SARTORIUS

Protocol

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

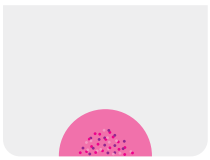
1 Harvest & resuspend organoids in Matrigel

Harvest and resuspend organoid cells or fragments in Matrigel (50–100%).

2 Pipette Matrigel in plate

Pipette Matrigel containing organoid fragments in the center of a 24- or 48-well plate (30–50 µL or 10 µL respectively).

3 Polymerize Matrigel



Incubate plate at 37°C for 10–15 minutes to polymerize Matrigel, forming a dome.

4 Add media

Overlay polymerized dome with recommended culture media.

5 Monitor organoid formation & growth

Place plate in Incucyte to monitor organoid formation & growth.

Protocol

Important:
In advance of experiments it is important to have:

a. Thawed Matrigel® Matrix vial overnight at 4° C. Keep on ice for duration of experiment.
b. Warmed tissue culture treated plates (24-well or 48-well) in a 37° C incubator for at least 30 minutes.
c. Stored Coolsink 96F accessory in a 37° C incubator for at least 4h.
d. 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 µL	50 or 100%	1	750 µL
48-well plate	10 µL	50 or 100%	1	250 µL

* Matrigel® (MG)

Creating Matrigel® Domes

1.1 Thaw, harvest and passage organoids of interest according to model-specific guidelines.
1.2 Resuspend pellet in Matrigel® (see table for recommended concentrations) or ECM of choice.
1.3 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).
1.4 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.
1.5 Place the plate in a 37° C incubator for 10–15 minutes to polymerize the Matrigel®.
1.6 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.
1.7 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.
1.8 Add sterile PBS to any unused wells.
1.9 Place plate in a 37° C incubator for 30 minutes prior to scanning.

2 Monitor Organoid Formation and Growth

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

3 Re-Feed Cultures

3.1 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.
3.2 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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