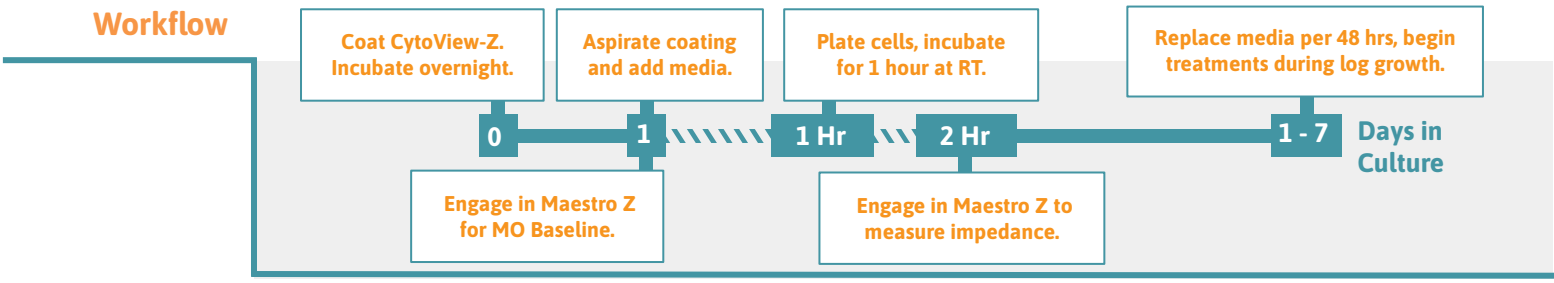
AXION BIOSYSTEMS

Cell Culture Protocol

Impedance - Non-Adherent Cell Lines



Preparing the CytoView-Z Plate - Antibody Coating

1. Pre-coat (100 µl per well is recommended) the entire well surface of the CytoView-Z plate using the relevant antibody for your cell type to anchor the non-adherent cells to the impedance plate surface. E.g. anti-CD40 for Daudi cells (4 µg/mL) or anti-CD71 for K562 cells (5 µg/mL).
2. Incubate the antibody-coated plate at 4°C, overnight.
3. Aspirate the antibody solution from the plate.
4. Add 100 µl 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 the relevant antibody in PBS for the cell line that is cultured in accordance to supplier recommendations.

Culturing Non-Adherent Cell Lines for Transfer to CytoView-Z Plate

6. Thaw and culture the cells of interest in accordance with supplier recommendations, passaging as needed.
7. Take the flasks of cultured non-adherent cells from the 37°C incubator, and mix the suspension gently, but thoroughly with a serological pipettor.
8. Remove a sample of the cell suspension and count the cells using a hemocytometer to determine both the viability and total number of viable cells.
9. Transfer the cell suspension to a 15 ml conical tube.
10. Centrifuge the cell suspension at 250 x g for 5 minutes.
11. Aspirate the supernatant, being careful not to disturb the cell pellet.
12. Dilute the cell suspension in complete medium to a working concentration of cells per 100 µl. The working concentration should be the number of cells per well necessary to achieve 100% confluence within 24 hours, however this may vary based on specific experimental goals.

Tip

Ensure the cells are evenly suspended before removing an aliquot to count.

Note: It is recommended to run a cell density sweep with a cell type first to determine the optimal number of cells for the working concentration and to inform future experiments.

Plating Non-Adherent Cells onto the CytoView-Z Plate

13. Undock the CytoView-Z plate from the Maestro Z once the MO baseline has been collected, and transfer the plate to a biosafety cabinet.
14. 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 µl addition per well.
15. After seeding cells on the plate, leave it to rest in the biosafety cabinet for 1 hour at room temperature.
16. Dock the plate and impedance measurements will begin automatically upon plate engagement.
17. For optimal cell health, 50% of the media should be changed after 48 hours.

Tip

Be sure to mix the cell suspension thoroughly before any addition to ensure even distribution of the cells. Dispense the cells directly in the middle of the well.

Tip

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

Impedance Electrode Diagram and Visualization of Typical Nonadherent Cell Results

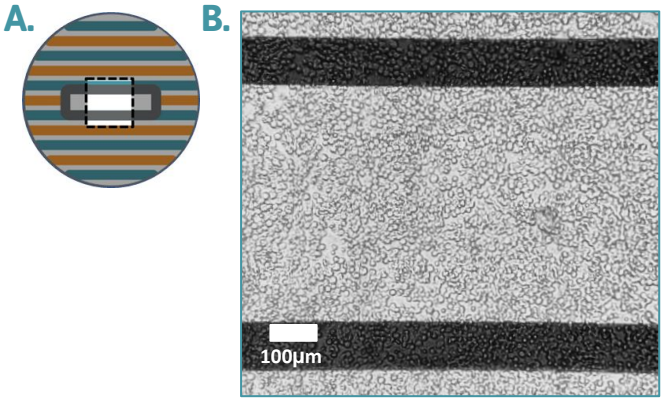


Figure 1: Daudi B-Cell Lymphoma cells on the CytoView-Z

(A) The layout above 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. Cells should be plated across the entire well. B) Immobilized Daudi B-Cells using an anti-CD40 antibody. Cells are shown plated at 150,000 cells per well, 24 hours post plating. Flattening of the cells will be observed when antibody attachment is sufficient.

Cell Density Sweep of Nonadherent Cell line

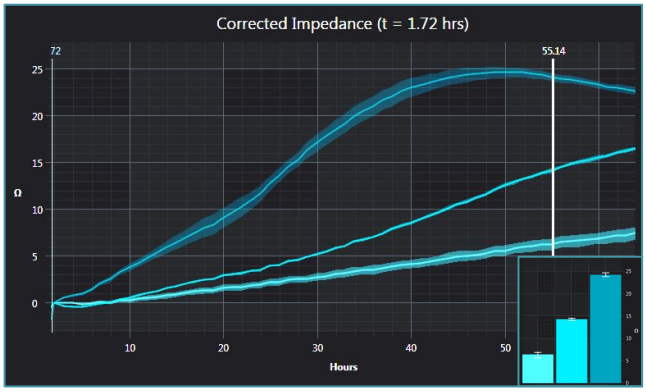


Figure 2: Example growth curves

Attachment and Immobilization of Daudi B-Cells using an anti-CD40 antibody. Shown here are 50,000, 150,000, and 300,000 cells per well over 48 hours. The Endpoint Cursor (white) can be dragged along the plot to report the measured impedance for any given time point.

Required Materials

Consumables

Item	Vendor
CytoView-Z Plate	Axion BioSystems
Antibody (*ex. R&D Systems, anti-CD40 - MAB6321, anti-CD71 - 2474-TR-050*)	Various
Cell Culture Media	Various
15 mL and 50 mL Centrifuge Tubes	Various
1 mL Pipette Tips	Various

Equipment

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