SARTORIUS

Protocol

Incucyte® Organoid Assay for Quantifying the Growth and Death of Organoids Embedded in Matrigel®

This protocol describes a solution for monitoring and quantifying the growth and death of organoids embedded in Matrigel® in 96-well flat bottom plates. The method utilizes the Incucyte® Live-Cell Analysis System and Incucyte® Organoid Analysis Software Module for image-based brightfield measurements.

General Guidelines

- Review manufacturer guidelines for thawing and storing of 100% Matrigel®. Thaw Corning® Matrigel® overnight by submerging the vial in ice cold water in the rear of a refrigerator (+4° C). Do not allow Matrigel® to warm to room temperature at any time as this will induce polymerization.
- Following cell seeding, media addition or replenishment, remove bubbles from 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 organoid seeding and all media changes, place the plate in the Incucyte® Live-Cell Analysis System and allow the plate to warm to 37° C for 30 minutes prior to scanning.

Required Materials

- 96-well flat bottom TC-treated microplate (Corning Cat. No. 3595)
- Matrigel® Growth Factor Reduced (GFR), Phenol Red-Free (Corning Cat. No. 356231)
- Organoids of interest
- Organoid specific growth medium
- Wet ice
- Manual multi- or single-channel pipettes
- Incucyte® Organoid Analysis Software Module, version 2021A (Sartorius Cat. No. 9600-0034-A00)

Optional Materials

- Biocision CoolBox System for Microplates with CoolSink (Cat. No. 1500-0078) CoolBox™ 96F System (Includes x1 Block with gelpack and CoolSink® 96F)
- Tacta® Mechanical Pipette, 12 Channel (Sartorius Cat. No. LH-729230)
- Tacta® Mechanical Pipette, Single Channel (Sartorius Cat. No LH-729050)

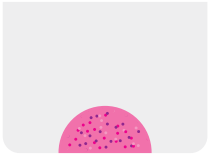
Quick Guide

1 Resuspend organoids in Matrigel® (Day 0)

Harvest and resuspend organoid fragments in 50% Matrigel®.

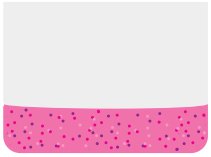
2 Add cells (Day 0)

Matrigel® Dome (Option 1)



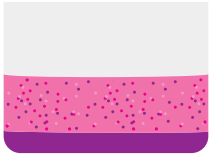
Pipette Matrigel® containing organoid fragments in the center of a 96-well plate (10 µL/well). Polymerize at 37°C for 20 minutes.

Embedded No Base (Option 2)



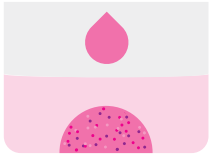
Seed organoid fragments into a 96-well plate (50 µL/well). Polymerize at 37°C for 20 minutes.

Embedded With Base (Option 3)



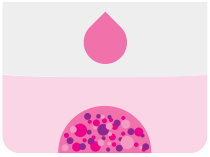
Seed organoid fragments into a pre-coated 96-well plate (50 µL/well). Polymerize at 37°C for 20 minutes.

3 Add media and monitor formation (Day 0–3)



Overlay polymerized Matrigel® with culture media (100 µL/well). Place inside the Incucyte® to monitor organoid formation.

4 Add treatments (Day 3)



Remove existing media and add treatments at 1X final assay concentration (100 µL/well). Monitor organoid growth and death.

Protocol

Important:

1. In advance of experiments it is important to have:
   a. Thawed Matrigel® overnight at 4° C. Keep on ice for duration of experiment.
   b. Stored CoolSink® 96F accessory at 4° C for at least 4 h.
   c. Warmed growth medium to ambient temperature (15–25° C).
   d. Warmed tissue culture treated plates in a 37° C incubator for at least 30 minutes.
2. Stored pipette tips used for dispensing Matrigel® at +4° C

Day 0:

1. Seed Cells

1.1 Harvest and dissociate organoids of interest according to model-specific instructions.

1.2 Aliquot culture media into a polypropylene tube and place on ice.

1.3 In a separate cold polypropylene tube, dilute 100% Matrigel® 1:1 in cold organoid specific culture media. Keep on ice.

1.4 Dilute cells in 50% Matrigel® solution at an appropriate density. Keep on ice.

Note: Seeding density will need to be optimized for each cell type used. As an example, and guide, we recommend a range of 500–2000 cells per well.

1.5 Pour diluted Matrigel® containing cells into a chilled sterile reagent reservoir (keep on ice).

1.6 Using pre-chilled pipette tips and reverse pipetting technique seed cells into each well of a 96-well plate. Utilize any of the following assay formats to establish cultures with desired formation, growth, and morphology.

a. Matrigel® Dome (Option 1)
   - To successfully form a dome, seed cells into the center of each well of a pre-warmed 96-well plate (10 µL per well).

   Tip: For ease, use a manual P100 single-or multi-channel pipette (e.g Sartorius Cat. No. LH-729050, LH-729230 respectively) to spot domes.

   Note: Use reverse pipetting technique to minimize generation of bubbles.

b. Cells Embedded in Matrigel® (Option 2)
   - Chill plate on a pre-chilled CoolSink® 96F within a CoolBox™ 96F box for 2–5 minutes. Seed cells into each well (50 µL per well).
   - While the plate is cold and Matrigel® is still liquid, gently rock the plate once within the CoolBox™ to ensure even well distribution.

c. Cells Embedded on Matrigel® Base (Option 3)
   - Place pre-coated plate on a cool sink (5 min) and seed cells on top of polymerized Matrigel® base (50 µL per well).
   - See Appendix for plate coating instructions.

1.7 Gently remove any bubbles using a wash bottle containing 70–100% ethanol, with the inner straw removed, to blow vapor over the surface of each well.

1.8 Place the plate in a 37° C incubator for 20 minutes to polymerize the Matrigel®.

1.9 Overlay polymerized layer with culture media (100 µL).

1.10 Place plate in a 37° C incubator for 30 minutes prior to scanning.

Day 0–3:

2. Monitor Organoid Formation

2.1 Place the cell plate into the Incucyte® Live-Cell Analysis System and schedule 24 hour repeat scanning:
   a. Objective: 4X (Corning® 96-well) 1 image per well
   b. Channel selection: Phase Contrast + Brightfield
   c. Scan type: Organoid, Assay
   d. Scan interval: Every 6 hours

Day 3:

3. Add Treatments

3.1 3 days post seeding or once organoids have reached desired size, remove the plate from the Incucyte® and carefully aspirate existing media using a manual multi-channel pipette.

Note: When removing media, keep the pipette tip at the edge of the well to avoid disrupting the polymerized Matrigel® layer.

3.2 Add appropriate treatments at 1X final assay concentration (100 µL per well).

3.3 Continue to monitor organoid growth and death (e.g., every 6 hours for 5 days).

4. Re-Feed Cultures

4.1 Maintain cultures by performing 100% media replenishment every 2 days.

4.2 Remove plate from Incucyte®. Carefully remove 100 µL of media per well and replace with 100 µL of media containing test agents (1X final assay concentration).

Note: When removing media, keep the pipette tip at the edge of the well to avoid disrupting the polymerized Matrigel® layer.

4.3 Return plate to the Incucyte® and continue to monitor organoid growth and death.

Appendix

5. Coating Plate With Matrigel®

5.1 In a cell culture hood, chill plates (10–15 minutes) on a pre-chilled CoolSink® 96F within a CoolBox™ 96F box.

5.2 In a cold polypropylene tube, dilute 100% Matrigel® 1:1 in cold serum-free culture media (keep all Matrigel® solutions on ice).

Note: To prevent incomplete gel formation, for coating we recommend using ≥ 4 mg/mL Matrigel®.

   a. Using a cold serological pipette, slowly pipette 100% Matrigel® into serum-free media and taking care to avoid bubbles, slowly mix by pipetting the solution up and down.

5.3 Pour prepared solution into a pre-chilled sterile reagent reservoir (keep on ice).

5.4 Using pre-chilled pipette tips and reverse pipetting technique, coat the pre-chilled 96-well plate by carefully adding diluted Matrigel® into the center of each well.
   a. The volume required to coat wells to encourage organoid formation and maturation will need to be optimized for each cell type of interest. We recommend performing a titration using a minimum of 20 µL and maximum of 40 µL Matrigel® per well.
   b. While the plate is cold and Matrigel® is still liquid, gently rock the plate once within the CoolBox™ to ensure even coating of each well.

Note: Use of reverse pipetting technique is important to minimize bubbles.

5.5 Remove any bubbles using a wash bottle containing 70–100% ethanol, with the inner straw removed, to blow vapor over the surface of each well.

5.6 Place the plate in a 37° C incubator for 20 minutes to polymerize the Matrigel®.

Analysis Guidelines

1. **Create a New Analysis Definition**
   - In the Analysis Wizard window, select 'Organoid' Analysis Type.
   - Select a set of representative images.
   - Adjust the Background/cells slider to determine the boundary of the organoid objects.
   - Evaluate the Brightfield (BF) mask and refine filter parameters accordingly. 'Preview All' to ensure parameters set appropriately mask all representative images within the collection.
   - Adjust the Edge split slider to delineate between individual organoid objects.
   - Evaluate the BF mask and refine filter parameters accordingly. 'Preview All' to ensure parameters set appropriately mask all representative images within the collection.
   - Once satisfied with all parameters, complete the Launch Wizard analysis by selecting the scan times and wells to be analyzed.

     Note: If your experiment is in progress you will have an option to check 'Analyze Future Scans' to perform real-time analysis.

2. **Data Interpretation**

Once the Analysis Job is complete the following primary metrics are provided:

- Organoid Object Count. This metric represents the number of objects per image (well).
- Organoid Object Total Area. This metric represents the total area of BF objects within the image (well) and is recommended for tracking organoid size over time.
- Organoid Object Avg. Eccentricity. This metric represents how round the organoids are.
- Organoid Darkness. This metric is available for tracking changes in organoid brightness over time.

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