Rapid, reliable measurement of cellular proliferation and toxic compound effects on 3D spheroid cultures grown from multiple cancer cell lines with ATPlite™ 3D and ATPlite 1step 3D

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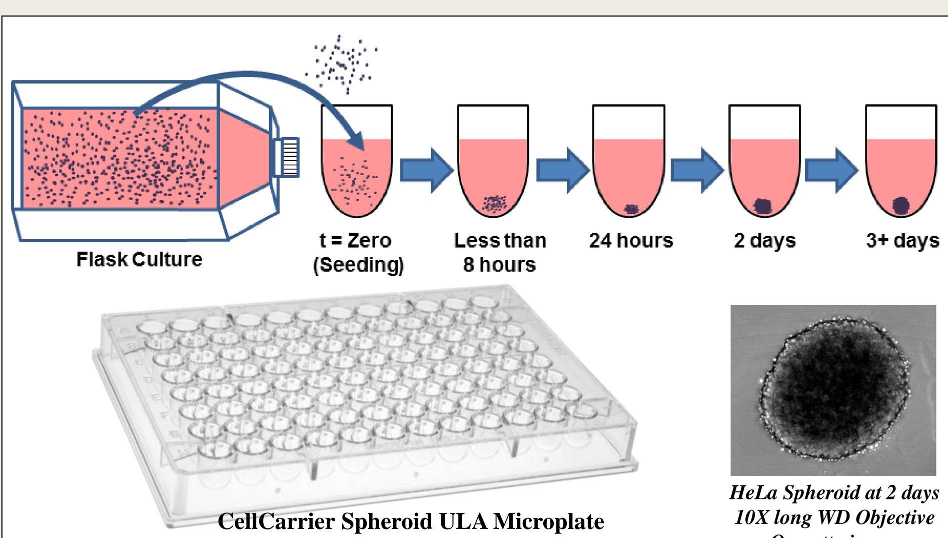
1 Introduction

Cellular viability and toxicity assays are fundamental tools in the drug discovery process – used to evaluate both the potency of compounds, as well as their toxicity profiles for early drug safety assessment. Toxicity is one of the major reasons for attrition during drug development and withdrawal from the market highlighting the need for the drug discovery process to utilize models that better represent clinical pathology. In many systems, three-dimensional (3D) cell culture methods can offer a more physiologically relevant context over traditional cell-culture models for the screening and identification of active compounds. One of the most common, simple, and efficient techniques for assaying cellular viability is using a luminescence-based assay that measures the amount of ATP present as an means of quantifying the number of metabolically active cells in a well. PerkinElmer's ATPlite products have been used by numerous scientists for several years to quantify ATP content in traditional 2D culture models. Cells cultured in 3D microtissues exhibit inherent differences in cellular adhesion and require substantially stronger lysis conditions than is sufficient for traditional monolayer or suspension cultures to access the cells deeper inside each spheroid. In order to address this need, we developed ATPlite 3D and ATPlite 1step 3D assay kits specifically for use with 3D cell cultures.

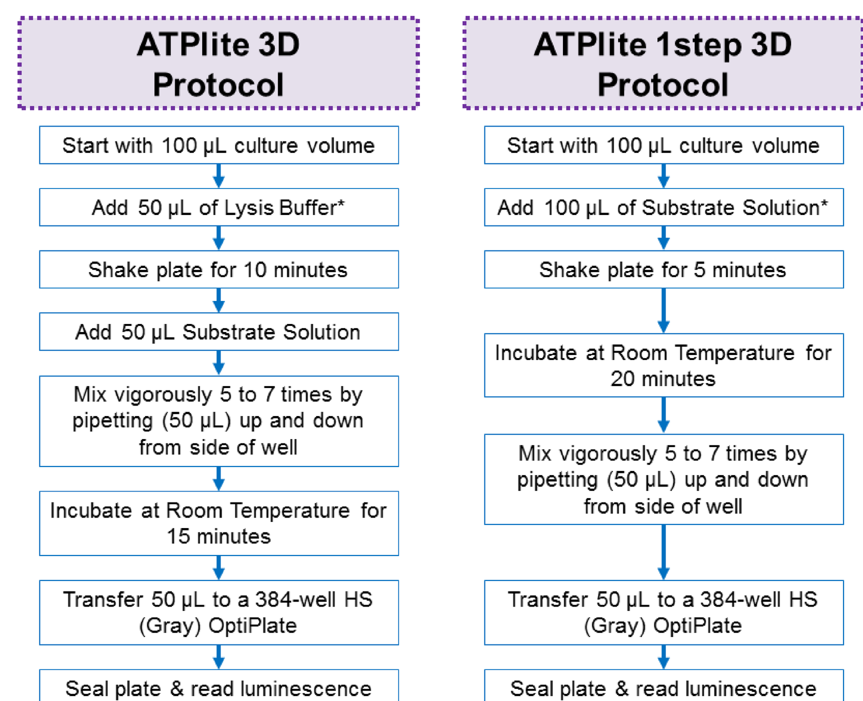
2 Materials & Methods



Workflow for seeding and growing cells in CellCarrier Spheroid ULA 96-well microplates

Cell Lines Used in these Experiments

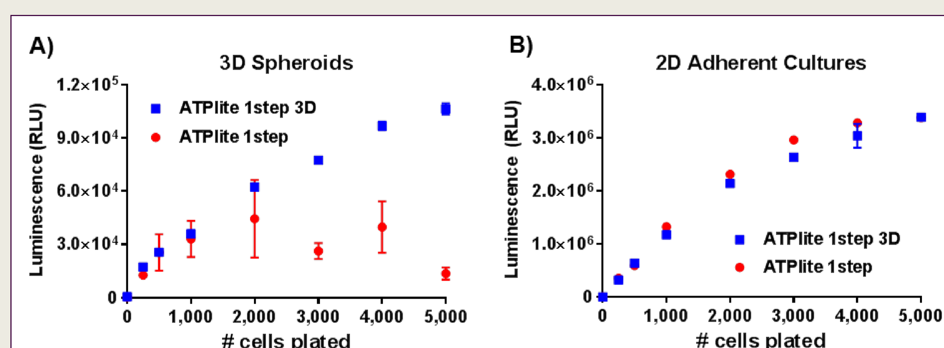
Cell Line	Description	ATCC Cat. No.
HeLa	Human Cervical Cancer model	CCL-2
DU 145	Human Prostate Cancer model	HTB-81
HEK293	Human Kidney Model	CRL-1573



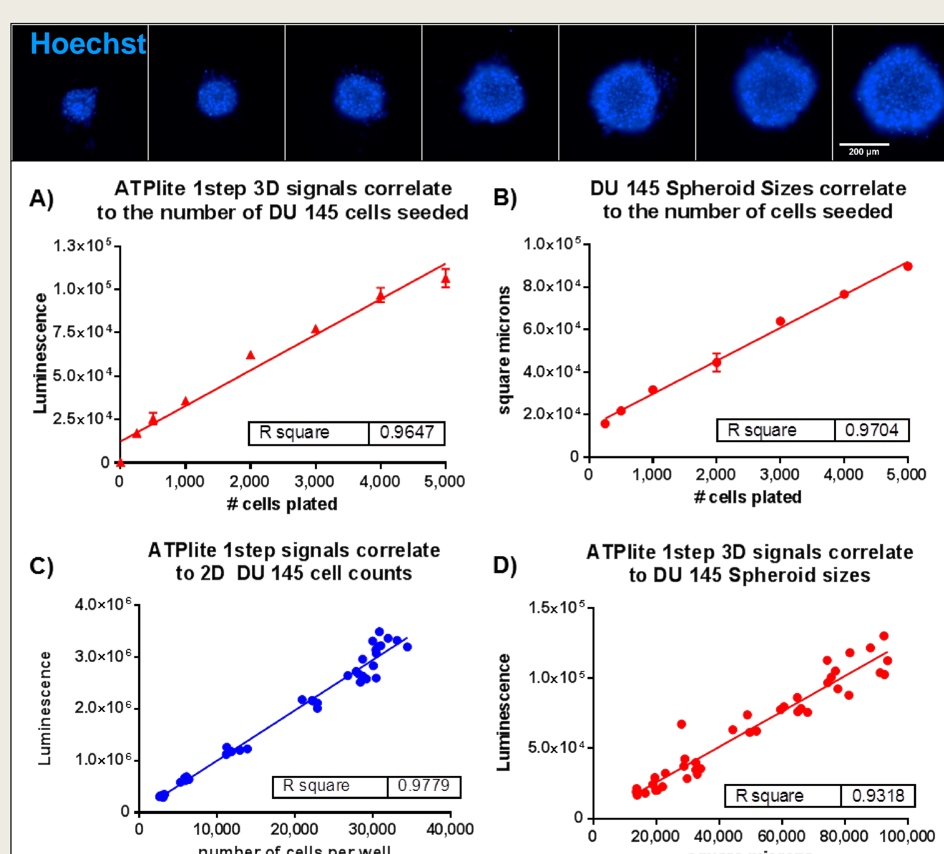
Overview of ATPlite 3D Protocols for use with 3D cultures grown in CellCarrier Spheroid microplates. Starting Cell Culture volume is 100 µL.
*Additional pipette-mixing steps can be added upon initial addition of Lysis Buffer or Substrate Solution for larger or more dense spheroid microtissues.

	Catalog Number	Pack Size
CellCarrier Spheroid ULA Microplates	6055330	10 pack
	6055334	Case of 40

3 Proliferation in 3D vs. 2D cultures

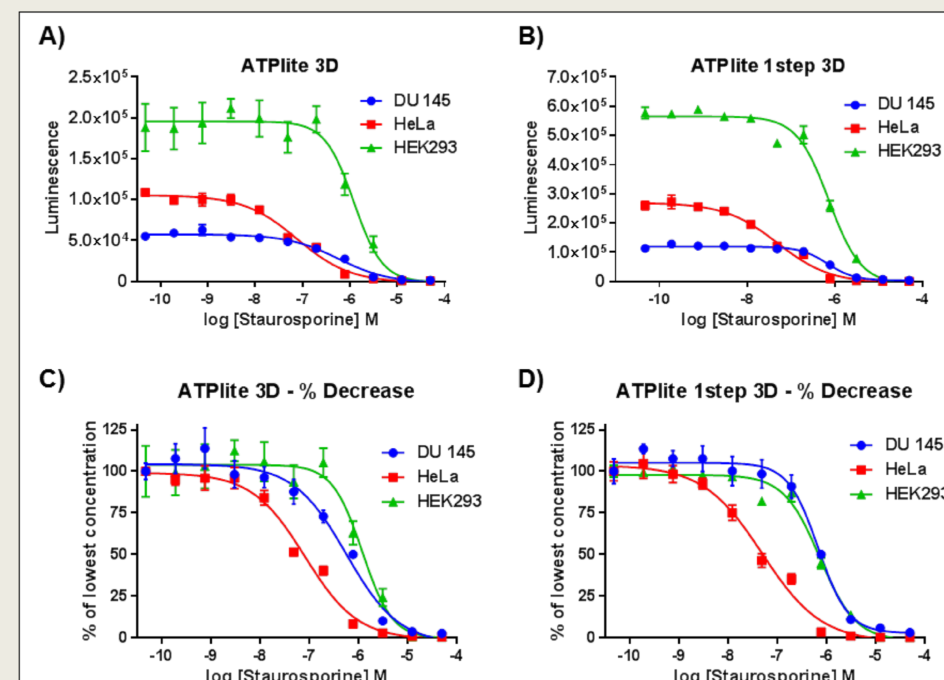


Comparison of ATPlite 1step and ATPlite 1step 3D in Spheroids and adherent (2D) cell cultures. DU 145 cells were seeded at the numbers indicated (x-axes) into CellCarrier Spheroid ULA (A) or standard CellCarrier 96-well microplates (B) and grown for 4 days. ATPlite 1step and ATPlite 1step 3D assays were run on both plate types with the final step being to transfer 50 µL of the reaction to a Gray OptiPlate-384 for luminescence reading on the EnSight (n=3, Error = SEM).

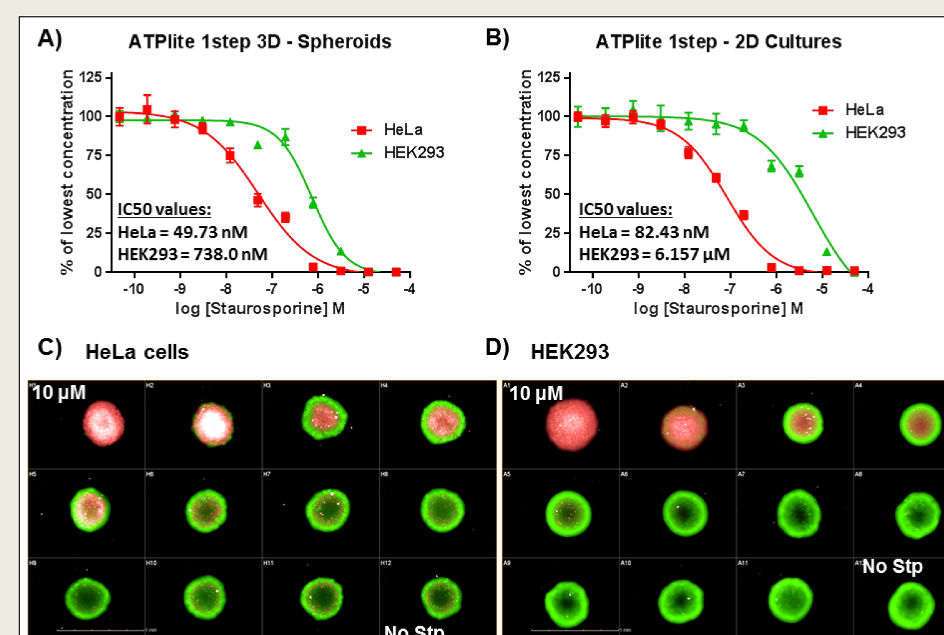


Data from ATPlite 1step 3D assays correlate with culture size. DU 145 cells were seeded into CellCarrier Spheroid ULA and standard 96-well black CellCarrier plates at the cell numbers specified and allowed to grow for 4 days. Cultures were labeled with Hoechst and 3D spheres were imaged in the UV channel with 10x long WD objective on the Operetta (images above graphs). Cross-sectional spheroid area was measured using an intensity cutoff. 2D cultures were imaged using the cellular imaging module of the EnSight plate reader. The "count nuclei" algorithm in Kaleido software was used to quantify the number of cells present in 2D cultures. (Luminescence data = average of 3 wells; Size data = average of 6 spheres, Error = SEM.)

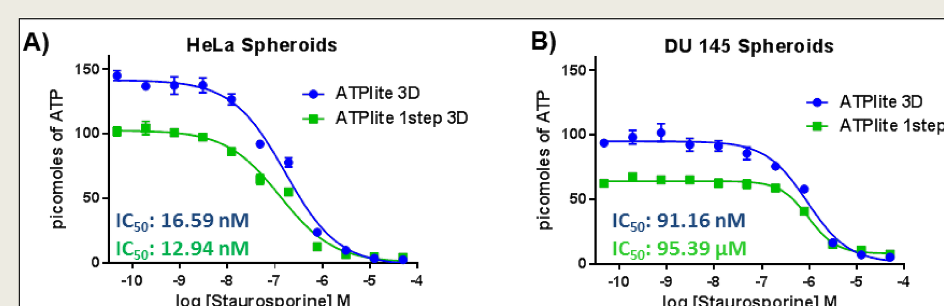
4 Toxicity Assessment



Toxic compound IC_{50} curves for 3 cell types. Cells were plated at 4,000 cells per well in CellCarrier Spheroid ULA plates and spheroids grown 3 days before overnight treatment with varying concentrations of Staurosporine (12 points) to induce a toxic response (A&B). To compare IC_{50} curves between cell types, data was normalized (C&D) to the average of wells with lowest concentration of compound (n=4, bars = SEM).



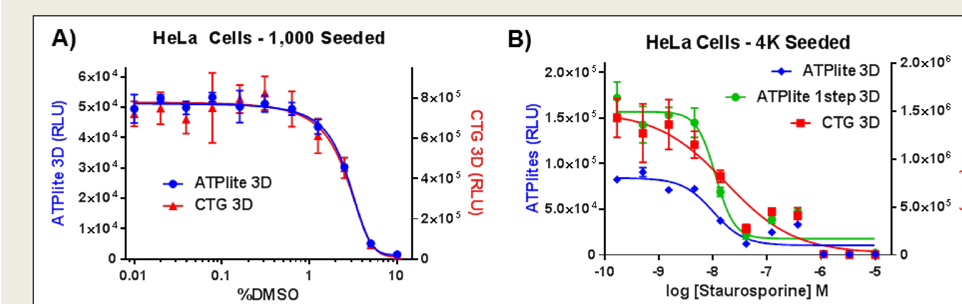
IC_{50} values compared between 3D Spheroid and 2D Cultures for HeLa and HEK293 cells plated at 4,000 cells per well in CellCarrier Spheroid ULA plates, grown for 3 days and treated overnight with Staurosporine. A subset of the wells (C, D) were stained with fluorescent dyes to identify live (Calcein AM; green) and dead (SyTox Red) cells, were imaged on the Operetta using the 10X long WD objective and fluorescence optics, and then assayed with ATPlite 1step 3D (A) or ATPlite 1step (B).



IC_{50} values compared between HeLa and HEK293 Spheroids. Cells were plated at 4,000 cells per well in CellCarrier Spheroid ULA plates, grown for 3 days and treated overnight with Staurosporine. A subset of the wells (spheroids) were stained with fluorescent dyes to identify live (Calcein AM; green) and dead (SyTox Red) cells; were imaged on the Operetta using the 10X long WD objective and fluorescence optics and then assayed with ATPlite 3D or ATPlite 1step 3D. The different cell types had a different sensitivity to Staurosporine, but both ATPlite 3D and ATPlite 1step 3D captured it similarly.

Assay Kit	Catalog Number	Kit Size (for 96-well plates)
ATPlite 3D	6066943	300 assay points
ATPlite 1step 3D	6066736	100 assay points

5 Assay Variability Comparison



Variability data for ATPlite 3D assays and a Competitor's assay. A) HeLa cultures were seeded at 1,000 cells per well in two CellCarrier Spheroid ULA plates and treated on day 2 with a DMSO titration overnight before running ATPlite 3D and a competitor's assay, CellTiter-Glo 3D. CellTiter-Glo 3D assay was run in the CellCarrier Spheroid plates as per the manual's instructions with the addition of a final transfer step of 50 µL to the Gray OptiPlate for luminescence measurement. While there was a difference in absolute signal level, data quality and the information generated by the ATPlite 3D assays were at least as good as that from the competitor's assay.

Assay Variability – CV's and Z-primes

Cell Type	Assay	mean 1	mean 2	S/B	n 1	n2	%CV1	%CV2	z'
HeLa	ATPlite 3D	296	37,129	*125*	16	16	20.10	7.01	**0.783**
HeLa	ATPlite 1step 3D	799	75,257	*94*	16	16	37.23	8.77	**0.722**
HeLa	CTG 3D	3,222	588,976	*183*	16	16	40.47	13.61	**0.583**
HEK293	ATPlite 3D	23,660	79,832	*3*	15	15	22.67	8.11	**0.368**
HEK293	ATPlite 1step 3D	37,818	181,486	*5*	16	16	15.29	4.27	**0.718**
HEK293	CTG 3D	493,647	1,361,293	*3*	16	16	53.48	9.42	**-0.356**
DU145	ATPlite 3D	840	21,516	*26*	16	15	16.00	9.73	**0.677**
DU145	ATPlite 1step 3D	3,818	53,908	*14*	16	16	14.21	5.75	**0.782**
DU145	CTG 3D	10,908	330,624	*30*	16	16	27.72	10.93	**0.633**

Variability data for ATPlite 3D assays and a Competitor's assay. Z-primes were calculated from raw luminescence signals from spheroids seeded at 1,000 cells per well from 3 cell types, grown for 3 days in CellCarrier Spheroid ULA plates and treated overnight with 5 µM Staurosporine or 0.5% DMSO.

6 Summary & Conclusions

- **Using CellCarrier Spheroid ULA microplates, we demonstrate a quick procedure for generating single, consistently round 3D microtissues from multiple cancer cell lines.**
- **We introduce here a reliable, straight-forward method for screening cytotoxicity in spheroid cultures with the new ATPlite™ 3D and ATPlite 1step 3D assay kits**
- **Data presented illustrate how ATPlite 3D kits can be used to evaluate and compare differences in proliferation and toxicity on cells grown in 2D versus 3D cell culture.**
- **We compared drug-responses of well-characterized toxic compounds on multiple cancer cell lines in 3D cultures.**
- **We demonstrate how ATPlite 3D kits compares well with a leading competitor assay with comparable or better penetration and with reduced variability.**

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