APPLICATION NOTE

Celigo provides an alternative method to Cell Titer-Glo for proliferation studies in suspension cells.

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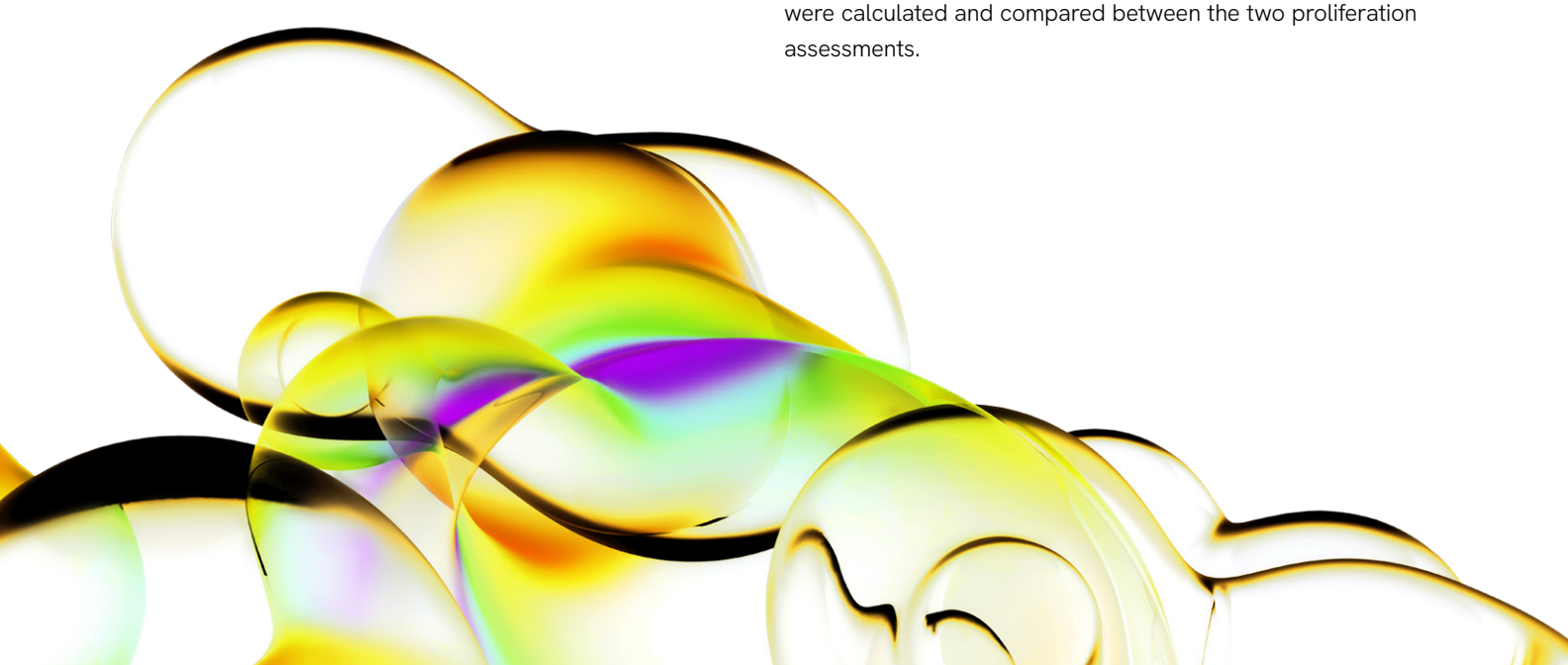
Introduction

Traditional cell proliferation assays involve multiple steps, expensive reagents, and are fixed end-point assays, prohibiting the same cells from being repeatedly assessed over a time course. Ignyta® was searching for a new, reagent-free proliferation detection method to determine the number of suspension cells per well. This new method should generate results comparable to their current one. Here, Ignyta® and Revvity carried out a reagent-free cell proliferation assay employing the Celigo® image cytometer[1] using suspension cells and proprietary compounds. The same experiment was performed using the Cell Titer-Glo® Assay[2], and the proliferation results were compared directly to Celigo.

Materials and methods

Four suspension cell types, including Ba/F3 parental cell line (Cell A), Ba/F3 expressing an oncogenic gene (Cell B), an oncogenic gene mutant A or B (Cell C and D respectively) were plated at a concentration of 5,000 cells/well and mixed in the presence of various concentrations of four drugs (1-4) at Day 0. On Day 3, the Celigo was used to image and analyze cell proliferation using the brightfield application, where whole-well images of each well were captured and analyzed in less than 5 minutes per plate. No reagent or additional incubation periods were required.

The same plate imaged and analyzed by Celigo was then used to evaluate cell proliferation following the standard Cell Titer-Glo® protocol, meaning the same wells were analyzed by both methods for a true comparison. Dose-response curves and IC_{50} values were calculated and compared between the two proliferation assessments.



Conclusions

Cell proliferation results between the two methods of Celigo and Cell Titer-Glo® were comparable and the generated data values were highly correlated (r^2=0.998)

- Celigo provides a simple, rapid, reagent-free way to determine cell proliferation in suspension cells
- Celigo provides images for visual verification of all results
- Celigo is not an end-point assay, meaning that the cells remain alive during the proliferation analysis, so cell proliferation can be assessed repeatedly over multiple time points throughout an experiment rather than single, fixed end points, thereby reducing time, supplies, and cost

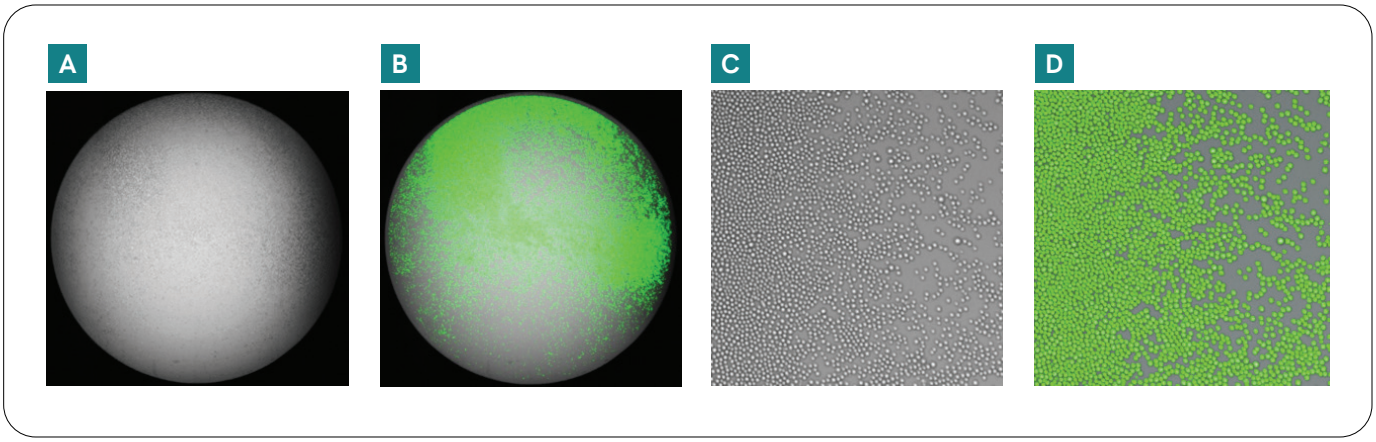
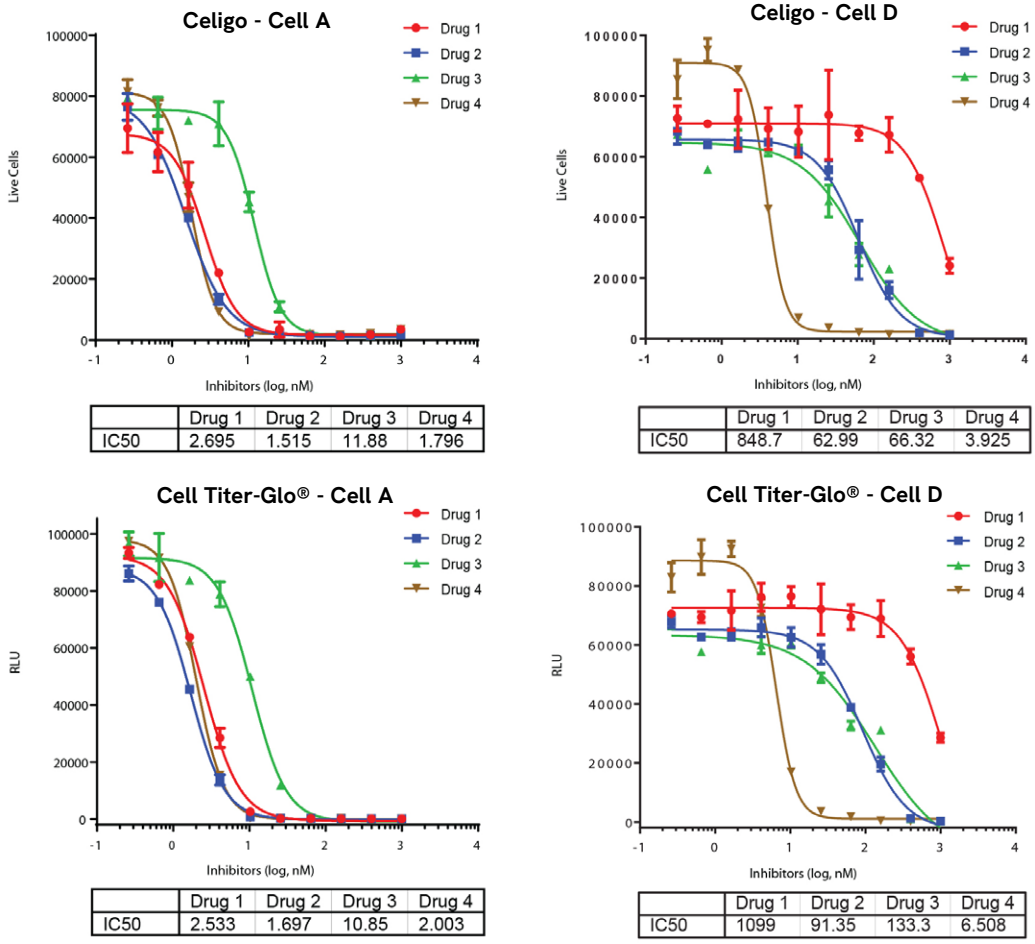


Figure 1: 1 Brightfield, whole-well images acquired and analyzed by Celigo. Celigo provides whole-well brightfield images and analysis that allow for user-visualization of the automatically generated cell counts, so images can be used to verify accuracy of the data. A= Wholewell brightfield. B= Whole-well brightfield analyzed. C= Zoom brightfield. D= Zoom segmented. Celigo resolution = 1 µm/pixel.

Table 1: Side-by-side comparison of IC_{50} values (nM) for cell types A-D with drugs 1-4 between the Celigo (dark gray columns) and Cell Titer-Glo® (light gray columns). CTG= Cell Titer-Glo®

	Celigo cell A	CTG cell A	Celigo cell B	CTG cell B	Celigo cell C	CTG cell C	Celigo cell D	CTG cell D
Drug 1	2.695	2.533	3.294	8.720	4.034	5.510	848.7	1099
Drug 2	1.515	1.697	1.493	3.300	1.863	2.234	62.99	91.35
Drug 3	11.88	10.85	51.60	45.32	14.39	14.27	66.32	133.3
Drug 4	1.796	2.003	~4.067	~4.239	2.391	3.269	3.925	6.508
Correlation across all cell types and drugs tested: r^2=0.9987								



	Drug 1	Drug 2	Drug 3	Drug 4
IC50	2.695	1.515	11.88	1.796

	Drug 1	Drug 2	Drug 3	Drug 4
IC50	848.7	62.99	66.32	3.925

	Drug 1	Drug 2	Drug 3	Drug 4
IC50	2.533	1.697	10.85	2.003

	Drug 1	Drug 2	Drug 3	Drug 4
IC50	1099	91.35	133.3	6.508

Figure 2: Side-by-side comparison of IC_{50} curves for cell types A (left) and D (right) treated with Drugs 1-4 between the Celigo (top row) and Cell Titer-Glo® (bottom row). All concentrations in nM.

References

1. Wang, YC et al. (2011) Different mechanisms for resistance to trastuzumab versus lapatinib in HER2-positive breast cancers--role of estrogen receptor and HER2 reactivation. Breast Cancer Res. 13(6):R121

2. Crouch, SP et al. (1993) The use of ATP bioluminescence as a measure of cell proliferation and cytotoxicity. J. Immunol. Methods 160, 81–8.

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