>> Real-Time, Label-Free Assessment of HER2-targeted Antibody-Drug Conjugate Therapies

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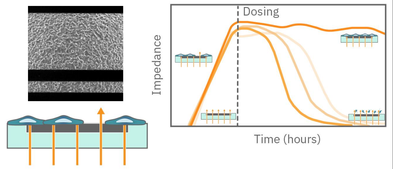
Maestro Z: Dynamic Cell Tracking

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.

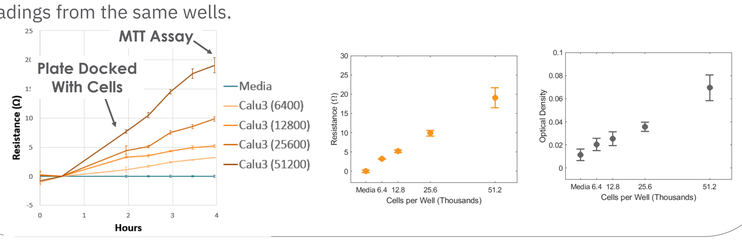
The Maestro Z Product Family

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*

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.

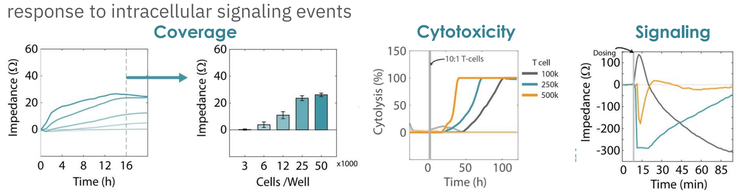


Real-time, Label-free Cytotoxicity Assay

Impedance Assay Measures Diverse Cell Properties

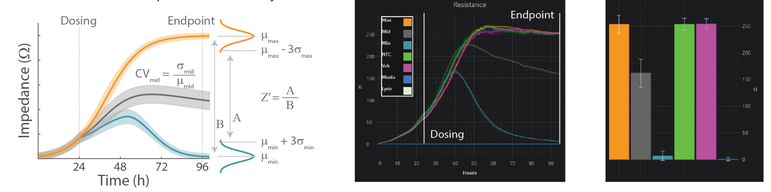
The Maestro Z records impedance at multiple frequencies simultaneously, enabling a thorough characterization of cell behavior, including:

- **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.
- **Morphology** – cell size, shape, and intercellular tight junctions significantly impact the measured impedance.
- **Signaling** – small changes in cell shape or cytoskeleton organization are detected in response to intracellular signaling events



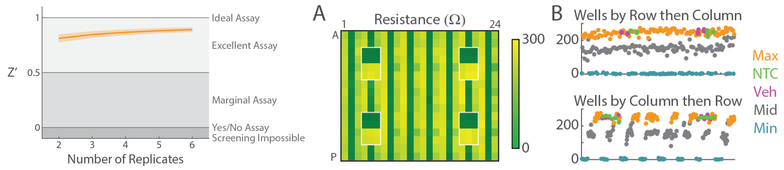
High throughput screening requires careful validation of assay performance. Z-prime (Z'), which defines the statistical separation between positive and negative controls in an assay, is a widely accepted metric for assessing assay performance. Higher Z-prime values indicate separation of positive and 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. The Z-prime was computed from the "Max" and "Min" treatment groups, corresponding to the lowest and highest concentrations of mitoxantrone, respectively, for each of three independent assays.



Maestro Z Cytotoxicity Assay is Sensitive and Consistent

Z' between 0.5 and 1 is considered an excellent assay, as the separation between "Max" and "Min" signals affords high sensitivity and reliability, with low risk of false positives or negatives. Each of the three plates well exceeded Z' of 0.5, with the average performance of Z' = 0.81 ± 0.03. The heat map shows the resistance as measured at 41.5 kHz at 96 hours (green represents background, yellow represents high resistance). No significant drift (e.g. from left to right) or edge effect (e.g., deviation of outer row/column) was observed.



- 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.

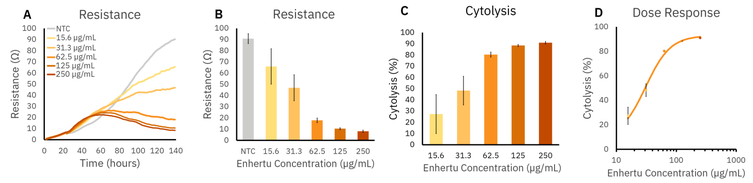
	Coeff. Var. (%)			Z'	Drift	Edge	Pass
	Min*	Mid	Max				
Criteria	<SD_{min}	<20	<20	>0.4	None	None	All
Plate 1	✓	11.2	4.4	0.83	✓	✓	✓
Plate 2	✓	7.6	4.6	0.83	✓	✓	✓
Plate 3	✓	8.9	5.7	0.77	✓	✓	✓

*SD_{min} is compared with SD_{min} because $\mu_{min} \sim= 0$

ADC Cytotoxicity Assay

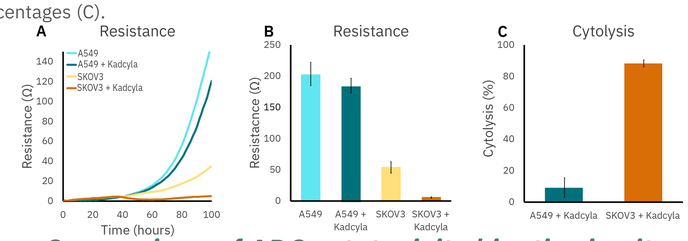
Antibody-Drug Conjugate (Enhertu®) Killing on Maestro Z

Antibody-drug conjugates (ADCs) have emerged as a promising avenue for the treatment of many cancers. To measure the *in vitro* killing of one such ADC, Enhertu (trastuzumab-deruxtecan), 5,000 HER2+ SKOV3 cells were dosed with increasing amounts of Enhertu and monitored on the Maestro Z. Resistance data (A and B) showed that SKOV3 cell death showed an Enhertu dose-dependent relationship. This same relationship was observed when the data was normalized to the no treatment control (NTC), and cytolysis was calculated (C). Using the data from the Maestro Z, a dose-response curve was generated using the Hill equation to illustrate the dynamics of cell killing over a range of doses.



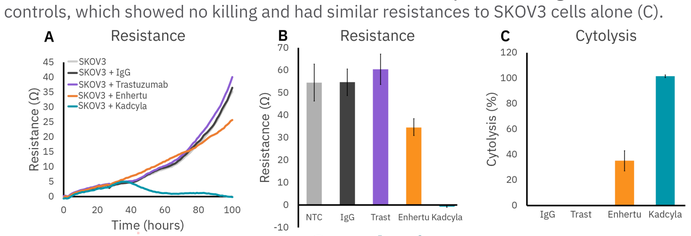
Differences in HER2+ Cell Sensitivity to Kadcyla® in vitro

The cytotoxic potential of trastuzumab-containing ADCs is dependent on the expression of HER2 on the target cell type. Here, we showed the difference in the killing of $HER2_{low}$ A549 cells and $HER2_{high}$ SKOV3 cells by Kadcyla (trastuzumab-emtansine) on the Maestro Z. As anticipated, Kadcyla killed a larger portion of SKOV3 cells due to their higher HER2 expression, as illustrated both in the resistance measurements (A and B) and the cytolysis percentages (C).



Comparison of ADC cytotoxicity kinetics in vitro

In vitro assays that reveal the efficacy and kinetics of ADC cytotoxicity can aid in which ADC formulations are the best therapeutic candidates against a given cancer type. Therefore, we dosed HER2+ SKOV3 cells with Enhertu and Kadcyla (at 50 µg/mL) and monitored cell killing using the Maestro Z. Resistance showed that both ADCs killed SKOV3s, with Kadcyla's killing happening at a much faster rate than that of Enhertu (A and B). Additionally, we added IgG and trastuzumab alone as controls, which showed no killing and had similar resistances to SKOV3 cells alone (C).



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.
- The cytotoxic potential of antibody-drug conjugates against different cancer cell types can be measured using the Maestro Z and help inform the development of therapeutic ADC products.