PROTOCOL



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)

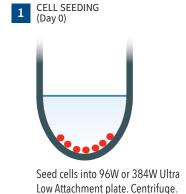
384-well round-bottom, ultra-low attachment plate (e.g., S-BIO Cat#MS-9384UZ)

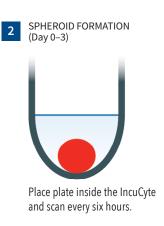
NOTE: Combination of cells expressing fluorescent proteins with cell health reagents (Cytotox, Annexin V) is NOT recommended.

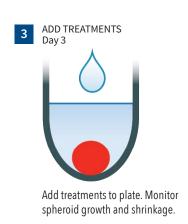
General Guidelines

- 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







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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 μ m), remove the ULA plate from the incubator and carefully add culture media supplemented with cell heath reagent (100 μ L per well for 96-well, 25 μ L 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)

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

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