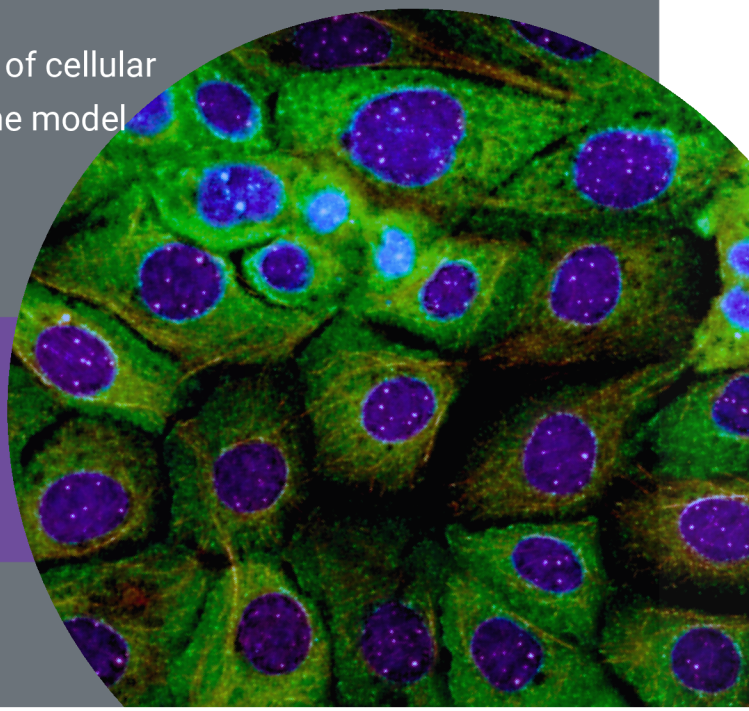
Paraza Pharma, Inc.

Cell Fate and Fitness Assays for Oncology

High-throughput viability assays are a cornerstone of screening cascades to guide SAR in oncology drug development. In the early stages of drug discovery, cell-based assays are indispensable for understanding the functional effects of new therapeutic agents. Our portfolio encompasses high-throughput assays to assess fundamental cell health indicators such as proliferation, viability and cytotoxicity, as well as more complex assays to quantify cell cycle alterations, senescence, apoptosis and more.

Leverage our team's experience in an extensive range of cellular assays to implement a cell fitness assay tailored to the model system of your choice.



- High throughput cell fitness assay
- Advanced readouts of cell fate
- Cell death measurements

Questions? Contact us

parazapharma.com/
info@parazapharma.com

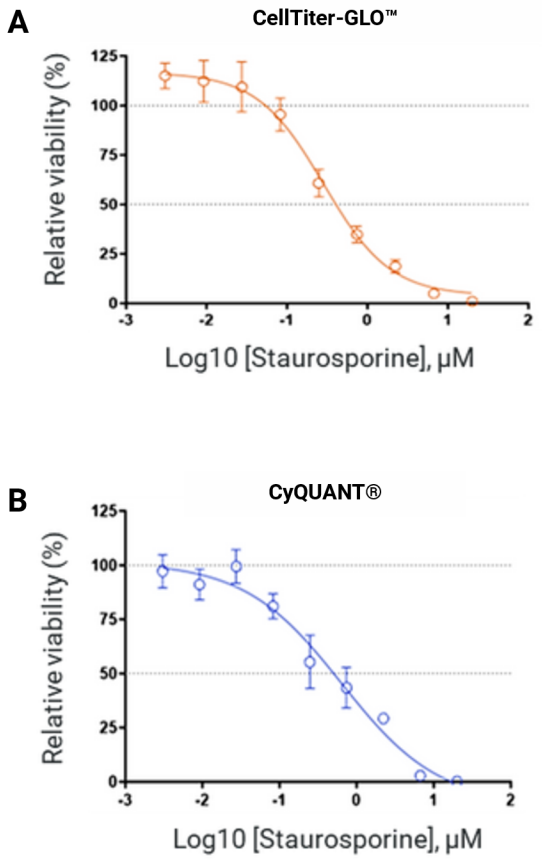
Canada: +1-514-395-4501
USA: +1-617-216-9459

High Throughput Cell Fitness assays

Viability and proliferation assays

High-throughput cell viability assays provide rapid and efficient insight into a compound's ability to kill or stop the growth of cancer cells, providing metrics of potency, efficacy and therapeutic window. Multiple readouts are available to assess cell viability/proliferation based on mitochondrial metabolic activity (XTT, MTT), enzymatic activity (LDH-Glo™, Calcein-AM), ATP content (CellTiter GLO™), protein (SRB) or DNA content (CyQuant®). In order to provide meaningful data, viability assays need to be carefully optimized in terms of duration, seeding density, re-feeding schedule and type of readout. Our scientists can guide you to design the most appropriate assay for your program and deliver accurate and reproducible results.

Fig 1. Comparative drug response in CTG (A) and CyQuant (B) assays.
Tecan D300e (Tecan) is used for dispensing compounds. Plates are read in luminescence or fluorescence mode with SpectraMax I3X reader (Molecular Devices).

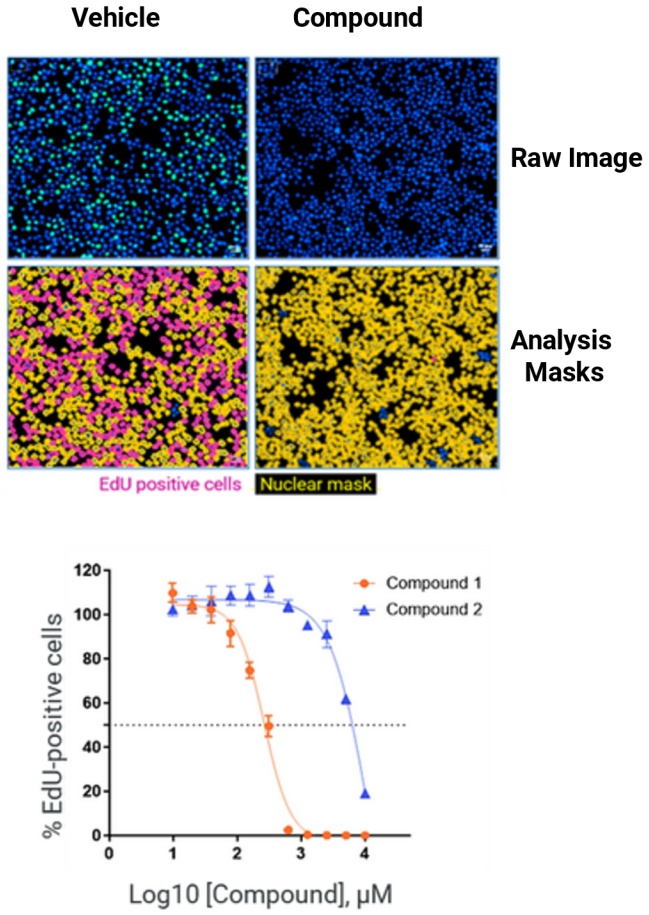


*CellTiter-GLO and LDH-GLO are registered trademarks of Promega Corporation.
**CyQUANT is a registered trademark of ThermoFisher Scientific.

EdU-incorporation assay

EdU (5-ethynyl-2'-deoxyuridine) incorporation, optimized for a plate-format imager, provides a high-throughput solution for studying compounds with a mechanism of action that impacts DNA replication. By integrating EdU into the DNA of actively dividing cells during S-phase, this assay enables precise quantification of actively proliferating cells (% EdU-positive cells). In addition, fluorescence intensity correlates with the rate of DNA synthesis [1]. In oncology programs, plate-format EdU incorporation can be used to generate dose-response curves of G1/S cell cycle inhibitors, DNA damaging agents, and DNA repair inhibitors using a mechanistically relevant read-out.

Fig 2. EdU incorporation assay and high-content image analysis.
Acquisition and analysis performed on BioTek Cytation5 (Agilent). Total cell count is obtained based on a nuclear mask (DAPI staining). EdU positive cell count is obtained based on GFP signal (Click-iT reaction) threshold within a primary mask.



Paraza Pharma, Inc.

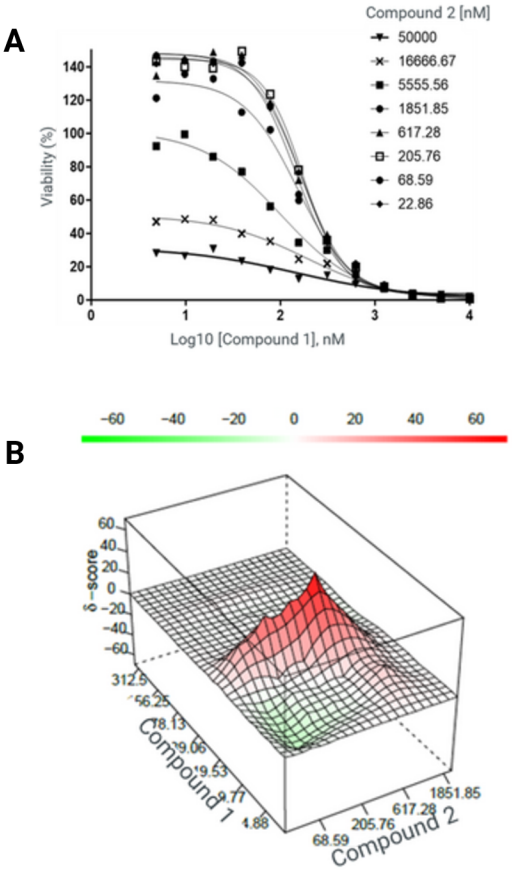
Advanced Readouts of Cell Fate

Matrix synergy assay

Drug combinations help to identify vulnerabilities and complementary pathways that, when targeted together, maximize therapeutic efficacy, overcome drug resistance, or reduce toxicity by allowing lower doses to be administered [2]. This approach fosters the development of more effective and personalized treatment regimens, ultimately improving patient outcomes. Matrix-type assays in plate format allow efficient and robust identification of synergistic drug interactions to enhance cancer cell killing beyond the effect of single agents. Combination studies are also central to the concept of synthetic lethality, a strategy particularly valuable in oncology drug discovery.

Fig 3. Matrix-type assay to measure synergistic interaction between two compounds in a viability assay.

Compounds matrix generated with Tecan D300e (Tecan). Image generated with the SynergyFinder 3.0 web application [3]. Dose-response curves for compound 1 in the presence of increasing concentrations of compound 2 ***(A)****. Matrix plot of Bliss synergy score* ***(B)****.*

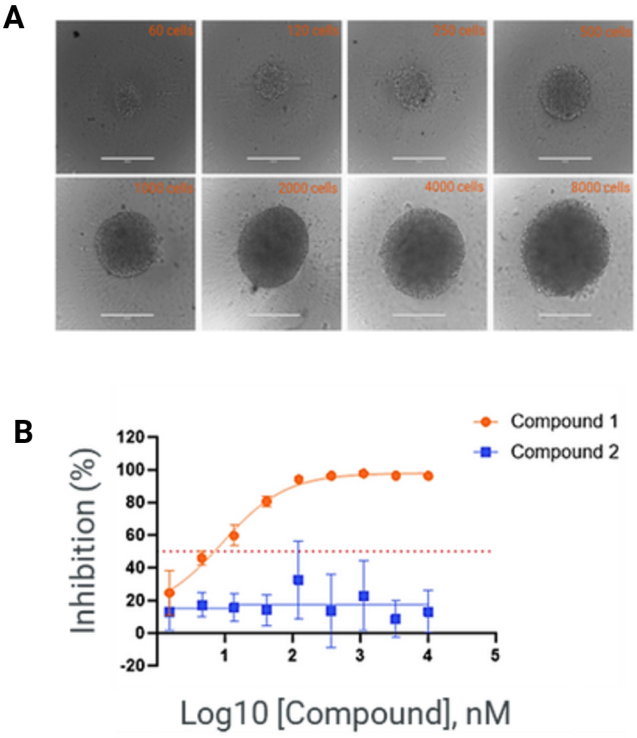


Viability in spheroid (3D) cultures

In the search for cell-based assays that better predict in vivo efficacy, there is growing interest in spheroids, which more accurately mimic tumor biology compared to monolayer cell cultures. Spheroids reproduce several aspects of the tumor microenvironment, such as nutrient and oxygen gradients, as well as tumor architecture, including the development of a necrotic core. Due to their tissue-like morphology, spheroid models can reveal reduced or enhance sensitivity to chemotherapeutic drugs compared to 2D cultures [4]. Spheroids are now amenable to high-throughput screening thanks to recent advances in hardware, consumables and reagents that make their growth and analysis more reliable and cost-effective. Our scientists have optimized spheroid growth assays for multiple cancer cell lines, and our expertise can be leveraged to develop new models of your choice.

Fig 4. Effect of two test compounds on spheroid viability.

Spheroids were seeded in 384-well ultra-low attachment plates and assessed by light microscopy ***(A)****. Example of dose response curves using Cell TiterGLO-3D™ (Promega)* ***(B)****.*



Paraza Pharma, Inc.

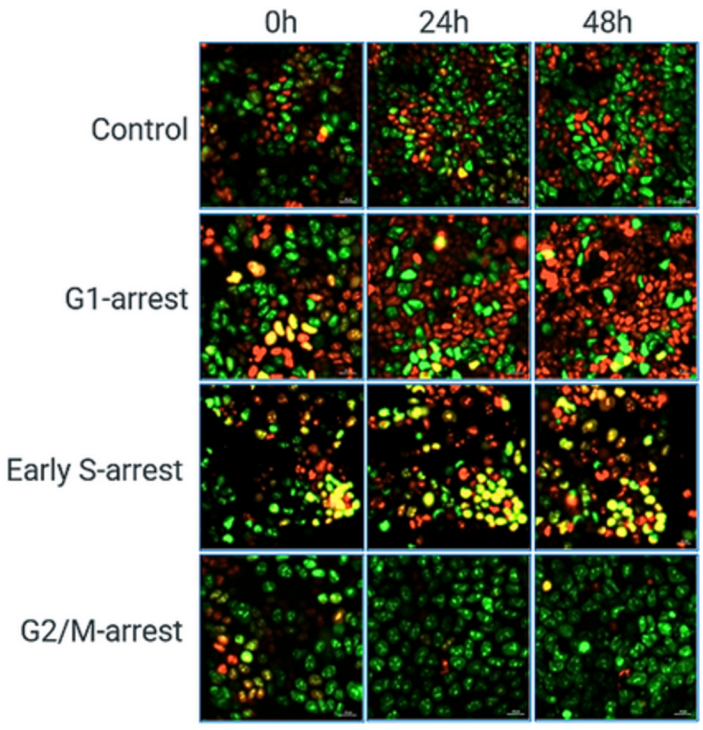
Advanced Readouts of Cell Fate (continued)

FUCCI cell cycle reporter

The FUCCI system (Fluorescent Ubiquitination-based Cell Cycle Indicator) is a tool designed to visualize the cell cycle dynamically in living cells. It leverages the oscillating levels of cell cycle-regulated proteins, specifically Geminin and Cdt1, fused to fluorescent proteins, to mark different phases of the cell cycle. It is amenable to multiplexing with additional fluorescent markers (e.g. for apoptosis), providing multiple layers of information simultaneously [5]. Our scientists have implemented several FUCCI models to study the effect of anticancer drugs on cell cycle regulation such as quantifying cell cycle arrest, describing tumor cell population heterogeneity, identifying subpopulations contributing to drug resistance, and tracking the kinetics of emerging resistance.

Fig 5. Cell cycle perturbations detected with FUCCI reporter.

Automated acquisition and analysis performed on BioTek Cytation5 (Agilent).

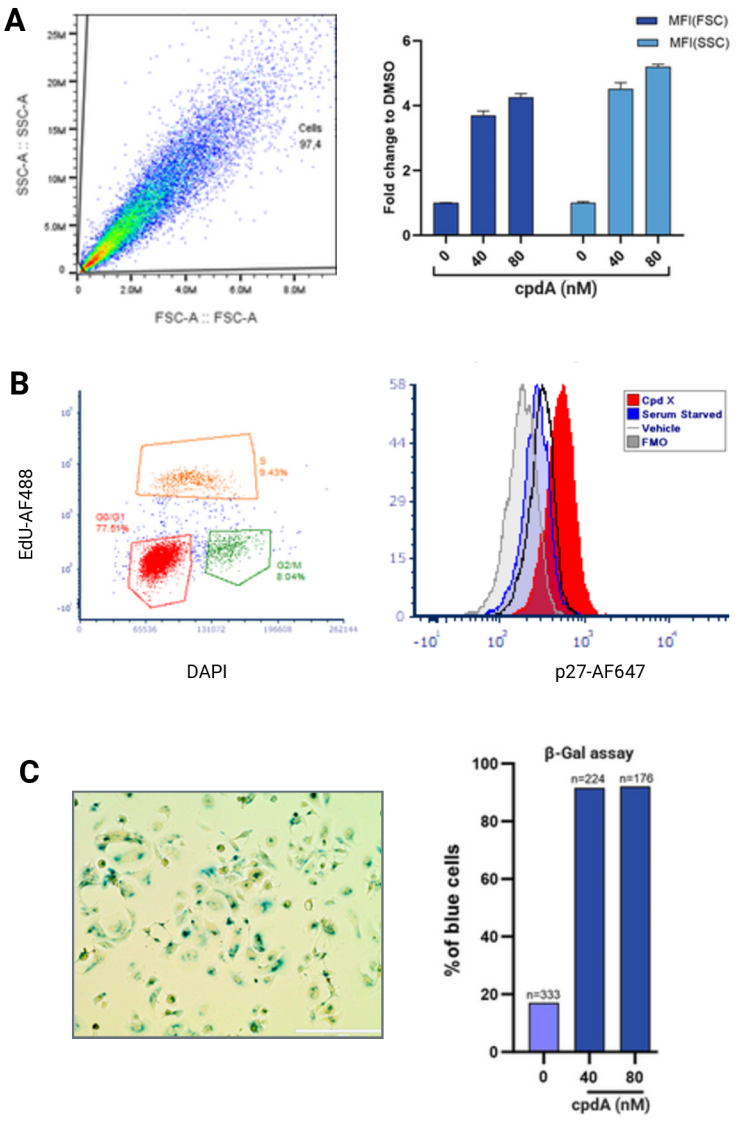


Senescence assays

Cellular senescence is a process by which cells exit the cell cycle and change their metabolism and phenotype. Anticancer therapy can induce cellular senescence, which can delay tumor growth but also constitute a mechanism of resistance. There is not a single assay that can unequivocally categorize cells as senescent, so a combination of readouts are needed to investigate this complex cell fate [6].

Fig 6. Analysis of cellular senescence.

*Senescent phenotype analyzed by flow cytometry by quantifying changes in cell size **(A)**, cell cycle arrest and p27 expression **(B)**. The appearance of senescence-associated beta-Gal activity was observed by light microscopy using a colorimetric substrate **(C)**.*



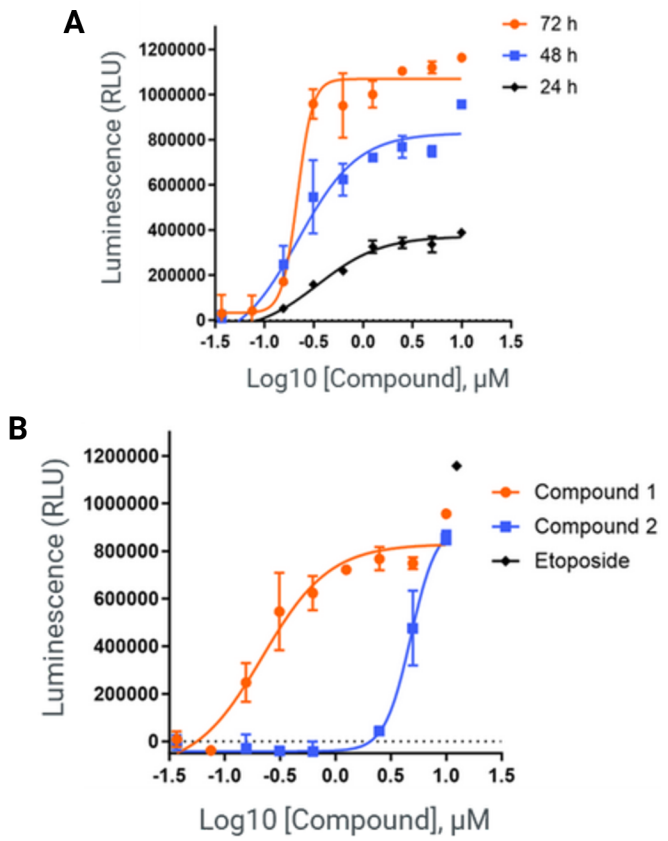
Cell Death Measurements

High-throughput cell death assays

Cell cytotoxicity can be measured using assays based on release of intracellular factors, like LDH, or dye penetration due to loss of membrane integrity. These assays do not discriminate between necrosis and other cell death mechanisms, but they do afford a fast and efficient way to measure the cytotoxic effect of a drug. Conversely, one may want to specifically measure induction of the apoptotic process, which is a key mechanism by which many anticancer drugs achieve their therapeutic effects [7]. The caspase-3/7 activity assay, with luminescent or fluorescent readout, is particularly well-suited for plate-based formats, enabling efficient automation and adaptation for high-throughput screening (HTS).

Fig 7. Caspase 3/7 activation kinetics for cpd 1 (A) and dose-response curve for two tested cpds (B).

*Caspase 3/7 activation determined in Caspase-GLO™ assay (Promega). Time-course analysis allows determination of time-point of maximal apoptotic signal **(A)**. Dose-response analysis of compounds at the optimal time **(B)**.*



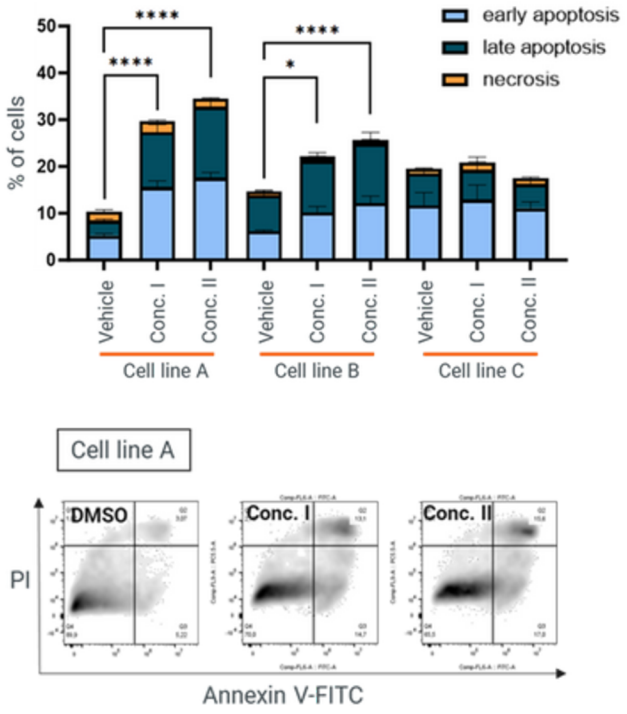
*Caspase-GLO is a registered trademark of Promega Corporation.

Annexin V /PI

Annexin V (AnnV) and propidium Iodide (PI) staining is the classic flow cytometry method to quantify apoptotic cells [8]. Early during the apoptotic process, cells flip phosphatidylserine to the outer layer of the cell membrane, where it can be bound by AnnV. As apoptosis progresses, loss of membrane integrity allows dyes like PI to enter the cell and bind to DNA. Flow cytometry analysis of AnnV-PI is particularly suited for analyzing immune cells, making it the method of choice for leukemia/lymphoma treatment evaluation. It can also be adapted for solid tumor samples using single-cell dissociation techniques.

Fig 8. Annexin V-PI analysis by flow cytometry.

Acquisition and analysis performed on CytoFLEX Flow Cytometer (Beckman Coulter) or with ViaStain Cell Fitness Panel on Cellaca MX (Sartorius).



References

[1] Stengl A. *et al*., SLAS Discov. 2017.
[2] Chen Y. *et al*., Clin Transl Med. 2023.
[3] https://synergyfinder.aittokallio.group/20250825211146597114/
[4] Selby M, et al., SLAS Discov. 2017.
[5] Fischer L. et al., Methods Mol Biol, vol 2644. 2023.
[6] Rodier F. et Campisi, J., J. Cell Biol. (2011) 192 (4): 547.
[7] Méry B. et al., J Cell Death. 2017.
[8] Crowley L. *et al*., Cold Spring Harb Protoc; 2016.

Paraza Pharma, Inc.