T cell cytotoxicity (xCELLigence platform)

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1 Works for me Share dx.doi.org/10.17504/protocols.io.sqkeduw

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ABSTRACT

This protocol outlines the steps for preparing cellular cytotoxicity assays using the xCELLigence platform.

In brief: For impedance assays, 10e5 tumor cells were plated overnight in 100mL media in plastic 96 well VIEW-E plates (ACEA) and grown to confluence (measured using the cell index readout on xCELLigence MP platform, RTCA software, ACEA). Transduced T cells were added at varying E:T ratios (as indicated), and cell index was monitored for up to 5 days. Impedance data was analyzed using xIMT software (v1.2, ACEA).

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MATERIALS TEXT

MATERIALS

xCELLigence RTCA MP – Bundle (complete system) **ACEA Biosciences, Inc Catalog #0088060140**

E-Plate View 96 (6 plates) **ACEA Biosciences, Inc Catalog #0647248001**

xIMT Immunotherapy Software **ACEA Biosciences, Inc Catalog #8100004**

1 Add 50ul media to each well of the E-VIEW plate (use 'inverse pipetting' technique to avoid bubbles in the wells), place the plate in the xCELLigence MP cradle, and run the setup step of the xCELLigence software to confirm all the wells and connections on the cradle are functioning properly.

2 Resuspend adherent tumor target cells at 10e5 cells/50ul T cell media.

3 Remove the plate from the cradle and plate 10e5 adherent tumor cells (target cells) to grow overnight in (100uL total volume per well) in plastic 96 well VIEW-E plates. Use 'inverse pipetting' technique to avoid bubbles in the wells. Let plate sit in the hood for 30 minutes for cells to settle. Return each plate to the xCELLigence cradle. Begin recording impedance values at desired time interval.

4 Grow cells to confluence (determine confluence using cell index readout on the xCELLigence MP platform using RTCA data acquisition software).

5 Prepare effector cells at desired concentrations for assy. Pause impedance readings and remove the plate from the cradle. Add transduced T cells, cytolysis control, or media only for growth control, in 50ul volume, to each well (use 'inverse pipetting' technique to avoid bubbles in the wells).

6 Return E-VIEW plate to the xCELLigence cradle and resume recording impedance values until desired stopping point. If running an assay for more than 2-3 days, pause the instrument, remove the plates and add additional media (25-50ul) so that the wells do not dry out.

7 Use RTCA and xIMT software for analysis.

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