SARTORIUS

Protocol

Incucyte® Immune Cell Killing of Tumor Spheroids Assay

For Measurement of Immune Cell-Mediated Cytotoxicity of Tumor Spheroids

This protocol provides an overview for the measurement of immune cell-mediated cytotoxicity of tumor spheroids. This assay format is suitable for cytotoxic T cell killing and antibody-dependent cell-mediated cytotoxicity (ADCC) assays using a co-culture methodology. This method utilizes changes in fluorescence of nuclear labeled target cells to determine immune cell-mediated cytotoxicity of tumor spheroids over time. Incucyte® image analysis software enables automated detection and selective quantitation of tumor cell death in real time.

General Guidelines

- Following seeding and after treatment addition, remove bubbles from all wells by gently squeezing a wash bottle containing 70-100% ethanol, with the inner straw removed, to blow vapor over the surface of each well.
- After placing the plate in the Incucyte® Live-Cell Analysis System, allow the plate to warm to 37° C for 30 minutes prior to scanning.
- 4x image acquisition is required for analysis of tumor spheroid cytotoxicity.

Required Materials

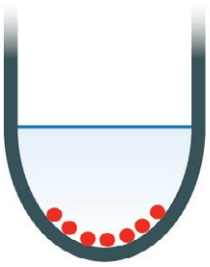
- Fluorescently labeled target cells of interest
- Immune (effector) cells of interest
- 96-well round-bottom, ultra-low attachment plate (e.g., Corning® Cat. No. 7007, BRANDplates® Cat. No. 781900, S-Bio Cat. No. MS-909602)
- Incucyte® Spheroid Analysis Software Module (Cat. No. 9600-0019)
- Incucyte® software version 2017B or later: required for spheroid scan type and additional supported vessels

Recommended Materials

- Incucyte® Nuclight Green, Red, Orange or NIR Lentivirus (Cat. No. 4475, 4476, 4771 or 4805)
- Incucyte® Cytolight Red or Green Lentivirus (Cat. No. 4481 or 4482)
- Matrigel® (Corning Cat. No. 356234)

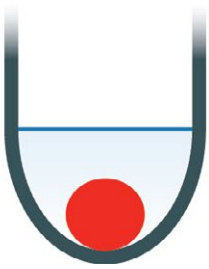
Quick Guide

1. Seed cells (day 0)



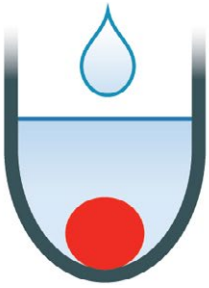
Seed target cells into 96-well Ultra-Low Attachment plate (100µL/well). Centrifuge.

2. Monitor spheroid formation (day 0–3)



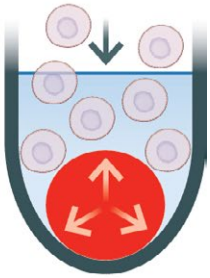
Place plate inside the Incucyte® Live-Cell Analysis System and scan every six hours.

3. Add treatments (day 3)



Add appropriate treatments (50 µL/well) at 4x final assay concentration.

4. Add immune cells (day 3)



Add chosen immune cells to plate (50 µL/well). Monitor spheroid proliferation and apoptosis.

Protocol

Day 0

1 Seed target cells

1.1 Seed fluorescently labeled target cells of interest (100 µL per well) at an appropriate density into a 96-well ultra-low attachment (ULA) plate such that by day 3, spheroids have formed with the desired size (e.g., 200–500 µm after 3 days). Seeding density will need to be optimized for each cell line used, however it is usually in the range of 1,000–5,000 cells per well (10,000–50,000 cells per mL seeding stock).

NOTE: Some cells lines may require the addition of a basement membrane extract, typically 2.5% v/v Matrigel®, to promote tight spheroid formation.

1.2 Centrifuge the ultra-low attachement (ULA) plate (125 g, 10 minutes) at room temperature.

Day 0–3

2 Monitor spheroid formation

2.1 Place the cell plate into the Incucyte® Live-Cell Analysis System and schedule 24 hour repeat scanning:

a. Objective: 4x (96-well ULA) 1 image per well
b. Channel selection; Phase Contrast + Brightfield + "Red" or "Green"
c. Scan type: Spheroid
d. Scan interval: Every 6 hours

Day 3

3 Add treatments

3.1 Once spheroids have reached desired size (e.g. 200–500 µm), remove the ULA plate from the incubator and carefully add appropriate treatments (e.g. T cell stimuli, antibodies or cytokines) at 4x final assay concentration (50 µL per well).

NOTE: For treatment controls, add 50 µL of assay medium.

4 Add immune cells

4.1 Carefully add chosen immune cells (e.g. T cells, PBMCs, NK cells) at an optimized effector-to-target cell ratio (50 µL per well) to achieve a final assay volume of 200 µL. It is recommended that different effector-to-target cell ratios are tested (e.g 2:1.5, 5:1).

NOTE: Assay duration may be reduced by pre-activating the effector cells before addition to assay plate.

4.2 Continue to monitor spheroid proliferation and cytotoxicity (e.g. every 6 h for up to 10 days).

Analysis Guidelines

Fluorescent measurements only

Result: Size and viability measurements

Suggested Metric: Integrated intensity

Secondary metric: Total Fluorescence Object area

NOTE: Brightfield measurements, including fluorescence within the brightfield boundary, cannot be used as segmentation is confounded by the presence of immune cells in the periphery of the spheroid.

A complete suite of cell health applications is available to fit your experimental needs.
Find more information at www.sartorius.com/incucyte

For additional product or technical information, please email us at AskAScientist@sartorius.com

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