells; shift in IC50 values. **(B)** High concentrations of Gambogic Acid drug are required to have an effect on spheroid health. Long incubation time (greater than 24 hours) is required with Gambogic Acid to effect Spheroid health.

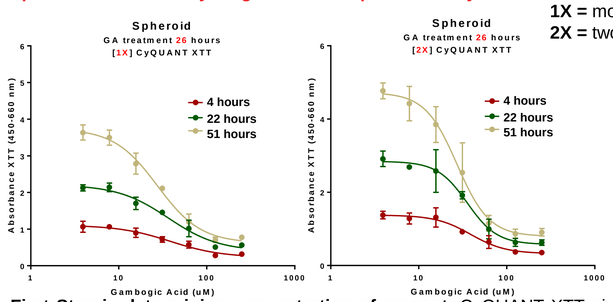
Enhanced Assay Performance

High Sensitivity Resazurin-based Reagents: An innovative purification process was developed that removes contaminants from the original PrestoBlue and alamarBlue products to make the High Sensitivity versions (HS) of both reagents.



Performance Comparison of resazurin-based reagents: (A) High Sensitivity reagents have a 100% increase in the signal-to-background ratio and greater dynamic range when compared to other resazurin-based reagents. **(B)** Cellular reduction kinetics on A549 spheroids **(C)** A549 spheroids were treated with Gambogic Acid for 24 hours and cell viability was measured with resazurin-based reagents (fluorescence response was measured at 60 minute).

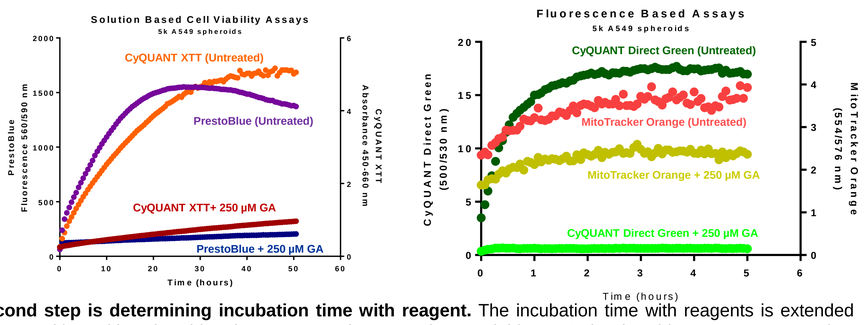
Optimization of viability reagents for 3D spheroid analysis



1X = monolayer working concentration
2X = two times monolayer working concentration

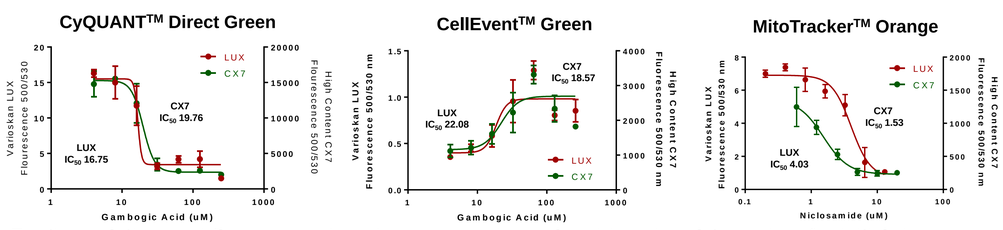
CyQUANT XTT incubation time	IC50s (µM)	
Concentration (µM)	1X	2X
4 hours	38.4	38.9
22 hours	36.5	35.5
51 hours	27.5	27.3

First Step is determining concentration of reagent. CyQUANT XTT gives a better signal/noise ratio and same IC50 value when used at twice the concentration on Spheroids when compared to one times the concentration on monolayer cellular models.

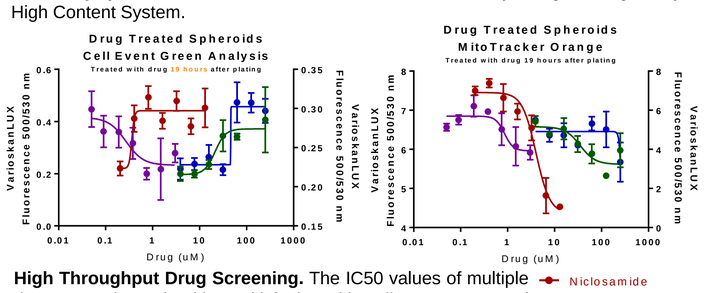


Second step is determining incubation time with reagent. The incubation time with reagents is extended when working with spheroids when compared to monolayer. Highly treated spheroids presents a very low signal turn-on, further confirming the presence of dead cells.

Using microplater reader Varioskan™ LUX to drug screen before image analysis on HCA System

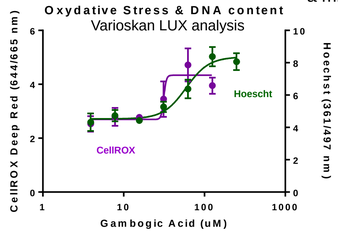


Existing solution- and fluorescence-based assays can be analyzed by microplate readers for initial drug discovery questions. Similar IC50 values are determined by using existing assays on the microplate reader and High Content System.

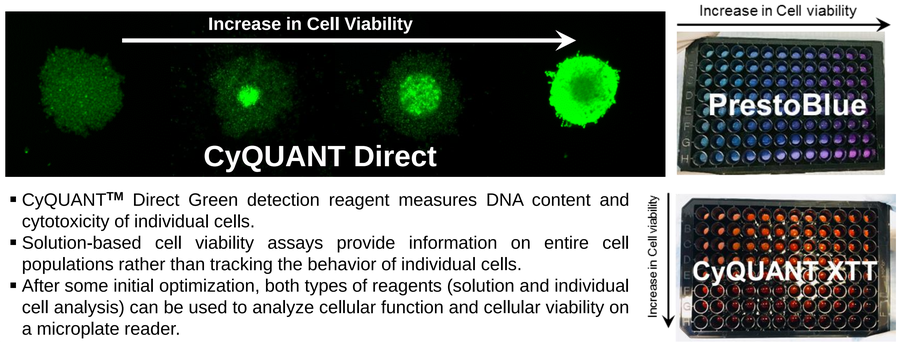


High Throughput Drug Screening. The IC50 values of multiple drugs were determined by multiplexing with CellEvent Green and MitoTracker Orange and analyzing on the Varioskan™ LUX microplate reader.

- Niclosamide
- Gambogic Acid Amide
- Gambogic Acid
- Nocodazole



Multiple Modes of cellular function can be monitored by microplate reader and existing cell functional assays



- CyQUANT™ Direct Green detection reagent measures DNA content and cytotoxicity of individual cells.
- Solution-based cell viability assays provide information on entire cell populations rather than tracking the behavior of individual cells.
- After some initial optimization, both types of reagents (solution and individual cell analysis) can be used to analyze cellular function and cellular viability on a microplate reader.

CONCLUSIONS

- Monolayer and Spheroids can be monitored across multiple platforms with multiple different reagents.
- Existing microplate assays can be used to quantify functional differences between 2D and 3D cellular models.
- Pharmaceutical drugs can be analyzed on a microplate as initial studies before further analysis on imaging platform using existing cell functional assays.

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