>> Development of a Faster Potency Assay for Evaluation of Immune Cell-Mediated Cytotoxicity In Vitro

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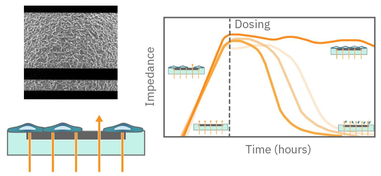
Maestro Z: Dynamic Cell Tracking

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

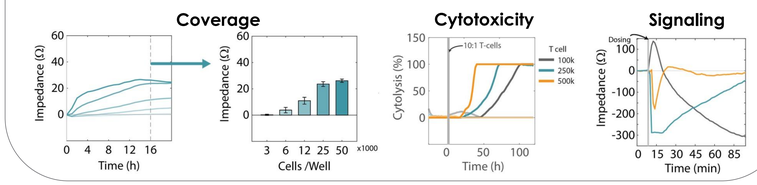
Features	*Maestro Z*	*Maestro TrayZ*	*Maestro ZHT*
Throughput:	96-well	Up to 8 x 96-well	384- and 96-well
Environmental Controls:	Built-in	External	Built-in
GxP Compatible:	✓	✓	✓
Barcode Plate Tracking:	✓	✓	✓
Automation API:	✓	No	✓
Dimensions *(WxDxH)*:	280 x 413 x 225 mm	440 x 450 x 60 mm	280 x 452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

Impedance Assay Measures Diverse Cell Properties

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

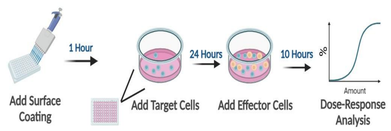
- Coverage/Density – the change in impedance is directly related to the quantity of cells in a 2D and 3D culture covering the electrodes.
- **Cytotoxicity** – dynamic monitoring of cell viability provides measures of the degree and speed of cell death.
- **Morphology** – cell size, shape, and intercellular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



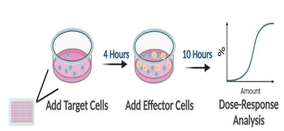
Real-time Label-Free Potency Assay

Development of a Rapid Potency Assay for the Maestro Z

The standard protocol for a potency assay on the Maestro Z involved coating CytoView-Z plates with fibronectin, adding target cells, and then adding CAR T cells at 24 hours post-target cell seeding. Here, we present a set of steps to reduce overall potency assay time. First, we show that the surface coating step can be removed with minimal reduction in target cell proliferation. After target cell seeding, CAR T cells are added at 4 hours and data analysis can be performed as early as 10 hours post CAR T addition.



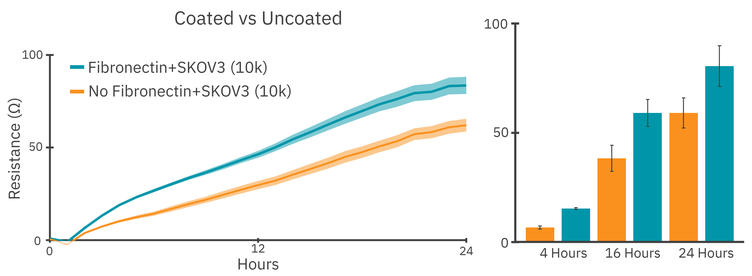
Standard Protocol – 35 Hours



Rapid Protocol – 14 Hours

ECM surface coating has Minimal Effect on Target Cell Proliferation

The Standard Protocol includes a one-hour incubation with FBN before target cell seeding, while the Rapid Protocol eliminates this step, reducing assay time. Although FBN enhances cell attachment, growth rates are similar for both protocols, indicating that cells can proliferate effectively without surface coating. Similar findings were noted with HeLa cells (data not shown).



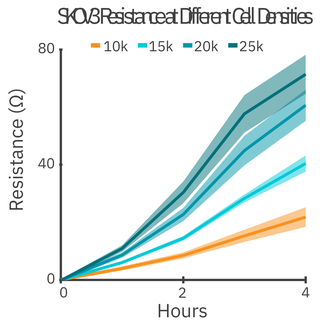
(Left) Resistance time course for SKOV3 with FBN (teal) and SKOV3 with No FBN (orange). (Right) Comparison of resistance of FBN and No FBN at 4-, 16-, and 24- hours.

SKOV3 Density Sweep Determines Optimal Target Seeding Density

The Rapid Protocol aims to identify a cell density that maintains a minimum resistance of over 10 ohms (above noise level) at the 4-hour mark, while preventing over-confluence that could cause cell death or signal plateauing.

We present a density sweep of SKOV3 cells in a CytoView-Z 96 plate without surface coating. Resistance levels at 4 hours were: 25k - 64.6 ± 6.65 ohms, 20k - 52.8 ± 5.26 ohms, 15k - 34.4 ± 2.08 ohms, and 10k - 18.6 ± 2.75 ohms.

Resistance measurements reveal a dose-dependent trend: higher cell densities result in increased resistance. However, the 10k cell density exhibits consistent growth while maintaining resistance levels within the desired range.

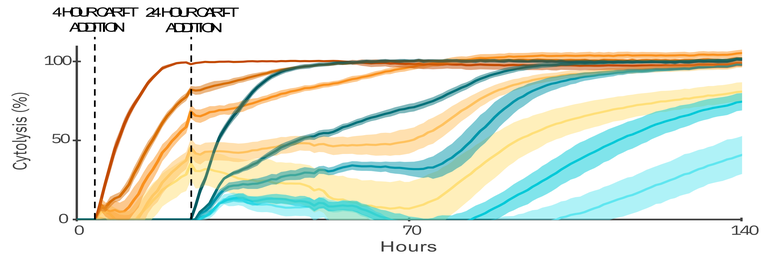


Resistance time course of SKOV3 comparing the optimal density, 10k (orange), against varying densities 15k, 20k, 25k (teal).

The Dynamics of CAR T cell killing

Timing of CAR T Cell Addition Influences Cytolysis

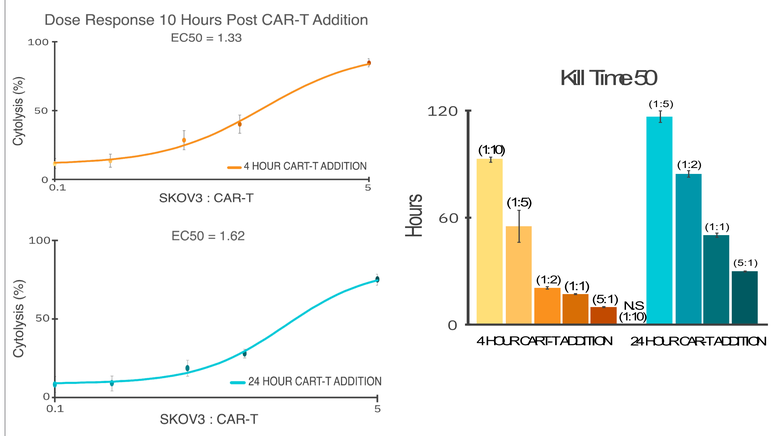
The timing of adding CAR T cells is vital for accurate effector-to-target (E:T) ratios in potency assays. Adding CAR T cells 4 hours after target cell seeding maintains the E:T ratio, leading to 100% cytolysis at a 5:1 E:T ratio by 30 hours. This early addition allows quicker cytolysis, reducing overall assay time. In contrast, the Standard Protocol, which adds effector cells after 24 hours, delays cytolysis as target cell proliferation alters the E:T ratio, though the overall trend remains the same.



% cytolysis of SKOV3 cancer cells by CAR T cells at different effector-to-target (E:T) ratios (1:10, 1:5, 1:2, 1:1, and 5:1) under two conditions: CAR T additions after a 4-hour resting period (Rapid Potency) and a 24-hours resting period (Standard Protocol).

Dose Response and KT50 Analysis for Rapid Potency

SKOV3 cells showed dose-dependent cytolysis 4 and 24 hours after CAR T cell addition, with similar EC50 values of 1.33 and 1.62, respectively. Kill Time 50 (KT50) decreased with increasing E:T ratios, with values at 4 hours ranging from 92.5 ± 1.4 hours at 1:10 to 9.8 ± 0.3 hours at 5:1, and at 24 hours from 154.3 ± 3.1 hours at 1:10 to 29.8 ± 0.3 hours at 5:1.



(Left) %cytolysis dose-response and EC50 for SKOV3 at 4- (top) and 24- (bottom) hours after CAR T addition.

(Right) Kill Time 50 (KT50), or the time required for 50% of the target cells to be killed for SKOV3 cells at 4- (Orange) and 24- (teal) hours after CAR T addition at E:T ratios 1:10, 1:5, 1:2, 1:1, and 5:1.

Conclusions

- The Rapid Protocol enables faster analysis on the Maestro Z platform without sacrificing reliability.
- It improves efficiency by introducing CAR T cells earlier while maintaining an accurate effector-to-target (E:T) ratio.
- Potency assays using the Rapid Protocol should be optimized for the specific target and effector cells involved.