SARTORIUS

Product Guide

Incucyte® 3D Nanowell Plate

For Organoid Assay with Incucyte® Live-Cell Analysis Systems

Product Information

Presentation, Storage and Stability

The Incucyte® 3D Nanowell Plate is a ready-to-use, hydrogel-based 96-well plate designed for reproducible and high-throughput 3D organoid culture. Its unique design ensures the robust generation of single organoids in each well, allowing for easy manipulation and imaging within the same plate. The plates are individually wrapped and provided at three sizes: single, 5- and 10-pack. The Incucyte® 3D Nanowell Plate should be stored at 2 – 8 °C and protected from direct sources of light and heat. Do not freeze the plate. Do not store upside down. The plates are stable for at least 6 months post receipt when stored at the recommended conditions.

Product Name	Cat. No.	Size	Storage	Stability
Incucyte® 3D Nanowell Plates 500 µm	BA-04878	Single pack	2 – 8 °C	6 months post receipt
	BA-04879	5-pack		
	BA-04880	10-pack		

Compatible with Incucyte® SX5, S3, or SX1 Live-Cell Analysis Systems.

Note: Safety data sheet (SDS) information can be found on our website at sartorius.com/incucyte-shop

Background

Incucyte® 3D Nanowell Plates are a valuable tool designed to facilitate high-throughput organoid studies, which are crucial for advancing research in developmental biology, disease modeling, and drug discovery. These plates are engineered to provide a three-dimensional (3D) microenvironment that closely mimics in vivo conditions, allowing for the growth and differentiation of organoids. Unlike traditional organoid culture platforms, these plates offer an array of hydrogel printed microwells, each designed to support the formation of uniform and reproducible organoids (cell seeding chamber, Figure1A). Each well also features a unique pipetting port (Figure 1B), that allows for seamless medium changes without disrupting the organoid culture and allows for automation of cell seeding, medium changing, and treatment additions via liquid handlers. This high-throughput capability enables researchers to conduct large-scale experiments efficiently, testing multiple conditions or compounds simultaneously. Together with the Incucyte® Live-Cell Analysis System and Incucyte® Organoid Analysis Software Module, thousands of organoids can be imaged and analyzed in real time to evaluate growth and viability.

Recommended Use

The seeding density in Incucyte® 3D Nanowell Plate depends on microwell size, cell size, proliferation rate, treatment, monitoring period, etc, and needs to be optimized for each organoid model. We recommend a starting range of 50 – 200 cells per 500 µm microwell. The recommended seeding volume is 50 µL per well. The seeding density is calculated as follows:

Seeding density (cells/mL) =
 # cells per microwell × # microwells/0.05 mL

Note: Each well of the Incucyte® 3D Nanowell Plate contains 73 microwells (500 µm diameter).

For example, to seed 100 cells per 500 µm microwell, the seeding density =
100 cells per microwell × 73 microwells/0.05 mL = 1.46×10^5 cells/mL

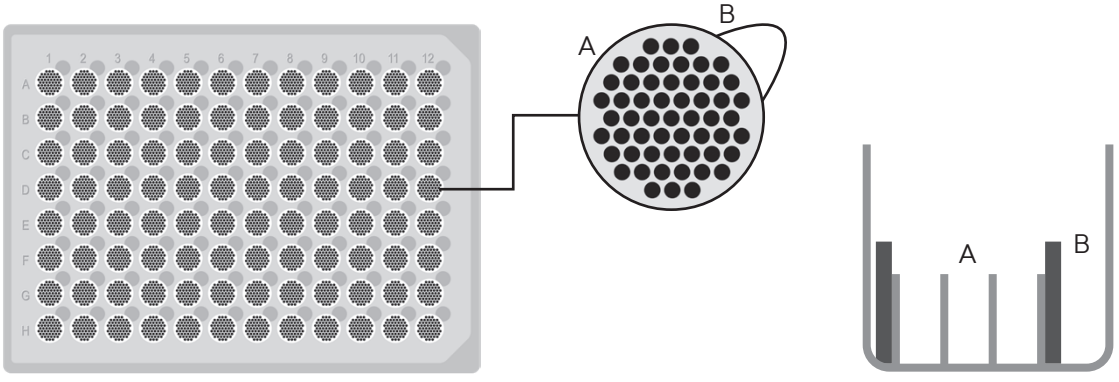
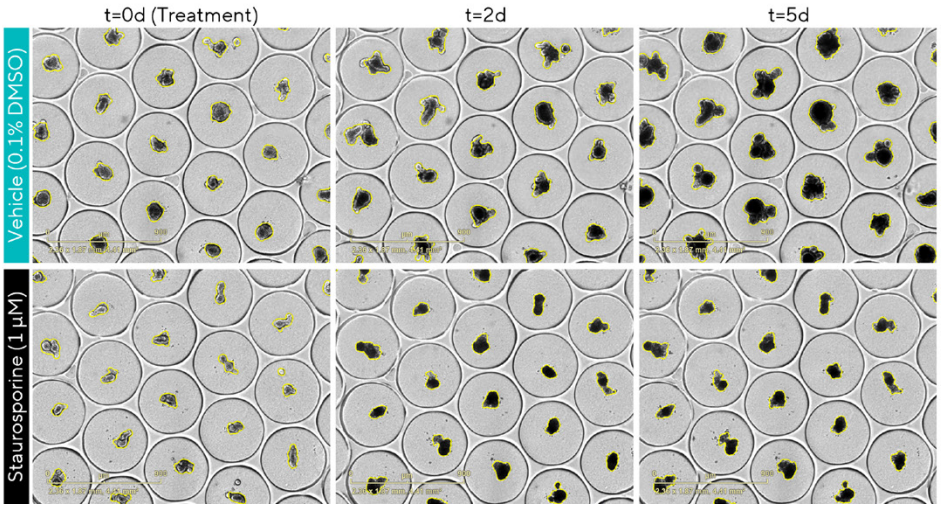


Figure 1
Unique Design of Incucyte® 3D Nanowell Plate
A. Cell seeding chamber of each well which contains numerous U-shaped microwells in hydrogel. For the 500 µm plate, each well has 73 microwells with diameter of 500 µm. B. The pipetting port for medium change and drug treatment.



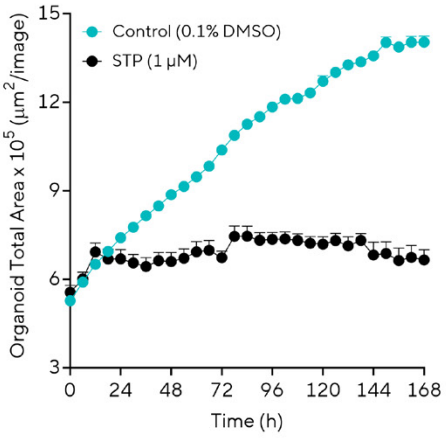


Figure 2

Example Data

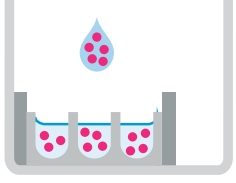
Brightfield images show mouse intestinal organoids (200 cells/µwell) grown in the Incucyte® 3D Nanowell Plate and treated for 5 days with Staurosporine (1 µM, STP). Images and time-course data of Total Organoid Area demonstrate an increase in size of the vehicle control treated organoids that is not present in organoids treated by STP.

Quick Guide

1. Prepare single cell solution

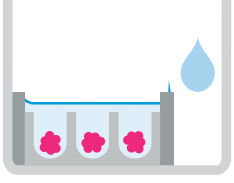
Harvest and dissociate organoids into single cells.

2. Seed cells



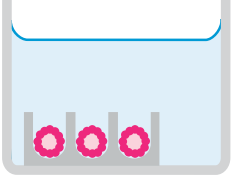
Add 50 µL cell suspension into cell seeding chamber.

3. Add culture medium



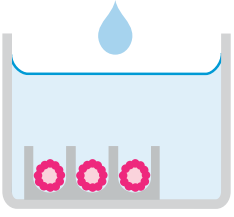
Allow cells to settle for 20 – 30 min in an incubator, then add 150 µL culture medium containing 1.5 – 2% Matrigel® into pipetting port.

4. Monitor organoid growth



Capture images in Incucyte® Live-Cell Analysis System. Change media every 2 – 3 days, adding 1 – 1.5% Matrigel® every other media change

5. Add treatment



Remove media, add treatments at 1.33X final assay concentration (150 µL/well). Continue to monitor organoid growth and death.

Protocols and Procedures

General Guidelines

- Review manufacturer guidelines for thawing and storing 100% Matrigel®. Thaw Corning® Matrigel® overnight by submerging the vial in ice in the rear of a refrigerator (2 – 8 °C). Do not allow Matrigel® to warm to room temperature at any time as this will induce polymerization.
- During media changes or treatment additions, ensure that the pipetting port is dry to avoid distrubance of organoids.
- Always use cell seeding chamber to add cells, use pipetting port for culture media and treatments. On the first day of culture, add ROCK inhibitor Y-27632 (10 µM) or Thiazovivin (2.5 µM) to organoid growth media and dissociation solution. ROCK inhibitor is not needed after the first media change.
- 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 – 60 minutes prior to scanning.
- Due to the inherent nature of bioprinting, there may be artifacts observed in a small subset of microwells within a portion of the wells, such as shallow microwells or trapped air bubbles. Many of these can be addressed during image processing and analysis and have not been found to significantly impact the population metrics captured.
- Flow lines may be observed as a result of the injecton molding process during the plate manufacture. This line can be exludued during analysis from most microwells and has not been seen to significantly impact population metrics gathered.

Required Materials

- Incucyte ® 3D Nanowell Plates - 500 µm (Sartorius Cat. No. BA-04878, BA-04879, BA-04880)
- Matrigel® Growth Factor Reduced (GFR), Phenol Red-Free (Corning® Cat. No. 356231)
- Basal culture medium: Advanced DMEM/F12 supplemented with 10mM HEPES, and 1X Glutamax
- Basal culture medium supplemented with 10% FBS
- Organoids of interest
- Organoid specific growth medium
- TrypLE Express (ThermoFisher Scientific Cat. No. 12605010)
- Y-27632 or Thiazovivin
- Wet ice
- Multi- or single-channel pipettes
- Incucyte® Organoid Analysis Software Module (Cat. No. 9600-0034), base software version 2024A or later.

Organoid Assay in Incucyte® 3D Nanowell Plate Protocol

Important:

Prior to the experiment, it is important to:

- Thaw an aliquot of Matrigel® on ice.
- Pre-cool basal culture medium on ice.
- Bring organoid growth medium to room temperature.
- Prepare basal culture medium supplemented with 10% FBS for TrypLE Express inactivation.
- Add Y-27632 (10 µM) or Thiazovivin(2.5 µM) to TrypLE Express dissociation solution.
- Add 10 µM Y-27632 or 2.5 µM Thiazovivin to organoid growth medium (prepare 40 mL for one full plate).

1. Prepare the plate

1.1 Before use, spray Incucyte® 3D Nanowell Plate in its outer plastic wrapping with 70% ethanol, open the plate under the hood, and remove the sealing layer inside the lid.

1.2 Aspirate the storage buffer from the wells, both in the pipetting port and the cell seeding chamber following the steps below:

a. With an aspirator and a Pasteur pipette, remove the liquid from the pipetting port first (Figure 1 B).

b. Carefully access the cell seeding chamber and aspirate the remaining buffer until the microwell arrays become visible (full buffer removal is not necessary). To do so, slide your pipette tip on the side of the well until resistance is felt at the ring, and aspirate from there without touching the hydrogel (Figure 1 A).

1.3 Add 150 µL of organoid growth medium in the pipetting port. Leave the plate for 30 – 60 minutes at room temperature or 15 – 30 minutes in the incubator to equilibrate the hydrogel.

Note: For precious medium, carefully add 50 µL of medium only to the cell seeding chamber.

2. Prepare single cell suspension

Harvest and dissociate organoids of interest according to model-specific instructions. Below is an example protocol to obtain single cells from organoids cultured in Matrigel® domes. The conditions may vary depending on the model.

2.1 Collect the organoids of interest by disrupting the Matrigel® domes using ice cold basal culture medium and collect them in a 15 mL conical tube.
2.2 Rinse wells with an additional 1 mL of ice cold basal culture media and add it to the 15 mL tube.
2.2 Centrifuge at 290 x g for 5 minutes at 4 °C.

Note: Centrifugation settings need to be adapted depending on the organoid model.

2.3 Discard the supernatant carefully without disturbing the cell pellet and wash the organoids once with 10 mL of ice-cold basal culture medium.
2.4 Centrifuge at 290 x g for 5 minutes at 4 °C.
2.5 Aspirate the supernatant, add the desired amount of dissociation solution with 10 µM Y-27632 or 2.5 µM Thiazovivin to the cell pellet and gently pipette up and down 5 – 10 times.
2.6 Incubate the tube at 37 °C for 5 – 10 minutes , mix every 3 – 5 minutes.

Note: For some cell types dissociation to single cells may take longer and needs to be optimized.

2.7 Add basal culture medium supplemented with 10% FBS to the tube in equal volume to the dissociation solution, gently pipette up and down 5-10 times to mix.
2.8 Centrifuge at 290 x g for 5 minutes at 4 °C.
2.9 Discard the supernatant carefully without disturbing the cell pellet.
OPTIONAL: Perform a second wash and centrifugation with the same volume of basal culture medium as step 2.8 if the pellet is not clear or to wash away the FBS.
2.10 Resuspend the cell pellet in 0.1 – 1 mL organoid growth medium containing 10 µM Y-27632 or 2.5 µM Thiazovivin and count the cells.
2.11 Prepare the appropriate cell seeding density in the same culture medium as step 2.10 (see Recommended Use section).

Note: To seed an entire plate, prepare at least 5mL of cell suspension.

3. Seed cells in Incucyte® 3D Nanowell Plate

3.1 Remove organoid growth medium from both the pipetting port and the cell seeding chamber of the plate following the same procedure as step 1.2 above.
3.2 Add 50 µL of cell suspension in the cell seeding chamber, on top of each microwell array.
3.3 Allow the cells to settle for 20 – 30 minutes in the incubator (37 °C, 5% CO_2).
3.4 Cool down the remained organoid growth medium containing 10 µM Y-27632 or 2.5 µM Thiazovivin (15 mL for the entire plate) on ice.
3.5 Add the appropriate amount of thawed Matrigel® to the growth medium (step 3.4) to have 1.5 – 2% as final concentration (corrected with the seeded volume of 50 µL, correction factor = 1.33). Mix gently and leave the Matrigel® containing medium at room temperature.
3.6 Remove the Incucyte ® 3D Nanowell Plate from the incubator and confirm under the microscope that the cells have settled to the bottom of the microwells. Add 150 µL of the growth medium with diluted Matrigel® carefully into the pipetting port.
3.7 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.
3.8 Place the plate in Incucyte® Live-Cell Analysis System in a 37 °C incubator with 5% CO_2 for 30 – 60 minutes prior to scanning.

4. Monitor Organoid Formation

4.1 Schedule repeat scanning in the Incucyte® for every 6 hours to monitor organoid formation.
   a. Scan type: Organoid Assay.
   b. Image Channels: Phase Contrast + Brightfield
   c. Objective: 4X.
   d. Plate: Incucyte® 3D Nanowell Plate – 500 µm

5. Change Media and Add Treatment

5.1 Change medium every 2-3 days as follows:

a. Aspirate medium from the pipetting port.
b. Add back 150 µL of organoid growth medium to the pipetting port.
c. Every other medium change (every 4 – 6 days), add 1 – 1.5% Matrigel® in the medium (to be adapted for each model).

Note: Always use the pipetting port for medium exchange and addition of treatments to avoid disturbing the organoid.

5.2 Follow the steps below to add treatments to the organoid culture if needed:

a. 2 – 3 days post seeding or once organoids have reached desired size, remove the plate from the Incucyte®.
b. Carefully aspirate medium from the pipetting port.
c. Add 150 µL appropriate treatments in organoid growth medium (corrected with the seeded volume of 50 µL, correction factor = 1.33) through the pipetting port.
d. Continue to monitor organoid growth and viability for the desired assay length.

6. Analyze images

6.1 In the Analysis Wizard window, select "Organoid" Analysis Type and a set of representative images to preview the analysis parameters.

6.2 Adjust the "Background/Cells" slider to determine the boundary of the organoid objects.

6.3 Evaluate the Brightfield (BF) mask and refine filter parameters accordingly.

6.4 "Preview All" to ensure parameters set appropriately mask all representative images within the collection.

6.5 Once satisfied with all parameters, complete the Launch Wizard analysis by selecting the scan times and wells to which the processing definition will be applied for analysis.

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

6.6 Once the image analysis is complete, use the following metrics to evaluate organoid growth and the effect of the treatments:

- Organoid Object Count: Total Number of Objects per Image.
- Organoid Object Total Area per Image: Total Area of BF Objects Within the Image.
- Organoid Object Average Area per Image: Average Area of BF Objects Within the Image.

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