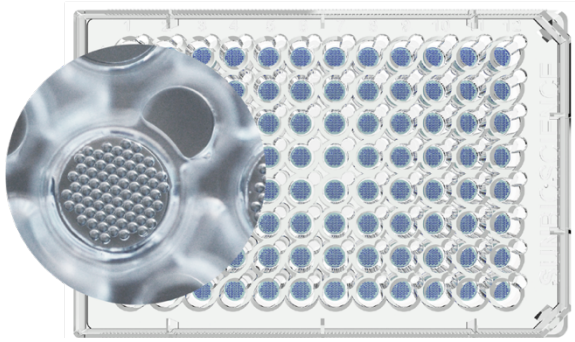
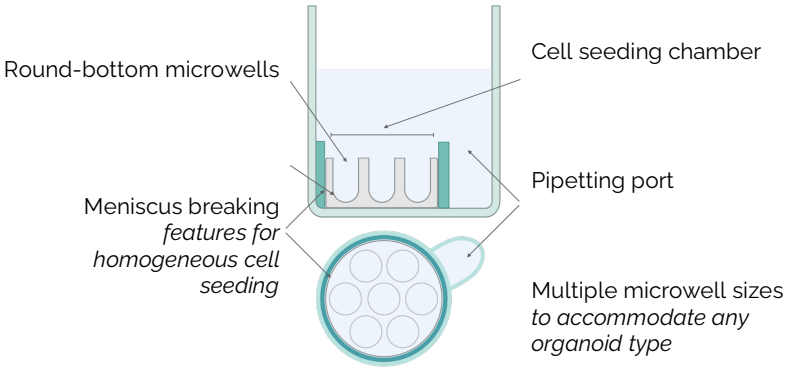
Data Sheet Gri3D®

SUNBIOSCIENCE
Gri3D-96IBI-S-96-500
73 microwells

FOR RESEARCH USE ONLY Store at: 4˚C





Background

Gri3D® is a ready-to-use platform for high-throughput and reproducible 3D organoid and spheroid culture. Based on a standard 96 microtiter plate, each well contains a microwell array patterned in a cell repellent polyethylene-glycol (PEG) hydrogel. On Gri3D®, microtissues are robustly generated in the microwells and are located in the same imaging plane. The high-water content of the hydrogel (>95%) renders it transparent and ideal for diffraction-less imaging. This greatly facilitates quantitative analyses in high content image-based screens. Furthermore, the uniquely designed pipetting port enables medium exchange without microtissue loss. The design allows automation of cell seeding, media exchange and compound incubation with liquid-handlers.

References

Brandenberg, N., Hoehnel, S., Kuttler, F. et al. High-throughput automated organoid culture via stem-cell aggregation in microcavity arrays. Nat Biomed Eng 4, 863–874 (2020). https://doi.org/10.1038/s41551-020-0565-2

Storage and Handling

Store all components of Gri3D® in the fridge at 4˚C (40˚ F), away from direct sources of light and heat. Do not freeze. Do not store upside down.

Product specifications

Gri3D® are 96-wellplate SLAS/ANSI standard 3D cell culture plates. Plate dimensions are 127.90 x 85.60 x 14.45 mm (l x w x h. Gri3D® plates are made of transparent polystyrene. Plate bottom can be either in polystyrene (1.5 mm thick) or imaging compatible IBIDI polymer (180 µm thick).

Instructions of use

This protocol describes the culturing of 3D microtissues in Gri3D® microwell arrays. The resulting spheroid or organoid arrays are homogeneous and can be used for a variety of applications, such as toxicity, efficacy, or antibody transport assays.

1. Gri3D® plate preparation

- Before use, spray Gri3D® in its outer plastic wrapping with ethanol, open the plate under the hood, and remove the sealing layer inside the lid. Aspirate the storage buffer from the wells, both in the pipetting port and the cell seeding chamber (Fig. 1 A).
    - *TIP:* With an aspirator and a Pasteur pipette, first remove the liquid from the pipetting port. Then, carefully access the cell seeding chamber and aspirate the remaining buffer until the microwell arrays become visible (full buffer removal is not necessary). For that, slide your pipette tip on the side of the well until you feel a resistance – the seeding ring; aspirate from there without touching the hydrogel.
- Add 150 µl of cell medium in the pipetting port (Fig. 1 C). Leave the plate for at least 30 minutes at room temperature or at least 15 minutes in the incubator to equilibrate the hydrogel.
    - For precious medium, carefully add 50 µl of medium only to the cell seeding chamber.

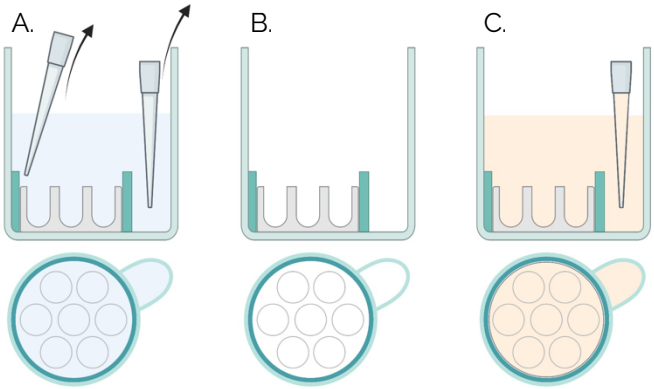


Figure 1. Gri3D® plate preparation. A-B. Remove storage buffer from the pipetting port. C. Add 150 µl of cell medium to the pipetting port and keep in incubator for 15-30 minutes.

2. Cell preparation and seeding

- Prepare the appropriate cell seeding density in medium[1]. We recommend a range of 100-300 cells starting population for organoid cultures (highly proliferative), and 500-6000 cells for spheroid cultures with cell lines or primary cells, but this will need to be adapted to each model (see Table 1). The recommended seeding volume is 50 µl:

  Seeding density (cells/ml) = # cells per microwell x # microwells x 20

  To seed an entire plate, prepare at least 5 ml of cell suspension (5% extra volume).

- Remove medium from both the pipetting port and the cell seeding chamber (Fig. 2 A-B).
- Add 50 µl of cell suspension in the cell seeding chamber, in the center, on top of each microwell array (Fig. 2 C).

[1] Seeding density will depend on the model.

Microwell diameter (in µm)	400	500	600	800
Microwell number (per well)	121	73	55	31
Cell density range (per microwell)	100 – 1500	150– 2000	200 - 4000	300 – 6000

Table 1. Microwell numbers per well and cell densities per microwell as a function of the microwell diameter (in µm).

- Let the cells sediment for 20-30 minutes in the incubator (37°C, 5% CO2).
- In organoid cultures needing extracellular matrix (ECM): place the left over medium on ice to cool down. Thaw on ice an aliquot with the desired volume of Matrigel (or other ECM of interest). Once the leftover medium is cold and the Matrigel thawed, add the appropriate amount of Matrigel[2] to the medium to have 1.5-2% as final concentration (corrected with the seeded amount, 50 µl; correction factor = 1.33). Homogenize to ensure proper ECM dilution and leave the medium at room temperature.
- Take the Gri3D® plate from the incubator and check under the microscope that the cells have sedimented to the bottom of the microwells. Then, add 150 µl of medium (with diluted Matrigel in the case of organoid cultures) carefully in pipetting port (Fig. 2 D).
- Incubate the cells at 37°C, 5% CO2.

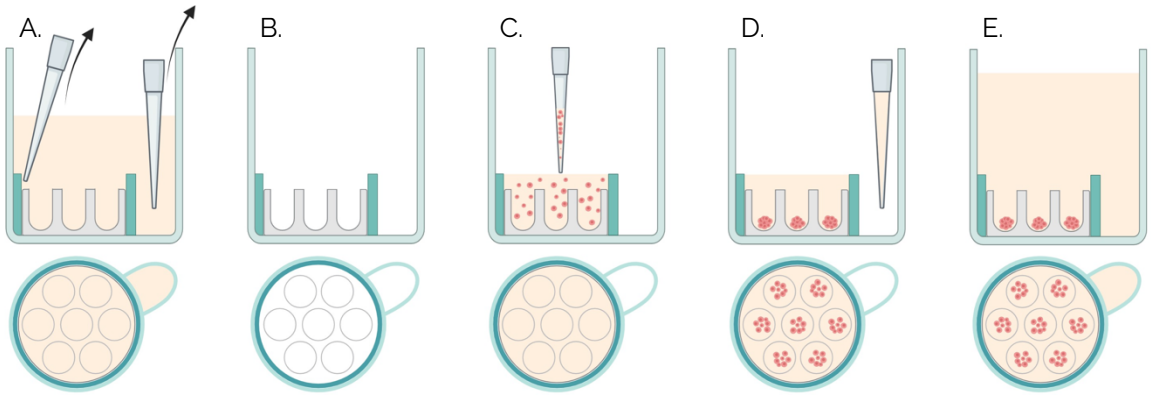


Figure 2. Cell seeding on Gri3D®. A-B. Remove medium from the pipetting port and cell seeding chamber. C. Add 50 µl of cell suspension to the cell seeding chamber. D-E. After cell sedimentation, add 150 µl of media to the pipetting port (with diluted ECM in organoid cultures).

[2] ECM concentration should be optimized or each organoid model.

3. Cell maintenance on Gri3D®

- Change medium every 2-3 days. For that, aspirate medium from the pipetting port, and add back 150 µl of medium (Fig. 4). Addition of half the initial concentration of Matrigel diluted in media is recommended in organoid cultures (to be adapted for each model).
- **IMPORTANT**: Do not touch the microwell array gels compartment, as that would disturb the forming microtissues. Use the pipetting port instead.

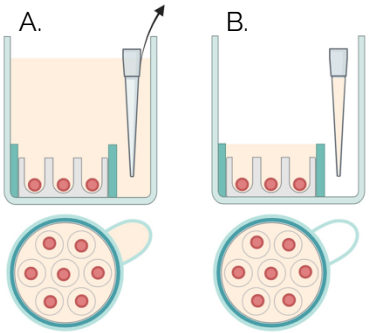


Figure 3. Cell maintenance on Gri3D®. A. Remove medium from the pipetting port. B. Add back 150 µl medium (if desired, supplemented with ECM) to the pipetting port.

4. Microtissue assays on Gri3D®

- Do your assays (dye, probe, or reagent incubation) by using the pipetting port to avoid disturbing the organoid. Cells can be directly imaged on Gri3D®.
- If a second cell type should be added to the microtissues to establish a co-culture, remove 150 µl medium from the pipetting port and carefully remove the desired volume from the cell seeding chamber (typically 20 µl). Pipette the cell solution in the cell seeding chamber on top of the microtissues (typically 20 µl; cell seeding chamber maximum volume = 50 µl). Let the cells sediment for 15-30 minutes in the incubator. Add 150 µl of medium in the pipetting port and proceed with cell culture as usual (see Fig. 7).
- If microtissue retrieval is required for further downstream analyses, use a 1000 µl pipette set at 150 µl approximately and pipette up and down gently in the cell seeding chamber 4-5 times (Fig. 5). The flow will allow microtissues to be resuspended in the medium, which can be harvested in a tube. A washing step may be needed to make sure all microtissues are recovered from the microwells.

> *NOTE*: To avoid sticking to pipette tips and tubes, we recommend pre-coating tips and tubes with 2% BSA in PBS or media. For tips, pipette the coating solution up and down a few times before collecting the microtissues. For tubes, add the coating solution and leave for at least 15 minutes on ice.

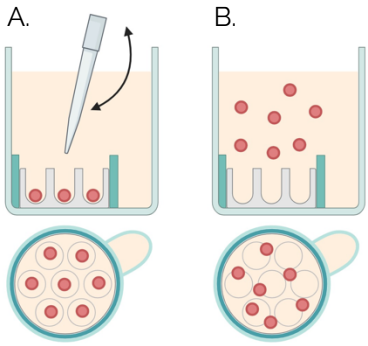
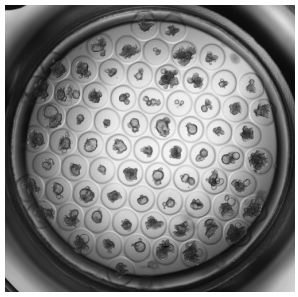


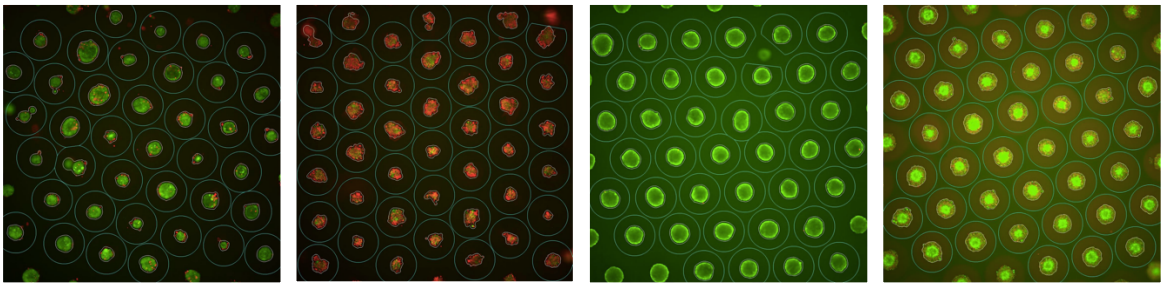
Figure 4. Microtissue retrieval from Gri3D®. Use a BSA-coated 1000 µl pipette tip to resuspend the microtissues by pipetting up and down in the cell seeding chamber.

Representative Data

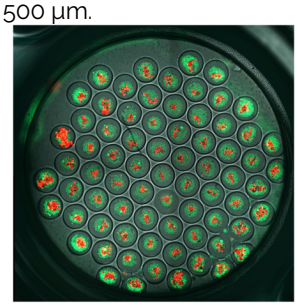
5-day old mouse adult stem-cell-derived intestinal organoids cultured in Gri3D® 96WP plastic-bottom 400 µm.



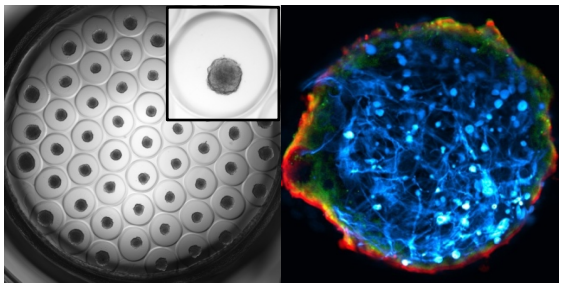
5-day old human adult stem cell-derived rectal organoids cultured in Gri3D® 96WP plastic-bottom 500 µm.



4X fluorescence microscopy images after Live/Dead assay of **A.** HCT-118 spheroids and **B.** colorectal cancer organoids in either control (left) or 10 µM gambodgic acid (right) conditions grown in Gri3D® 96WP plastic-bottom 500 µm.



Human colorectal cancer tumoroids (green) in co-culture with autologous tumor-infiltrating lymphocytes (red) on Gri3D® 96WP plastic-bottom 500 µm to evaluate the immune killing capacity.



Blood-brain barrier organoids grown on Gri3D® 96WP imaging-bottom 600 µm. Immunofluorescence of endothelial cells (P-gp - red), pericytes (NG2 - green) and astrocytes (GFAP – blue.