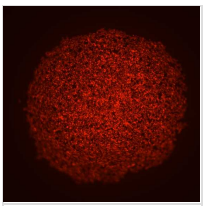

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Time-lapse killing assay (spheroid - IncuCyte)

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ABSTRACT

Many tumors exist *in vivo* as three-dimensional masses. In order to better model the dynamics of three-dimensional tumor growth and immune cell invasion, cancer cell lines are grown in low-adhesion plates that force them to interact with each other, forming a spheroid. After spheroids form in culture, immune effectors and drugs are added to the wells and their effect on the tumor mass is measured by transmitted light or fluorescence microscopy in a live-cell image system (IncuCyte, Sartorius Inc).

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1 *Optional:* If target cells that form a spheroid are to be themselves monitored by fluorescence, perform this labeling/transfection in advance (see *Time-lapse killing assay - monolayer - Incucyte*)

2 Resuspend target cells in their preferred media. Seed $2x10^4$ target cells in 100 µL/well into a 96 well U-bottom low adhesion plate (ULA plate, Cat. No. 7007, Corning).

3 Spin the plate at 40 *g* (500 RPM) in one orientation and then 80 *g* (700 RPM) in the opposite orientation without any brake applied to the centrifuge.

4 Capture an image of the plate using the live-cell image system (Incucyte S3 or Zoom, (Incucyte S3) to ensure the cells are in the center of the well. If cells are at the edges, resuspend them with a multichannel pipette (breaking up the pellet) and repeat the seeding, altering the centrifugal forces until a central pellet is achieved.

5 After allowing spheroids to form for 1-5 days, add effector cells and treatments on top of the spheroids. **It is important to do this very slowly and precisely as the spheroids are not attached to the plate.**

6 Replace in the plate in the live-cell image system and take images of the wells at regular intervals for 5 days.

7 Image analysis is performed in the IncuCyte software. The size of each spheroid, as detected by edge analysis of the transmitted light image or fluorescence detection of the spheroid mass, is measured for each time point and normalized to the size of the spheroid at the time effectors and drugs were added (t = 0 h).

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