>> Label-free, High-throughput Functional Assays For Evaluating The Potency And Safety Of Cell Therapies

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Maestro: Label-Free Live-Cell Analysis

The Maestro Product Family

Maestro Z | Maestro TrayZ | Maestro ZHT

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 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.*

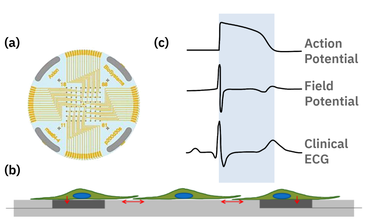
Maestro Pro | Maestro Edge | Maestro Volt

Features	*Maestro Pro*	*Maestro Edge*	*Maestro Volt**
Throughput (well format)	6, 24, 48, 96, 384**	6, 24, 96**	6
MEA Mode	✓	✓	✓
MEA Viability	✓	✓	
Impedance Mode	✓	✓	
Environmental Control	✓	✓	✓
Automation API	✓	✓	
Stimulation	Electrical & Optical	Electrical & Optical	Electrical
Omni Compatible	✓	✓	✓

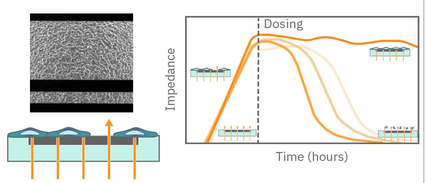
*Maestro Volt only available in Europe and Asia
**Well format available in impedance only

- *Label-free, non-invasive tracking extracellular voltage from cultured electro-active cells.*
- *Integrated environmental control provides a stable benchtop environment for short- and long-term studies*
- *Fast data collection rate (12.5 kHz) accurately quantifies the depolarization waveform*
- *Sensitive voltage resolution detects subtle extracellular action potential events*
- *Industry-leading array density provides high quality data from across the entire culture*
- *Scalable format (6-, 24-, 48- and 96-well plates) meets all throughput needs on a single system*

MEA and Impedance Technology



A planar grid of microelectrodes (a) interfaces with cultured neurons or cardiomyocytes (b) to model complex, human systems. Electrodes detect changes in raw voltage and record extracellular field potentials (c). The same microelectrodes can track cell viability using impedance measurements.

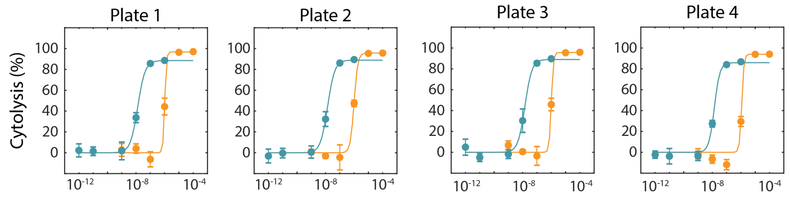


The impedance is measured from electrodes embedded in the bottom of each well. As cells cover more of the electrode, impedance increases in proportion to the number of viable cells. If a perturbation kills the attached cells, impedance decreases as the cells lyse.

Multi-Plate Dose Response Analysis

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

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.



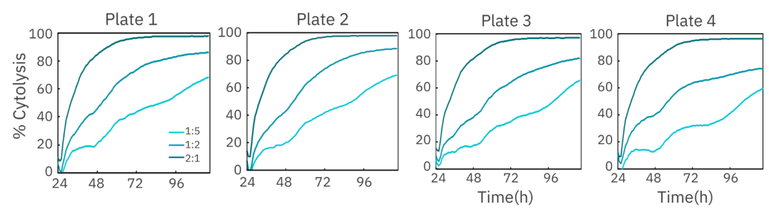
EC50 Values

	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.

Scalable Dose Response Analysis with TrayZ Cytotoxicity Assay

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. 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). 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.



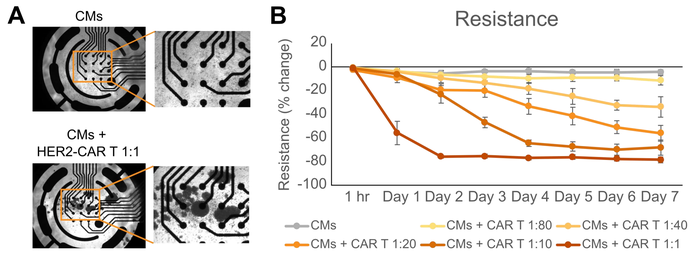
KT50 Values

	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

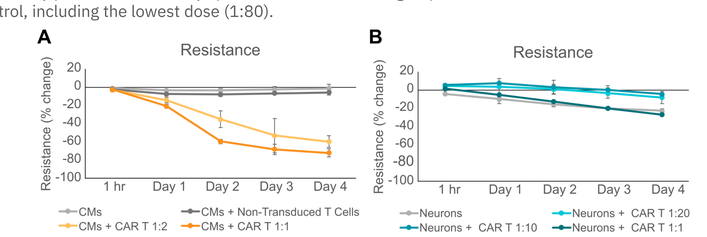
- SKOV3 cells were dosed with HER2-targeted CAR T cells at three different E:T ratios, and cytolysis was measured in real-time over multiple days.
- Dose response analysis (above, right) measured the KT50 values consistently across all four plates on the TrayZ system.

A Cardiotoxicity Assay of Cell Therapy

Evaluation of On-Target, Off-Tumor Effects using MEA Viability

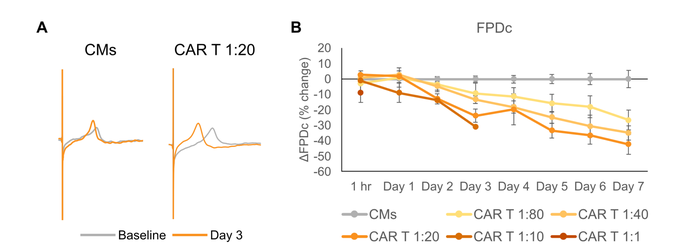


We confirmed the expression of HER2 in iPSC-derived cardiomyocytes (iPSC-CMs) using flow cytometry (data not shown) and then monitored killing of iPSC-CMs by HER2-CAR T cells at effector-to-target (E:T) ratios ranging from 1:80 to 1:1 on the Maestro Pro. Imaging showed that at 7 days post-dose, CAR T cells formed clusters, indicating significant cell activation after culture with the iPSC-CMs (A). MEA Viability (resistance) measurements from the iPSC-CMs decreased in a CAR T cell dose-dependent manner from baseline over 7 days (B). In the highest dose (1:1), significant decreases in resistance were seen as early as one day post-dose. At 7-days post dose, all treatment groups had lower resistance than the untreated control, including the lowest dose (1:80).



We treated iPSC-CMs with either HER2-CAR T cells or non-transduced control T cells. As expected, CMs dosed with HER2-CAR T cells at 1:2 and 1:1 ratios led to significant decreases in iPSC-CM resistance, while dosing with non-transduced T cells led to a much smaller decrease in resistance (A). We further tested for antigen-specific killing of HER2-CAR T cells by dosing iPSC-derived neurons which do not express HER2. There was no significant difference between the changes in resistance of untreated neurons and those treated with the 1:1 CAR T cell dose (B).

HER2-CAR T cells change iPSC-CM electrophysiology following co-culture



To evaluate the functional electrophysiology of CMs, we again dosed iPSC-CMs with HER2-CAR T cells and monitored electrophysiological outputs such as the Fridericia-corrected field potential duration (FPDc). Representative waveforms recorded from untreated iPSC-CMs and a representative treatment group (CAR T 1:20) at baseline and at Day 3 post-dose are shown in (A). CAR T dosing generally induced a leftward shift in repolarization, indicating a shorter FPD. Longitudinal data (B) shows that FPDc gradually decreased over time in a CAR T dose-dependent manner, and that the 1:1 CAR T and 1:10 CAR T groups completely lost electrophysiological activity by Day 3.