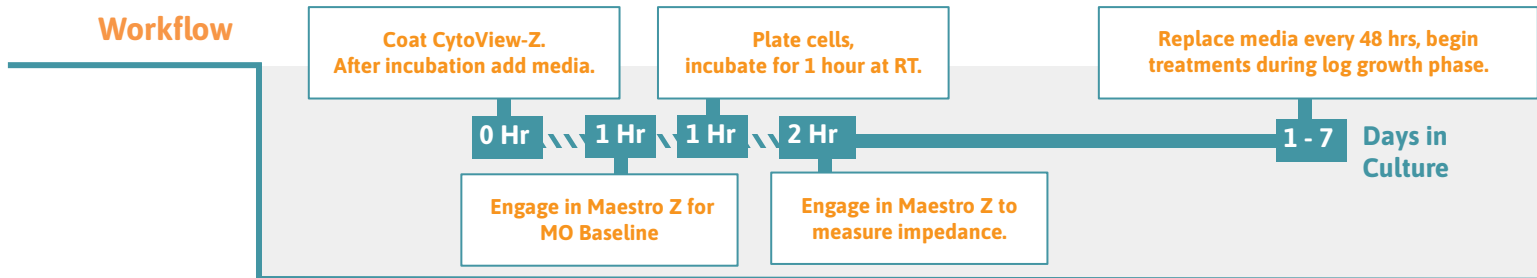


Cell Culture Protocol

Impedance - Adherent Cells and Cell Lines



Preparing the CytoView-Z Plate

1. Pre-coat (100 μ l per well is recommended) the entire well surface of the CytoView-Z plate using an extracellular matrix molecule (ECMM), such as fibronectin.

Note: A working concentration of fibronectin of 1 μ g/ml is recommended, however optimization of concentration or use of a different surface coating may be necessary.
2. Incubate the surface-coated plate in a cell culture incubator at 37°C and 5% CO₂ for at least 1 hour.
3. Aspirate the surface coating solution from the plate.
4. Add 100 μ l of complete medium to the plate, and add 8 mL of sterile water to the on-plate reservoirs to increase humidity.
5. Dock the plate in the Maestro Z to measure the media only (MO) Baseline. Transfer the plate to a biosafety cabinet when the Baseline is complete.

Tip

Reconstitute fibronectin, or ECM of choice, in sterile PBS at 1 mg/ml. Aliquot and store at -20°C then dilute to working concentration as needed.

Culturing Adherent Cells and Transfer to CytoView-Z Plate

6. Thaw and culture the cells of interest in accordance with supplier recommendations, passaging as needed.
7. Remove flasks of cultured cells from the incubator, aspirate the media and rinse with warmed PBS. Using trypsin, or another cell dissociating agent, detach and collect the cells from the flasks as per reagent recommendations.
8. Remove a sample of the cell suspension and count the cells using a hemocytometer to determine both the cell viability and total number of viable cells.
9. Transfer the cell suspension to a 15 ml conical tube and centrifuge the cell suspension to pellet.
10. Aspirate the supernatant, being careful not to disturb the cell pellet.
11. Dilute the cell suspension in complete medium to a working concentration of cells per 100 μ l. The working concentration should be the number of cells per well necessary to achieve 100% confluence within 24 hours.

Tip

Gently mix with a pipette to ensure the cells are evenly suspended before removing an aliquot to count.



Plating Adherent Cancer Cells onto the CytoView-Z Plate

12. Undock the CytoView-Z plate from the Maestro Z once the MO baseline has been collected, and transfer the plate to a biosafety cabinet.
13. Transfer the cell suspension to a trough for easy access by a multichannel pipette. Alternatively, divide the cell suspension evenly into microcentrifuge tubes that can be used with a multichannel pipette, providing enough volume to seed the cells with a 100 μ l addition per well.
14. After seeding cells on the plate, leave it to rest in the biosafety cabinet for 1 hour at room temperature.
15. Dock the plate and impedance measurements will begin automatically upon plate engagement.
16. For optimal cell health, 50% of the media should be changed after 48 hours.

Tip

Gently mix the cell suspension before any addition to ensure even distribution of the cells. Dispense the cells directly in the middle of the well with the pipette tip below the surface of the liquid.

Tip

High well-number microtiter plates are sensitive to thermal gradients, which can cause edge effects

Cell Coverage

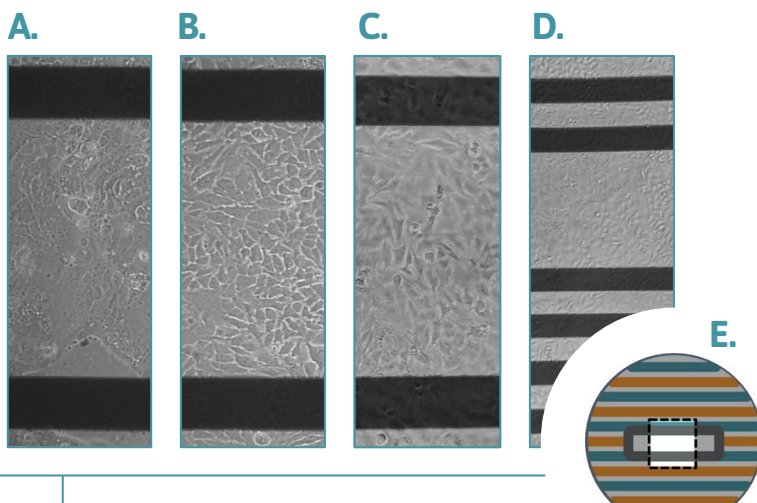


Figure 1: Various Cell Lines plated on the CytoView-Z plate

Microscopy images of Calu-3 (A), HeLa (B) and A549 (C&D) cells observed through the built-in window on the CytoView-Z plate at (A-C) 10x and (D) 4x magnification. The layout in the lower right (E) represents the bottom surface of a well of the CytoView-Z plate. The window in the center can be used for expanded visualization of cells in the absence of electrodes.

Visualization of Typical Cell Results

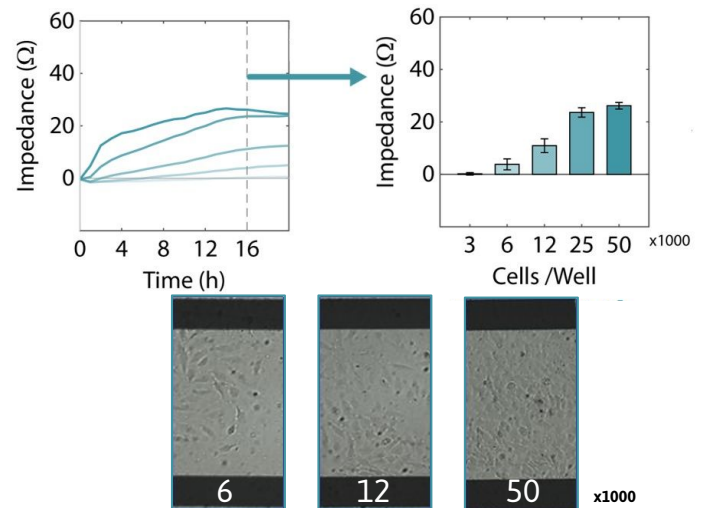


Figure 2: Cell Density Sweep and Growth Curves for HeLa cells.

HeLa cells were plated using a serial dilution across columns of an impedance plate from 3000 up to 50000 cells per well. The electrodes in the CytoView-Z plate can be seen and the cells can be observed through the built-in window.

Required Materials

Consumables

Item	Vendor	Catalog #
CytoView-Z	Axion BioSystems	
Fibronectin	Roche	11051407001
Dulbecco's PBS without Ca2+/Mg 2+	Thermo Fisher	14040
Kimwipes	Various	
Trypsin or similar cell detachment agent	Various	
15 mL and 50 mL Centrifuge Tubes	Various	
Pipette Tips	Various	

Equipment

Item	Vendor	Catalog #
Maestro Z	Axion BioSystems	
AxIS Z	Axion BioSystems	NA
Microscope	Various	
1 mL Micropipettor	Various	
8- or 12-channel Multiwell pipettor	Various	