Corning® Spheroid Microplates and Promega CellTiter-Glo® 3D Cell Viability Assay provide a novel approach for high throughput screening of multicellular spheroids.

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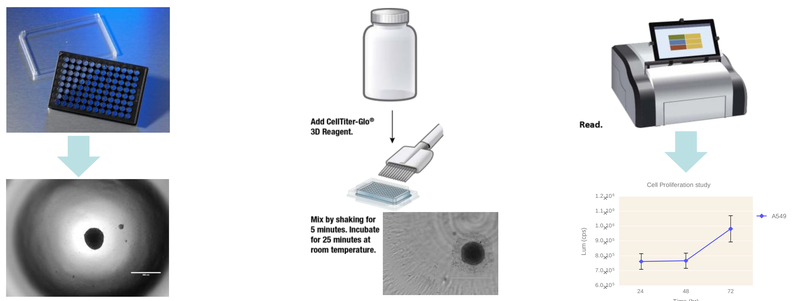
Introduction

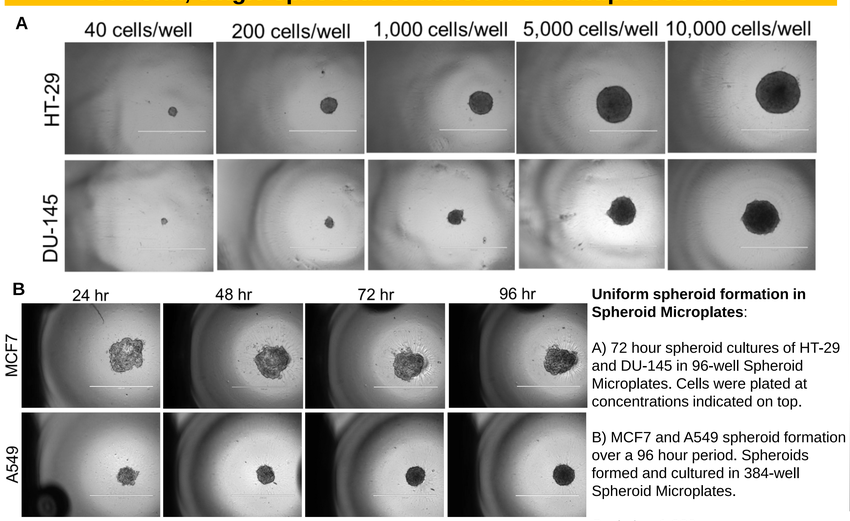
Three dimensional (3D) cultures are recognized as more physiologically relevant models since they provide a more accurate reflection of the intricate microenvironment of *in vivo* tumor models[1]. However, adoption in high throughput screening (HTS) platforms has been slow due to the limitations of the current technologies. Problems include increased variability, low throughput, and automation issues[2]. The new Corning Spheroid Microplates (Cat. Nos. 4520 and 3830) combine Corning Ultra-Low Attachment surface and an innovative well geometry to provide an ideal tool for generating, culturing, and assaying 3D multicellular spheroids in the same plate, without the need for a transfer step. Another issue limiting adoption of HTS spheroid assays is the inability of reagents to penetrate the multiple layers of 3D spheroids, leading to inaccurate data collection[3]. The new Promega CellTiter-Glo® 3D Cell Viability Assay (Cat. No. G9683) is a ready-to-use, one-step reagent specially formulated for improved cell lysis and extraction of ATP. The assay measures ATP to quantify viable cells, making it ideal for cell proliferation and cytotoxicity assay screening. In this study we demonstrated the ease of generating reliable, quantifiable data for high-throughput screening assays from multicellular spheroid tumor models using Corning Spheroid Microplates and Promega CellTiter-Glo® 3D Cell Viability Assay.

Easy to use, one-step addition homogeneous assay



Easy screening of multicellular spheroids: Single, uniform spheroids were generated, cultured, and assayed in Corning Spheroid Microplates. To quantify viable cells, Promega CellTiter-Glo® 3D reagent was added directly to wells. The microplates were read using an EnVision® Multilabel Plate Reader (PerkinElmer). The luminescent signal measured was proportional to the number of viable cells present in the culture.

Uniform, single spheroid formation with multiple cell lines



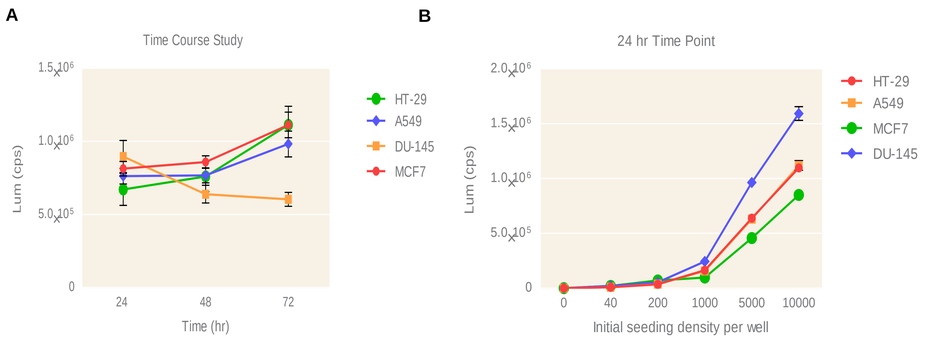
Uniform spheroid formation in Spheroid Microplates:

A) 72 hour spheroid cultures of HT-29 and DU-145 in 96-well Spheroid Microplates. Cells were plated at concentrations indicated on top.

B) MCF7 and A549 spheroid formation over a 96 hour period. Spheroids formed and cultured in 384-well Spheroid Microplates.

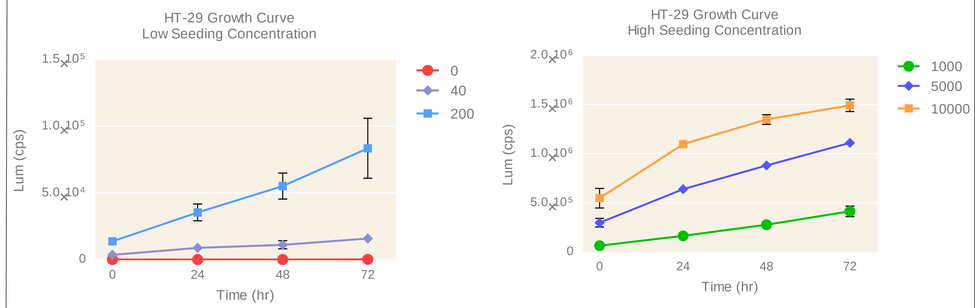
Scale bar 1,000 µm

Consistent and reproducible results



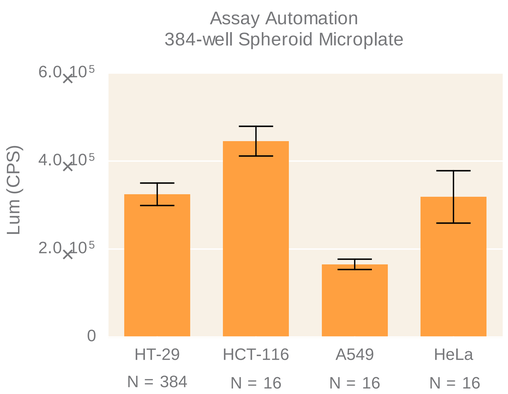
Consistent and reproducible results over a broad range of multicellular spheroids: Spheroid formation of four different cell lines in 96-well Spheroid Microplates was evaluated by adding CellTiter-Glo® 3D reagent to wells in a 1:1 ratio. A) Spheroid formation over a 72 hour period. All cells plated at 5,000 cells per well (data from 2 independent studies, n= 54 wells). B) Spheroid formation capabilities when using different seeding concentrations (representative data from one study, n=8 wells per cell concentration).

Accuracy and linearity of data



Linear and sensitive assay method: Four different cell lines were plated in 96-well Spheroid Microplates at different seeding concentrations. Spheroid growth was evaluated daily by adding CellTiter-Glo® 3D reagent straight to assay wells in a 1:1 ratio. CellTiter-Glo® 3D Cell Viability Assay demonstrated robust and consistent signals over a wide range of spheroid sizes. Representative data (n=8 wells per cell concentration).

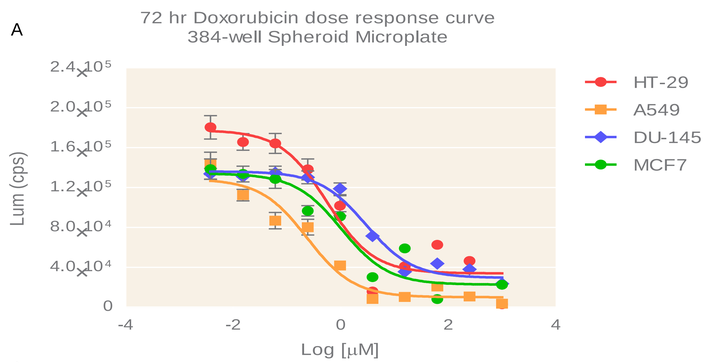
Automation for spheroid screening



	Z'	%CV
HT-29 (n=384)	0.763	7.84
HCT-116 (n=16)	0.772	7.57
A549 (n=16)	0.784	7.10
HeLa (n=16)	0.478	17.33

Automation friendly: To demonstrate automation friendliness, four different cell lines were plated into 384-well Spheroid Microplates using a Multidrop™ Combi Reagent Dispenser (Thermo Scientific). After 72 hours in culture, spheroids were washed once with PBS using an AquaMax® DW4 Microplate Washer (Molecular Devices). To quantify viable cells, a 1:1 mixture of CellTiter-Glo® 3D reagent and media were added directly to wells. Z `values >0.5 and %CVs of less than 20%, indicate an acceptable quality assay with very little variability.

Drug screening



Functionality of assay: A dose response study was used to demonstrate the functionality of the Spheroid Microplates and CellTiter-Glo® 3D Cell Viability Assay for multicellular spheroid drug screening. Four different cell lines were plated in 384-well Spheroid Microplates; HT-29 and A549 at 1,000 cells per well, DU-145 and MCF7 at 5,000 cells per well. Spheroids were cultured for 24 hours prior to treatment in a dose dependent manner with Doxorubicin. Drug toxicity was evaluated over a 72 hour period by adding CellTiter-Glo® 3D reagent in a 1:1 ratio into wells. A) Dose response curves of multicellular spheroids after 72 hour treatment. B) Screen captured shift in dose response of DU-145 spheroids over a 72 hour period. (Representative data, n=8 wells per concentration).

Summary

- Corning Spheroid Microplates are an optimal tool for generating, culturing, and assaying of multicellular spheroids.
  - Uniform, single spheroid formation in every well aids in maintaining reproducibility and accuracy of assay.
  - The design of the Spheroid Microplates allows for incorporation of automation and high-throughput screening.
- Promega CellTiter-Glo® 3D Cell Viability Assay is ideal for generating quantifiable data from multicellular spheroids.
  - Superior lytic ability and signal sensitivity allows for use over a broad range of spheroid types and sizes.
  - Homogenous one step reagent is optimal for automation and high-throughput screening.

References:
1. F. Pampaloni, et al., The third dimension bridges the gap between cell culture and live tissue. *Nat. Rev. Mol. Cell Biol.* 8(10), 839–845 (2007).
2. I. Vinci, et al., Advances in Establishment and Analysis of Three Dimensional Tumor Spheroid-Based Functional Assays for Target Validation and Drug Evaluation. *BMC Biology* 10:29 (2012).
3. G. Mehta, et al., Opportunities and challenges for use of tumor spheroids as models to test drug delivery and efficacy, *J. Control. Release* (2012), doi:10.1016/j.jconrel.2012.04.045

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