>> A Reproducible, Label-Free Platform for Real-Time Cytotoxicity and Hepatotoxicity Screening

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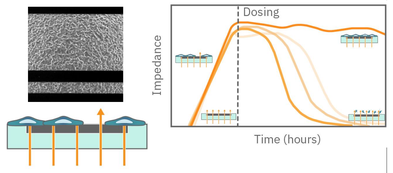
Maestro Z: Dynamic Cell Tracking

Impedance Technology

Cell-based *in vitro* cytotoxicity assays are a valuable tool for screening compounds for toxicity evaluation. Many in vitro cytotoxicity assays rely on dyes, or labels, to measure cell death at a single timepoint after a predetermined exposure time. Assessing the cytotoxicity of a compound label-free, *in vitro,* and at high throughputs is vital for toxicology evaluation.

Axion BioSystems' Maestro Z platform offers impedance-based cell analysis for real-time, label-free monitoring of cell viability, morphology, cytolysis, and signaling. Here, we used the Maestro Z to characterize a cytotoxicity assay for high-throughput screening and dose response analysis.



The impedance is measured from electrodes embedded in the bottom of each well. As cells cover more of the electrode, impedance increases in proportion to the number of viable cells. If a perturbation kills the attached cells, impedance decreases as the cells lyse.

The Maestro Z Product Family

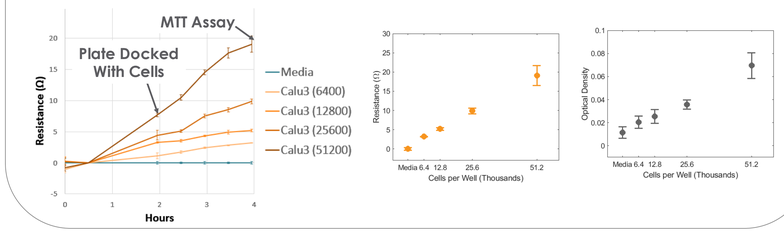
Features	*Maestro Z*	*Maestro TrayZ*	*Maestro ZHT*
Throughput:	96-well	Up to 8 x 96-well	384- and 96-well
Environmental Controls:	Built-in	External	Built-in
GxP Compatible:	✓	✓	✓
Barcode Plate Tracking:	✓	✓	✓
Automation API:	✓	No	✓
Dimensions *(WxDxH)*:	280 x 413 x 225 mm	440 x 450 x 60 mm	280 x 452 x 225 mm

- ***Label-free, non-invasive tracking*** *of cultured cells or spheroids/organoids*
- ***Integrated environmental control*** *provides a stable benchtop environment for short- and long-term toxicity studies*
- ***Automatic and continuous cell monitoring*** *from 96 or 384 wells simultaneously*
- ***"One button setup"*** *automatically docks the plate and adjusts temperature and CO2 levels*
- ***Powerful data analysis*** *to focus on the science, while AxIS Z handles the details with simple setup and automatic experiment tracking*
- ***See your cells*** *with the viewing window included in each well of the CytoView-Z 96-well plate.*
- ***State-of-the-art electrode processing chip (BioCore v4)*** *offers stronger signals, ultra-low frequency content, and enhanced flexibility*

BioCore v4

Direct Correlation of Impedance Assay with Cell Number

To validate impedance-based monitoring of cell viability, Calu-3 cells were added to a CytoView-Z plate with varying number of cells per well and monitored for four hours on the Maestro Z platform. The change in resistance was correlated with the number of cells initially seeded, and the resistance continued to increase as the cells adhered and flattened on the surface. At four hours post-seeding, the plate was removed and an MTT assay was performed in the CytoView-Z plate. The resistance measured with the Maestro Z platform was linear with respect to cell number and directly correlated to the MTT assay readings from the same wells.

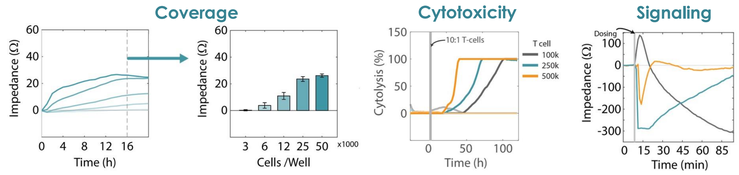


Real-time, Label-free Cytotoxicity Assay

Impedance Assay Measures Diverse Cell Properties

The Maestro Z records impedance at multiple frequencies simultaneously, enabling a thorough characterization of cell behavior, including:

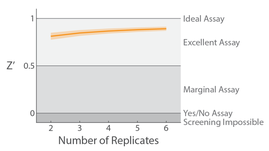
- **Coverage/Density** – the change in impedance is directly related to the quantity of cells in a 2D and 3D culture covering the electrodes.
- **Cytotoxicity** – dynamic monitoring of cell viability provides measures of the degree and speed of cell death.
- **Signaling** – small changes in cell shape or cytoskeleton organization are detected in response to intracellular signaling events

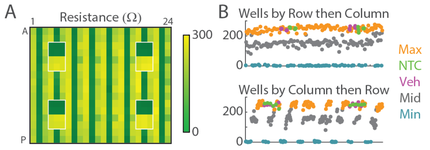


Maestro Z Cytotoxicity Assay is Sensitive and Consistent

High throughput screening requires careful validation of assay performance. Z-prime (Z'), which defines the statistical separation between positive and negative controls in an assay, is a widely accepted metric for assessing assay performance. Higher Z-prime values indicate separation of positive and negative compounds.

Here, we performed a validation study on an impedance-based cytotoxicity assay using the Maestro ZHT platform and CytoView-Z 384-well plates. A549 cells were cultured and treated with mitoxantrone at three concentrations to create "Max", "Mid", and "Min" treatment groups that were then used to calculate Z' values.





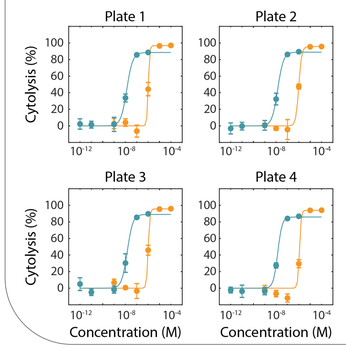
- The Maestro Z cytotoxicity assay classified as an "excellent" assay, with Z' = 0.81 ± 0.03.
- Further, the coefficient of variation was ~5% for the "Max" condition and consistent across plates.
- No significant drift or edge effect was observed.

	Coeff. Var. (%) Min*	Mid	Max	Z'	Drift	Edge	Pass
Criteria	<SD_{max}	<20	<20	>0.4	None	None	All
Plate 1	✓	11.2	4.4	0.83	✓	✓	✓
Plate 2	✓	7.6	4.6	0.83	✓	✓	✓
Plate 3	✓	8.9	5.7	0.77	✓	✓	✓

*SD_{min} is compared with SD_{max} because $\mu_{min} \approx 0$

Scalable Dose Response Analysis with TrayZ Cytotoxicity Assay

The TrayZ platform can perform dose response analysis from four CytoView-Z 96-well plates simultaneously with label-free impedance measurements inside a standard incubator. Here, we show the EC50 for Paclitaxel and Doxorubicin, dosed onto SKOV3 cells, across four plates measured simultaneously. We observed strong agreement in the dose response analysis across plates for each compound.



EC50 Values

	Plate 1	Plate 2	Plate 3	Plate 4
Paclitaxel	13.2 nM	13.9 nM	14.5 nM	13.9 nM
Doxorubicin	1.02 µM	0.99 µM	1.01 µM	1.09 µM

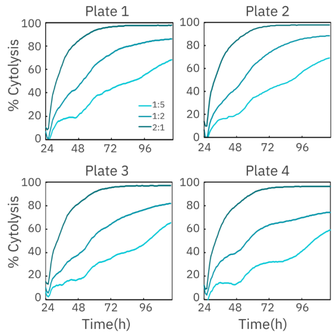
- **SKOV3 cells were treated with paclitaxel and doxorubicin (left) in a 6-point concentration sweep.**
- **Dose response analysis (above, right) measures the EC50 consistently across plates.**

Multi-Plate Dose Response Analysis

Multi-Plate CAR T Cell Potency Dose Response with the TrayZ

Not only can the TrayZ platform be used to study the dose response of anti-tumorigenic compounds, but it also can be used to study the cytotoxic potential of chimeric antigen receptor (CAR) T cells delivered at multiple E:T ratios. CAR T cells are engineered to target a specific antigen that, once engaged, leads to robust CAR T cell activation and subsequent killing of the target cell population.

Here, we cocultured HER2-positive SKOV3 target cells treated with CAR T cells targeted towards the HER2 antigen. We measured SKOV3 cytolysis caused by the CAR T cells at various E:T ratios (1:5, 1:2, and 2:1) across 4 different plates docked simultaneously on the TrayZ platform. We measured the Kill Time 50 (KT50) values (i.e., the time it took each CAR T cell dose to induce 50% cytolysis) and found consistent results across all four plates measured on the TrayZ.



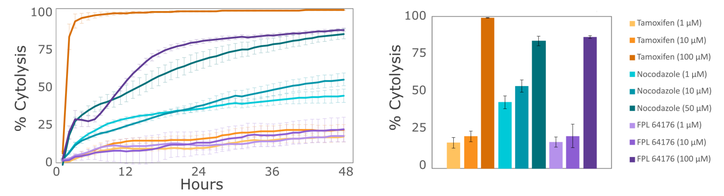
KT50 Values

	Plate 1	Plate 2	Plate 3	Plate 4
1:5	67.7	68.6	79.0	76.8
1:2	29.1	31.0	34.4	36.2
2:1	9.82	10.2	10.7	10.8

- SKOV3 cells were dosed with HER2-targeted CAR T cells at three different E:T ratios, and cytolysis was measured in real-time over multiple days.
- Dose response analysis (above, right) measured the KT50 values consistently across all four plates on the TrayZ system.

Label-Free Impedance Monitoring Reveals Dose-Dependent Hepatotoxicity Profiles

Next, we cultured HepaRG cells, an immortalized cell line that retains some of the characteristics of primary human hepatocytes, treated with various cytotoxic compounds. Time-course analysis over 48 hours demonstrates clear, dose-dependent cytotoxic responses across compounds. Tamoxifen induced rapid and near-complete cytolysis at 100 µM, with lower concentrations producing modest effects. Nocodazole elicited intermediate, concentration-dependent cytolysis, reaching substantial but incomplete cell loss at higher doses. FPL 64176 produced a delayed yet pronounced cytolytic response at 100 µM, while lower concentrations showed limited activity. Cytolysis at 48 hours confirms distinct potency and efficacy profiles, highlighting the platform's sensitivity for resolving kinetic and concentration-dependent differences in compound-induced cytotoxicity.



Conclusions

- Overall, the Maestro Z platform enabled continuous, dynamic, label-free quantification of cell attachment and proliferation, along with the potency and kinetics of drug-induced cytolysis.
- An assay validation study was performed on the Maestro Z cytotoxicity assay. The assay exhibited low coefficient of variation (~5%) and Z' = 0.81, without evidence of spatial effects (e.g. drift or edge effects) across the plate.
- Integrated dose response analysis within the AxIS Z software provides easy quantification of potency for drugs, effector cells, or other treatments.
- Overall, the hepatocyte impedance data demonstrate compound-specific, dose-dependent cytolytic responses, that highlight differential hepatotoxic liability across treatments.
- The TrayZ multi-plate system consistently measures the cytotoxicity of anti-tumorigenic compounds CAR T cells across plates, allowing for accurate, high-throughput dose response analysis.