

IncuCyte[®] S3 Spheroid Viability Assay - Cell Health Reagent

For the quantification of cell viability and spheroid size of parental cell lines.

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 health reagents are used to report cell viability (Cytotox Green or Red) and apoptosis (Annexin V Green or Red) in parental (non-transduced) cells.

Required materials

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

Cell health reagents and consumables

IncuCyte[®] Cytotox Red Reagent (Essen Cat#4632)
IncuCyte[®] Cytotox Green Reagent (Essen Cat#4633)
IncuCyte[®] Annexin V Red Reagent (Essen Cat#4641)
IncuCyte[®] Annexin V Green Reagent (Essen Cat#4642)
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)

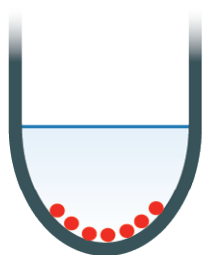
NOTE: Combination of cell health reagents (Cytotox, Annexin V etc.) with NuLight or CytoLight cells is NOT recommended.

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.

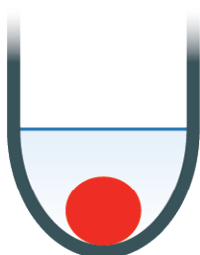
Protocol

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 AND CELL HEALTH REAGENT 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” depending on cell health reagent to be added post spheroid formation.
 - c. Scan type: Spheroid.
 - d. Scan interval: Every 6 hours. **Add treatments and cell health reagent (optional)**

Day 3:

3 Add treatments and cell health reagent (optional)

- 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 health 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. Cell Health reagents suggested concentration (optimize for specific conditions)

IncuCyte® Cytotox Red Reagent
(Essen Cat#4632) – 250 nM

IncuCyte® Cytotox Green Reagent
(Essen Cat#4633) – 25 nM

IncuCyte® Annexin V Red Reagent
(Essen Cat#4641) – 1:100 dilution

IncuCyte® Annexin V Green Reagent
(Essen Cat#4642) – 1:100 dilution

- 3.3. 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.

4 Day 7 onwards (optional, for 96-well plates)

1. Re-feed spheroid every 96 h. Remove ULA plate from the IncuCyte®. Carefully remove 100 µL of media per well and replace with 100 µL of culture media supplemented with cell reagent and containing test material at 1x final assay concentration.
2. Return plate to the IncuCyte® and continue imaging (e.g. every 6 hours).

Analysis Guidelines

NOTE: Utilize the IncuCyte® S3 Spheroid Software module in the Brightfield channel to identify spheroid boundaries and analyze fluorescent label(s) 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 parental cells with cell health reagent(s) -**
Fluorescent and Brightfield Boundary Measurements

Result: Size, viability and mechanism of action based on the cell health reagent used (e.g. Apoptosis: Caspase, Annexin V, or Cell Cytotox reagents)

Suggested 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

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