

Incucyte® Organoid Culture QC

For the Quantification of Organoid Growth in Matrigel® Domes

This protocol provides an overview for culturing, monitoring and measuring organoid growth, counts and morphology of Brightfield images in Matrigel® domes utilizing the Incucyte® Live-Cell Analysis System and Incucyte® Organoid Analysis Software.

General Guidelines

- Follow manufactures 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 dome creation (in 50–100% Matrigel®) and media addition, remove bubbles from dome or wells respectively 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.

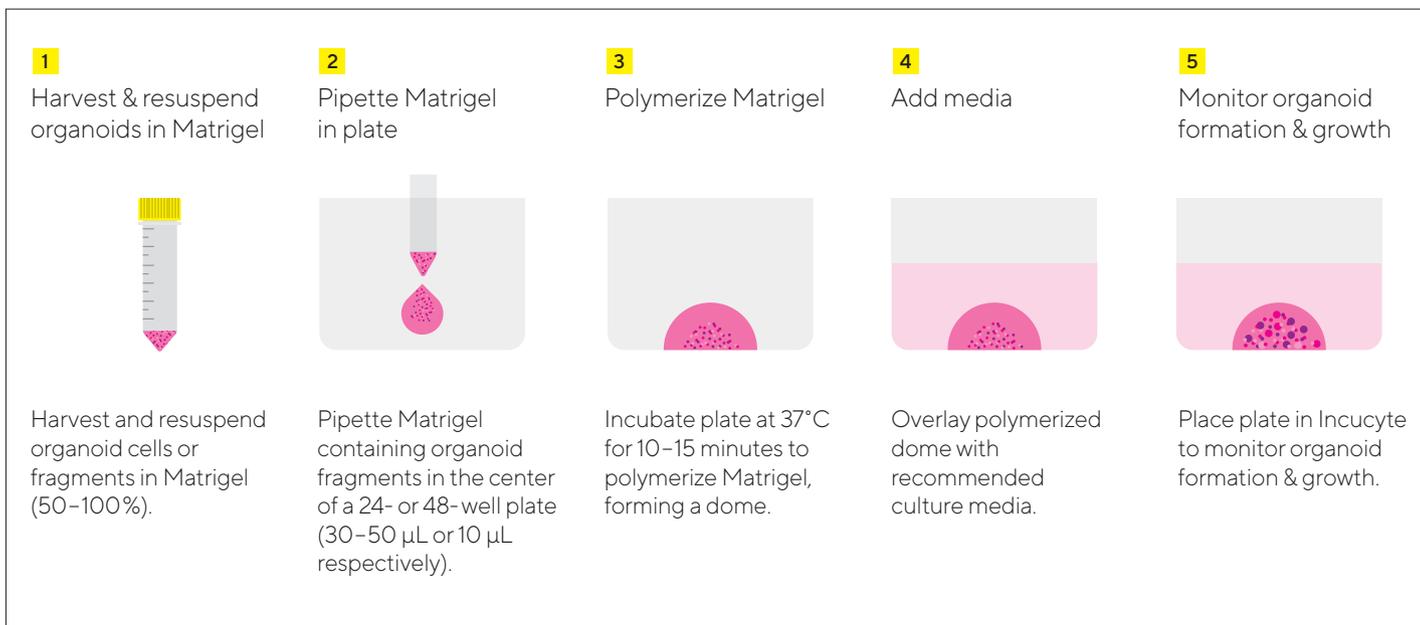
Required Materials

- 24-well or 48-well flat bottom TC-treated microplate (Corning Cat. No. 3526, Cat. No. 3548 respectively)
- Matrigel® Growth Factor Reduced (GFR), Phenol Red-Free (Corning Cat. No. 356231)
- Organoids of interest
- Organoid Specific Growth Medium
- Manual single-channel pipettes
- Wet Ice
- Incucyte® Organoid Analysis Software Module, version 2020B (Cat. No. 9600-0034-A00)

Optional Materials

- Incucyte® Coolsink 96F (Cat. No. 1500-0080)
- StemCell Technologies, Mouse Intestinal Organoids Cat. No. 70931; Mouse Pancreatic Organoids Cat. No. 70933; Mouse Hepatic Organoids Cat. No. 70932
- IntestiCult™ OGM Cat. No. 06005; PancreaCult™ OGM Cat. No. 06040; HepatiCult™ OGM Cat. No. 06030

Quick Guide



Protocol

Important:

In advance of experiments it is important to have:

- Thawed Matrigel® Matrix vial overnight at 4° C. Keep on ice for duration of experiment.
- Warmed tissue culture treated plates (24-well or 48-well) in a 37° C incubator for at least 30 minutes.
- Stored Coolsink 96F accessory in a 37° C incubator for at least 4h.
- Warmed growth medium to room temperature (15–25° C).

Stored pipette tips used for dispensing Matrigel® at 4° C

1 Culturing Organoids in Matrigel® Domes

Vessel Type	MG* Dome Vol.	MG* Dome Concentration	No. of Domes/Well	Growth Media Vol/Well
24-well plate	30 or 50 μ L	50 or 100%	1	750 μ L
48-well plate	10 μ L	50 or 100%	1	250 μ L

* Matrigel® (MG)

Creating Matrigel® Domes

- Thaw, harvest and passage organoids of interest according to model-specific guidelines.
- Resuspend pellet in Matrigel® (see table for recommended concentrations) or ECM of choice.
- Pipette Matrigel® containing organoid cells or fragments into the centre of the well of a 24-well or 48-well plate to form a dome (see table for recommended volumes).

- Remove any bubbles using a wash bottle containing 70–100% ethanol, with the inner straw removed, to gently blow vapor over the surface of each dome.
Tip: Blow vapor from a distance to minimize dispersion of dome.
- Place the plate in a 37° C incubator for 10–15 minutes to polymerize the Matrigel®.
- Gently add model-specific growth medium to each well (see table for recommended volumes).
Tip: Pipette medium down the sidewall of the well to avoid disrupting dome.
- Remove any bubbles using a wash bottle containing 70–100% ethanol, with the inner straw removed, to gently blow vapor over the surface of each well.
- Add sterile PBS to any unused wells.
- Place plate in a 37° C incubator for 30 minutes prior to scanning.

2 Monitor Organoid Formation and Growth

- Place plate into the Incucyte® Live-Cell Analysis System and schedule 24 hour repeat scanning:
 - Objective: 4X (24- or 48-well) 1 image per well
 - Channel selection; Phase Contrast + Brightfield
 - Scan type: Organoid
 - Scan interval: Every 6 hours

3 Re-Feed Cultures

- Maintain cultures by performing medium exchanges as required (e.g. 2 -3 days a week depending on organoids of interest).
Note: When removing media keep the pipette tip at the edge of the well bottom to avoid disturbing the dome.
- Continue to monitor organoid growth (e.g. every 6 hours for up to 10 days).

Analysis Guidelines

1 Create a New Analysis Definition

- a. In the Analysis Wizard window select 'Organoid' Analysis Type.
- b. Select a set of representative images.
- c. Adjust the Background/cells slider to determine the boundary of the organoid objects.
- d. Evaluate the Brightfield (BF) mask and refine filter parameters accordingly. 'Preview All' to ensure parameters set appropriately mask all representative images within the collection.
- e. Adjust the Edge split slider to delineate between individual organoid objects.
- f. Evaluate the BF mask and refine filter parameters accordingly. 'Preview All' to ensure parameters set appropriately mask all representative images within the collection.
- g. 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:

- a. Organoid Object Count. This metric represents the number of objects per image (well).
- b. 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.
- c. Organoid Object Average Eccentricity. This metric represents how round the organoids are.
- d. Organoid Darkness. This metric is available for tracking changes in organoid brightness over time.

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