

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.

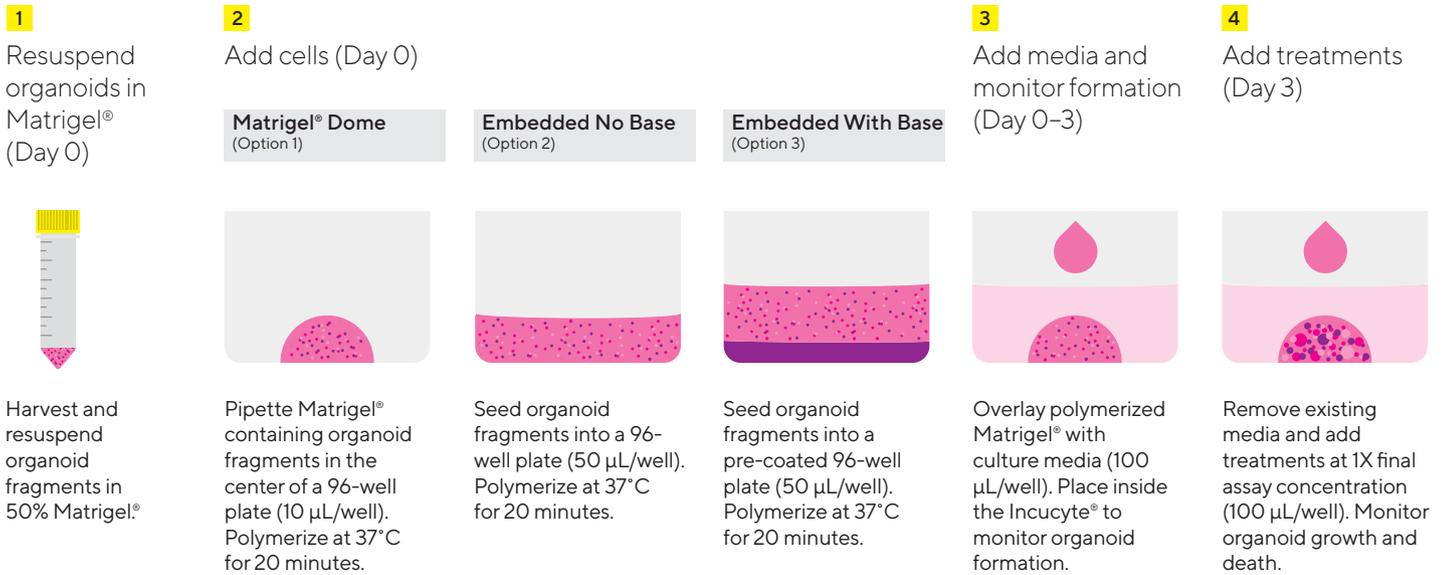
Required Materials

- 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



Protocol

Important:

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

Day 0:

1. Seed Cells

- Harvest and dissociate organoids of interest according to model-specific instructions.
- Aliquot culture media into a polypropylene tube and place on ice.
- In a separate cold polypropylene tube, dilute 100% Matrigel® 1:1 in cold organoid specific culture media. Keep on ice.
- 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.

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

- 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.
- Place the plate in a 37° C incubator for 20 minutes to polymerize the Matrigel®.
- Overlay polymerized layer with culture media (100 µL).
- 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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