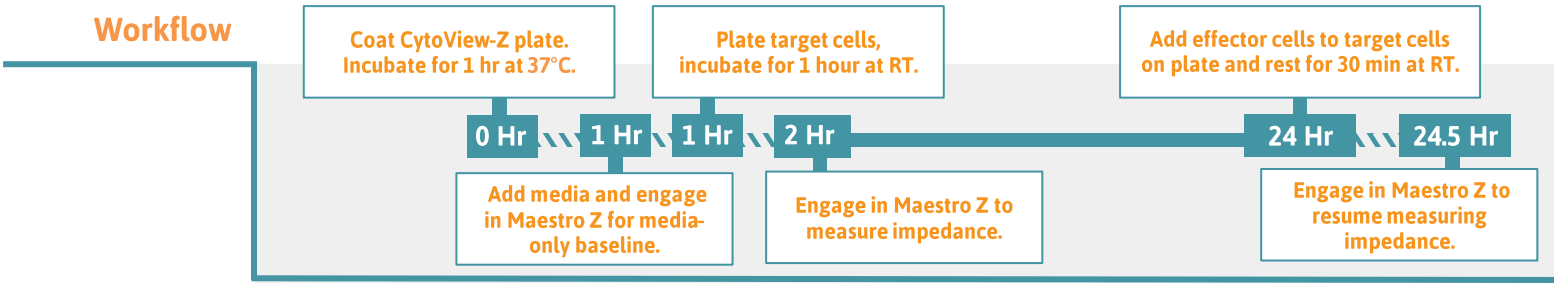
AXION BIOSYSTEMS

Cell Culture Protocol

Immuno-oncology Potency Assay



Preparing the CytoView-Z Plate

1. Pre-coat 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 µg/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% CO_2 for at least 1 hour.
3. Aspirate the surface coating 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 and 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 Target Cells and Transferring to CytoView-Z Plate

6. Thaw and culture the target 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 phosphate-buffered saline (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 the Exact FL or 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 the desired working concentration of cells per 100 µl.

Tip

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

Plating Adherent Target Cells onto the CytoView-Z Plate

12. Transfer the cell suspension to a reservoir for easy access by a multichannel pipette. Add 100 µl of cell suspension to the 100 µl of media already present in each well.
13. Leave the plate seeded with target cells to rest in the biosafety cabinet for 1 hour at room temperature.
14. Dock the plate and impedance measurements will begin automatically upon plate engagement.

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.

Dosing Target Cancer Cells with Effector Cells in the CytoView-Z Plate

15. Thaw and culture the cells of interest in accordance with supplier recommendations.
16. At 24 hrs following target cell seeding, take the flasks of cultured effector cells from the 37°C incubator and mix the suspension gently but thoroughly with a serological pipettor.

    Note: The desired timing of effector cell dosing may vary depending on the application.
17. Remove a sample of the cell suspension and count the cells using the Exact FL or a hemocytometer to determine both the viability and total number of viable cells.
18. Transfer the cell suspension to a 15 ml conical tube and centrifuge the cell suspension to pellet.
19. Aspirate the supernatant, being careful not to disturb the cell pellet.
20. Dilute the cell suspension in complete effector cell medium to the desired working concentration of cells per 22.2 µl and add 22.2 µl of effector cell suspension to each well.

    Note: The volume of the effector cell dose and optimal effector:target cell ratios may need to be determined experimentally.
21. Leave the plate dosed with effector cells to rest in the biosafety cabinet for 30 minutes at room temperature.
22. Dock the plate and impedance measurements will automatically resume upon plate engagement.

Tip

High well-number microtiter plates are sensitive to thermal gradients, which can cause edge effects if the rest step is skipped.

Tip

Resting reduces variability in effector cell cytotoxicity due to edge effects.

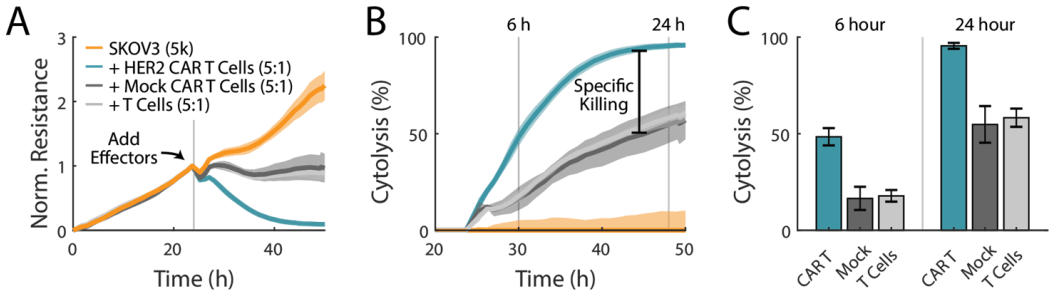


Figure 1: Example Potency Experiment
(A) Resistance and (B) cytolysis time courses for SKOV3 killing by CAR T cells and a (C) comparison of cytolysis at 6 and 24 hours following effector cell addition at E:T = 5:1. HER2-targeted CAR T groups demonstrated approximately twice as much target cell killing as mock CAR T cells and non-transduced T-cells at the same E:T ratios.

Required Materials

Consumables

Item	Vendor	Catalog #
CytoView-Z 96	Axion BioSystems	Z96-IMP-96B
Fibronectin	Roche	11051407001
Dulbecco's PBS without Ca2+/Mg 2+	Thermo Fisher	14040
Kimwipes	Various	
Trypsin or similar cell dissociation agent	Various	
15 mL and 50 mL Centrifuge Tubes	Various	
Pipette Tips	Various	
Reagent Reservoir	Various	
Trypan Blue	Various	

Equipment

Item	Vendor	Catalog #
Maestro Z	Axion BioSystems	
AxIS Z	Axion BioSystems	
Hemocytometer (such as the Exact FL)	Various	
Microscope	Various	
1 mL Micropipettor	Various	
8- or 12-channel Multiwell pipettor	Various	