IncuCyte® S3 Spheroid Viability Assay - Fluorescent Label

For the quantification of fluorescently labeled spheroid growth and shrinkage.

This protocol describes a solution for creating single spheroids using a 96- or 384- well round-bottom, ultra-low attachment plate. This method utilizes the IncuCyte® live-cell analysis system for image-based Brightfield and fluorescence within the Brightfield boundary of spheroid area measurements. Cell lines expressing fluorescent protein can be used to monitor spheroid health.

Required materials
IncuCyte® S3 Spheroid Software Module (Essen Cat # 9600-0019)
IncuCyte® S3 Spheroid software version 2017B

Cell fluorescent label reagents and consumables
IncuCyte® NucLight Red or Green BacMam 3.0 Reagent for nuclear labeling (Essen Cat # 4621 or 4622)
IncuCyte® NucLight Red or Green Lentivirus Reagent (EF-1 α, Puro) for nuclear labeling (Essen Cat # 4624 or 4625)
IncuCyte® CytoLight Red or Green Lentivirus Reagent (EF-1 α, Puro) for cytoplasmic labeling (Essen Cat # 4481 or 4482)
Matrigel® (Corning Cat#356234), optional
96-well round-bottom, ultra-low attachment plate (e.g., Corning® Cat#7007, S-BIO Cat#MS-9096UZ, BRANDplates® Cat#7816 60, 7819 00, 7819 60)
384-well round-bottom, ultra-low attachment plate (e.g., S-BIO Cat#MS-9384UZ)

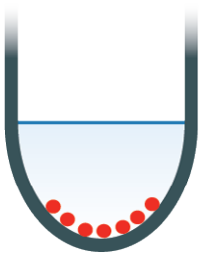
NOTE: Combination of cells expressing fluorescent proteins with cell health reagents (Cytotox, Annexin V) is NOT recommended.

General Guidelines

- 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.

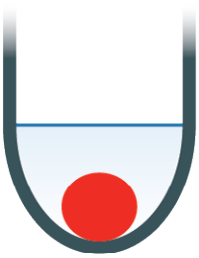
Protocol

1 CELL SEEDING (Day 0)



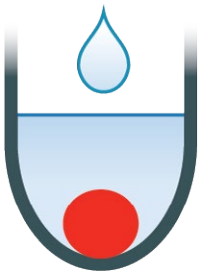
Seed cells into 96W or 384W Ultra Low Attachment plate. Centrifuge.

2 SPHEROID FORMATION (Day 0–3)



Place plate inside the IncuCyte and scan every six hours.

3 ADD TREATMENTS Day 3



Add treatments to plate. Monitor spheroid growth and shrinkage.

Day 0:

1 Seed cells

1.1. Seed cells of interest (100 µL per well for 96-well, 50 µL for 384-well) at an appropriate density into a 96- or 384-well 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, we recommend a range of 1,000 – 5,000 cells per well (10,000 – 50,000 cells per mL seeding stock).
NOTE: Some cell 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 ULA plate (125 g, 10 minutes) at room temperature (20-25°C).

Day 0–3:

2 Spheroid formation

2.1. Place the cell plate into the IncuCyte live-cell analysis System and schedule 24 hour repeat scanning:

a. Objective: 4x or 10x (96-well ULA) or 10x (384-well ULA), 1 image per well

b. **Channel selection:** Phase Contrast; Brightfield; "Green" or "Red" if fluorescent label OR if a cell health reagent will be added post spheroid formation.

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 culture media supplemented with cell heath reagent (100 µL per well for 96-well, 25 µL per well for 384-well) containing test material (e.g. small molecules, antibodies; prepared at 2x final assay concentration for 96-well, 3x final assay concentration for 384-well).

3.2. Continue to monitor spheroid growth (e.g. every 6 h for 10 days).

NOTE: It is not recommended to change media in this assay as it will disrupt spheroids containing necrosing or apoptotic cells.

Analysis Guidelines

NOTE: Utilize the IncuCyte® S3 Spheroid Software module in the Brightfield channel to identify spheroid boundaries and analyze fluorescence as needed. See "Guidelines for Analysis," which can be accessed from the IncuCyte® S3 Technical Notes folder as part of the GUI installer.

1. **For parental (non-transduced) cells –**
Brightfield Boundary Measurements
Result: Size of spheroid measurement
Suggested Metric: Largest Brightfield object (avoid segmentation of small fragments)

2. **For cells expressing fluorescent protein –**
Fluorescent and Brightfield Boundary Measurements
Result: Size and viability measurements
Suggested Metric: Integrated intensity
Secondary metric: Mean intensity

For additional product or technical information,
please e-mail us at **AskAScientist@essenbio.com**
visit our website at **essenbioscience.com**
or call
1-734-769-1600 (USA),
+44 1707 358688 (Europe)
+81-3-5579-6200 (Japan)

A complete suite of immuno-oncology applications is available to fit your experimental needs. Find more information at **essenbioscience.com/cellhealth**