IncuCyte® Spheroid Assay

For the quantification of spheroid formation, growth and shrinkage

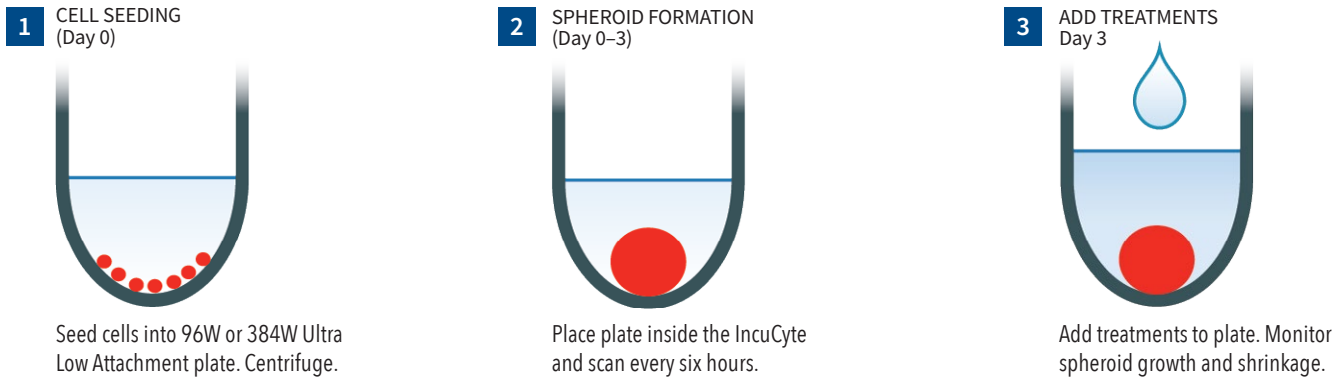
This protocol describes a solution for creating single spheroids using a 96- or 384-well round-bottom, ultra-low attachment plates. This method utilizes the IncuCyte® live-cell analysis system for image-based fluorescent measurements of spheroid formation, growth and shrinkage.

Required materials
Matrigel® (Corning Cat# 356234), optional
96-well round-bottom, ultra-low attachment plate (e.g., Corning® Cat #7007, S-BIO Cat# MS-9096UZ)
384-well round-bottom, ultra-low attachment plate (e.g., S-BIO Cat# MS-9384UZ)
IncuCyte® software version 2016B: required for spheroid scan type and additional supported vessels

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



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 it is usually in the range 1,000 – 5,000 cells per well (10,000 – 50,000 cells/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 (1000 RPM, 10 minutes) at room temperature.

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 + “Green” or “Red” if fluorescent label or cell health reagents are used
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 (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).

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

1. Re-feed spheroids every 96 h. Remove ULA plate from the IncuCyte. Carefully remove 100 µL of media per well and replace with 100 µL culture containing test material.

2. Return plate to the IncuCyte and continue imaging.

Related products and applications

A comprehensive range of fluorescent nuclear labeling reagents are available for use with the IncuCyte® live-cell analysis system to enable multiplexed measurements of cytotoxicity and apoptosis alongside spheroid formation, growth and shrinkage.

Product	Cat No.	Amount
IncuCyte® NucLight™ Red BacMam 3.0 Reagent for nuclear labeling	4621	1 mL
IncuCyte® NucLight™ Green BacMam 3.0 Reagent for nuclear labeling	4622	1 mL
IncuCyte® NucLight™ Green Lentivirus Reagent (EF-1 α, Puro) for nuclear labeling	4624	0.2 mL
IncuCyte® NucLight™ Red Lentivirus Reagent (EF-1 α, Puro) for nuclear labeling	4625	0.2 mL
IncuCyte® NucLight Green Lentivirus Reagent (EF-1 α, Bleo) for nuclear labeling	4626	0.2 mL
IncuCyte® NucLight Red Lentivirus Reagent (EF-1 α, Bleo) for nuclear labeling	4627	0.2 mL
IncuCyte® NucLight Green Lentivirus Reagent (EF-1 α, Puro) for nuclear labeling	4475	0.6 mL
IncuCyte® NucLight Red Lentivirus Reagent (EF-1 α, Puro) for nuclear labeling	4476	0.6 mL
IncuCyte® NucLight Green Lentivirus Reagent (EF-1 α, Bleo) for nuclear labeling	4477	0.6 mL
IncuCyte® NucLight Red Lentivirus Reagent (EF-1 α, Bleo) for nuclear labeling	4478	0.6 mL
IncuCyte® Caspase 3/7 Reagent for apoptosis	4440	20 µL
IncuCyte® Cytotox Red Reagent for counting dead cells	4632	5 µL x 5
IncuCyte® Cytotox Green Reagent for counting dead cells	4633	5 µL x 5
IncuCyte® Annexin V Red Reagent for apoptosis	4641	100 test
IncuCyte® Annexin V Green Reagent for apoptosis	4642	100 test

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

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)