



Gri3D® Hydrogel Microcavity Plate

Product Manual



PM015, October 2025

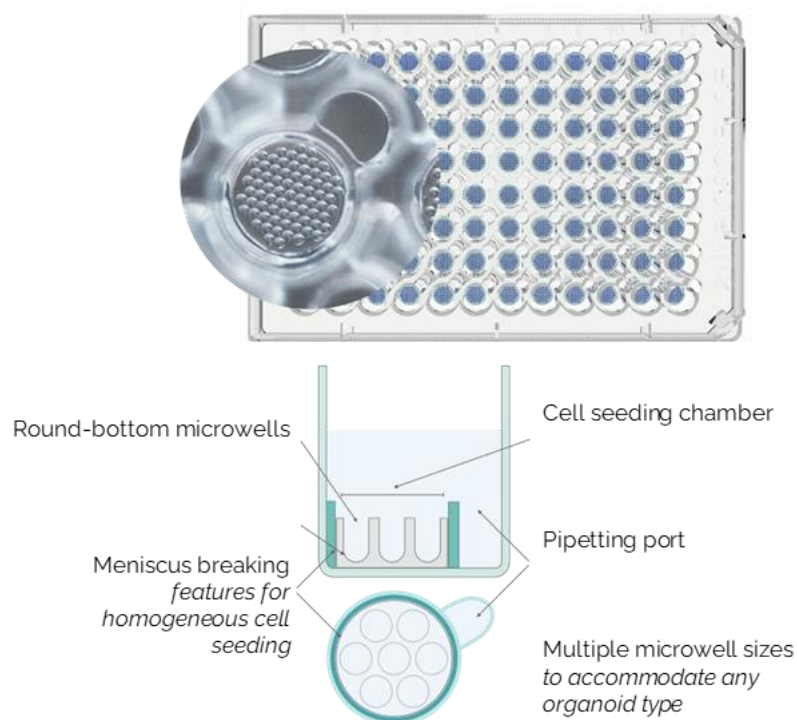
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Introduction

Gri3D® is a ready-to-use plate for high-throughput and reproducible 3D organoid and spheroid culture. Based on a standard 96 microtiter plate, each well contains a microcavity array patterned in a cell repellent polyethylene-glycol (PEG) hydrogel. On Gri3D®, organoids 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 organoid loss. The design allows automation of cell seeding, media exchange and compound incubation with liquid-handlers.

The Gri3D® Plate is designed to generate several 3D cell model types, such as organoids, spheroids and microtissues. For the remainder of the document, we refer to these models as 'organoids'.



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-well plate 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 organoids in the Gri3D® Hydrogel Microcavity Plate. The resulting organoid arrays are homogeneous and can be used for a variety of applications, such as toxicity, efficacy, or antibody transport assays.

Gri3D® Plate Preparation

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 both 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 microcavity 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.
 - **NOTE:** if you are working with automated-liquid handling devices, it is sufficient to aspirate the medium from the pipetting port only!
- 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.

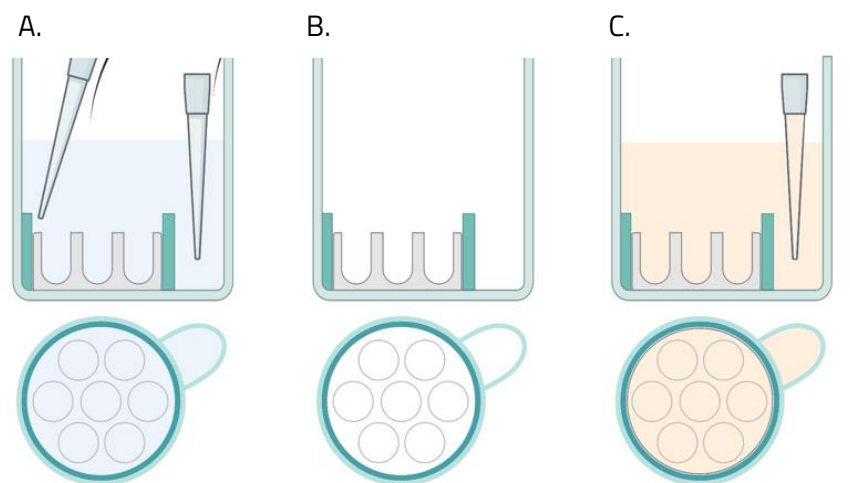


Figure 1: Gri3D® Plate preparation. A-B. Remove storage buffer from the pipetting port. C. Add 150 μ l of cell medium to the pipetting port and keep in incubator for 15-30 minutes.

2. Cell preparation and seeding

- Prepare the appropriate cell seeding density in medium¹. 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 μ l:

$$\text{Seeding density (cells/ml)} = \# \text{ cells per microwell} \times \# \text{ microwells} \times 20$$

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 μ l of cell suspension in the cell seeding chamber, in the center, on top of each microwell array (Fig. 2 C).

¹ Seeding density depends on the model.

Standard microcavity sizes (in μm)	400	500	600	800
Microcavity number (per well)	121	73	55	31
Cell density range (per microcavity)	100 – 1500	150 – 2000	200 – 4000	300 – 6000

Table 1: Microcavity numbers per well and cell densities per microcavity as a function of the microcavity 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 leftover 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 Matrigel² 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 microcavities. 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.

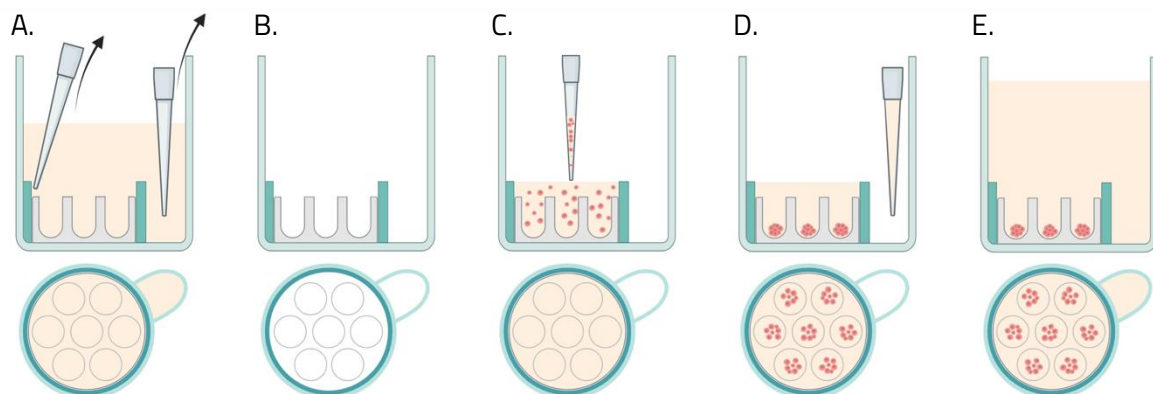


Figure 2: Cell seeding on Gri3D®. A-B. Remove medium from the pipetting port and cell seeding chamber. C. Add 50 μl of cell suspension to the cell seeding chamber. D-E. After cell sedimentation, add 150 μl of media to the pipetting port (with diluted ECM in organoid cultures).

¹ ECM concentration should be optimized for 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 μ l of medium (Fig. 3). 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 microcavity array gels compartment, as that would disturb the forming organoids. Use the pipetting port instead.

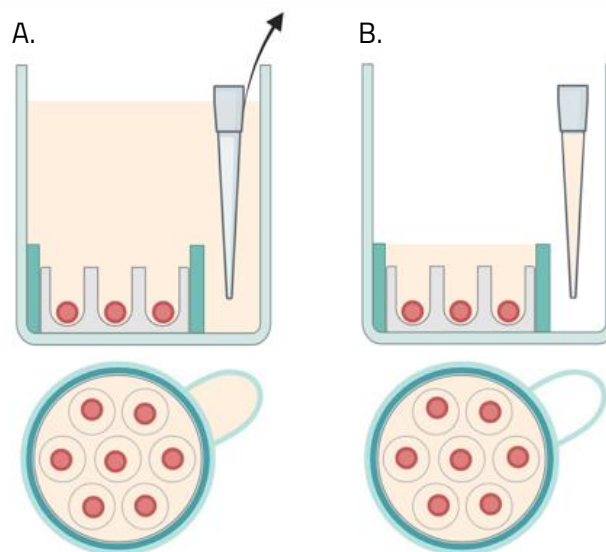


Figure 3: Cell maintenance on Gri3D®. A. Remove medium from the pipetting port. B. Add back 150 μ l medium (if desired, supplemented with ECM) to the pipetting port.

4. Organoid 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 organoids to establish a co-culture, remove 150 μ l medium from the pipetting port and carefully remove the desired volume from the cell seeding chamber (typically 20 μ l). Pipette the cell solution in the cell seeding chamber on top of the organoids (typically 20 μ l); cell seeding chamber maximum volume = 50 μ l). Let the cells sediment for 15-30 minutes in the incubator. Add 150 μ l of medium in the pipetting port and proceed with cell culture as usual (see Fig. 3).
- If organoid retrieval is required for further downstream analyses, use a 1000 μ l pipette set at 150 μ l approximately and pipette up and down gently in the cell seeding chamber 4-5 times (Fig. 4). The flow will allow organoids to be resuspended in the medium, which can be

harvested in a tube. A washing step may be needed to make sure all organoids 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 organoids. For tubes, add the coating solution and leave for at least 15 minutes on ice.

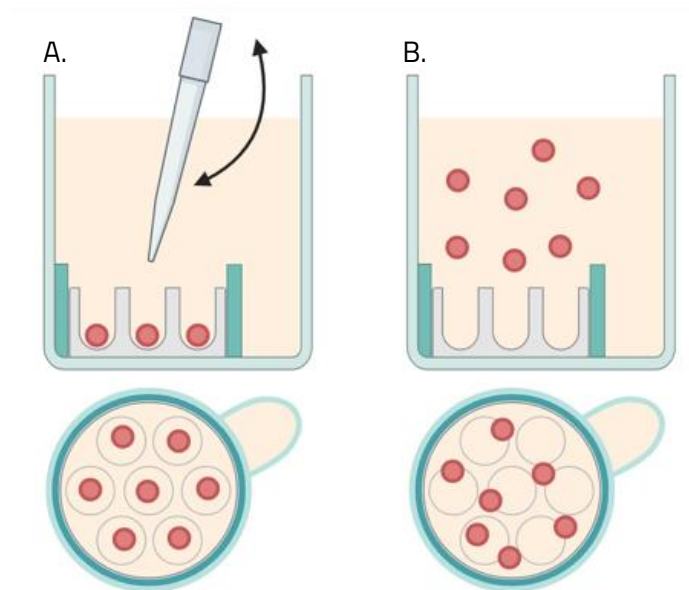
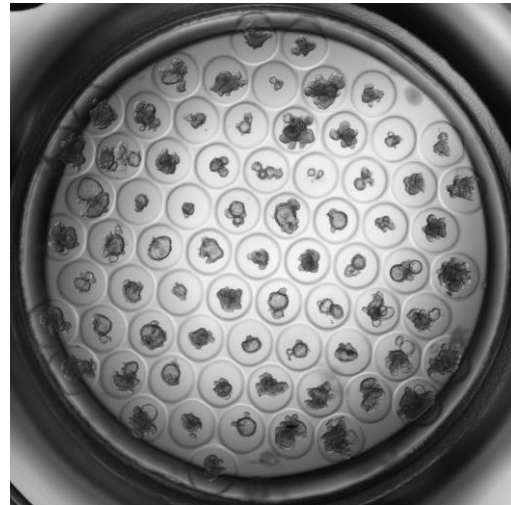


Figure 4: Organoid retrieval from Gri3D®. Use a BSA-coated 1000 μ l pipette tip to resuspend the microtissues by pipetting up and down in the cell seeding chamber.

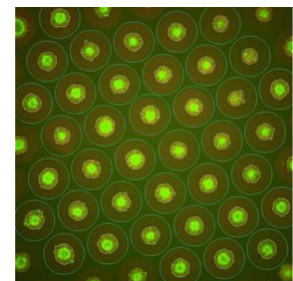
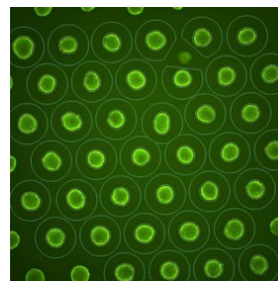
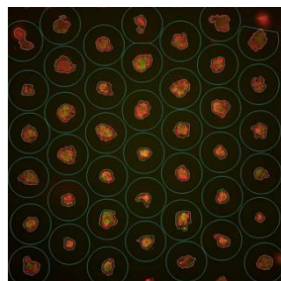
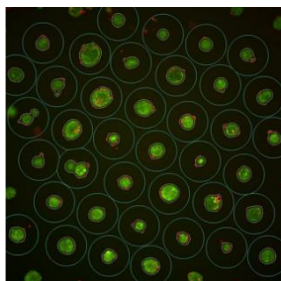
Representative Data



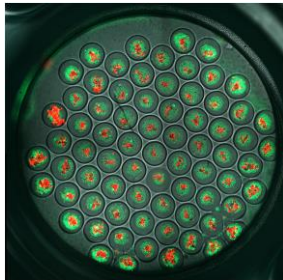
5-day old mouse adult stem-cell-derived intestinal organoids cultured in Gri3D® 96 plastic-bottom 400 µm.



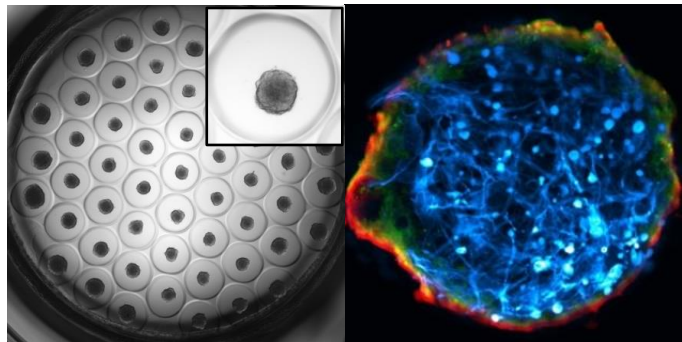
5-day old human adult stem cell-derived rectal organoids cultured in Gri3D® 96 plastic-bottom 500 µm.



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 gambogic 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® 96 plastic-bottom 500 μm to evaluate the immune killing capacity.



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

Annex A: License Agreement

License Agreement Gri3D® Hydrogel Microcavity Plates

This License Agreement (the „License Agreement“) is a legal agreement between the end user („End User“) and InSphero AG or its subsidiaries („InSphero“) to use the Gri3D® Hydrogel Microcavity Plates covered by patents owned or controlled by InSphero which are provided to you.

1. Warranties: The End User hereby irrevocably warrants to keep and use the Gri3D® Hydrogel Microcavity Plates in accordance with the restrictions and limitations contained in this License Agreement.
2. Proprietary rights of the Gri3D® Hydrogel Microcavity Plates may be covered by one or more of the following patents: EP 3237597 B1, US 11583860 B2, CA 2972057 C, JP 7089240 B2, EP 3515600 B1, JP 7318156 B2, CN 112534038 B and other pending patent applications. By entering into this License Agreement, End User acknowledges that the Gri3D® Plates are so covered.
3. Excluded Fields: No permission is granted hereunder for the use of the Gri3D® Plates:
 - a. for selling cell-based products generated using the Gri3D® Plates to third parties
 - b. provision of commercial testing services for 3rd parties without prior permission of InSphero
 - c. in veterinary applications, in diagnostics, *in vivo* use in humans and/or uses related to food products
4. Use by the End User Subject to Clause 3 above End User will use the Gri3D® Hydrogel Microcavity Plates solely for in vitro research in-house for the discovery and development of compounds outside the Excluded Fields by End User. End User will not sell, transfer, disclose or otherwise provide access to the Gri3D® Plates to any third party or entity. End User will not sell, or transfer cell-based products generated using the Gri3D® Plates to any third party or entity.

Annex B: Frequently Asked Questions Regarding the Gri3D® Hydrogel Microcavity Plates

Gri3D® Product Specifications

How should the plate be stored once received?

Upon receipt, please store the plates at 4 °C (32 °F)

How long can plates be stored at recommended condition?

Plates are stable at 4 °C (32 °F) for at least 6 months.

How long can the hydrogel be used for before it “degrades” or can no longer be used?

The hydrogel needs to stay hydrated in buffer to be stable. If the hydrogel dries out, it can no longer be used. This will not happen during the first 6 months of storage at 4 °C (32 °F) if the plate remains unopened.

Is Gri3D® automatable?

Yes. You can establish automated organoid workflows including hydrogel equilibration, cell seeding, medium exchange and compound exposure with liquid handlers.

What are the recommended well volumes?

50 µl seeding chamber + 150 µl pipetting port.

What material is Gri3D® made of?

Plastic plate: Polystyrene / Imaging plate: COP

Hydrogel microcavity: poly-ethylene glycol (PEG).

What are the microwell sizes available?

Our Gri3D® plates come with various microcavity sizes ranging from 400 µm to 800 µm.

How do I choose microwell size?

The purpose of having different diameters of microcavities is to accommodate different organoid types and sizes. If your organoids are small or develop in a short time from low cell amounts, smaller microcavity diameters will allow you to get a higher microcavity density per area, therefore resulting in more datapoints per well. If you plan to grow your organoids for longer times or to larger sizes, you can opt for larger microcavity sizes.

Gri3D® Plate Use

What cells have been tested in Gri3D®?

Our Gri3D® plates have been used to grow various models including but not limited to intestinal organoids, liver organoids, colorectal and pancreatic cancer organoids, blood-brain barrier organoids.

How many cells do I need to seed in the microcavities?

You need 50 µl of a single cell suspension per well. The cell density needs to be optimized for your application and depends on the growth rate of cells and desired final size of the organoid. You can control the organoid size by adjusting the starting cell seeding density. See details in the protocol provided with product or check our resources for more information on our established models.

What is the typical seeding uniformity?

The seeding ring surrounding the hydrogel allows separation from the pipetting port and has a meniscus breaking effect which enables homogeneous cell seeding within a well. Variations in cell numbers between microcavities in the same well are less than 5%.

How long do organoids take to form?

The formation time of the organoids strongly depends on the type of cells used, their proliferation rates and the cell seeding density used. These parameters need to be optimized for your application. It can take 2-5 days before the cells are fully compact.

What is the size distribution of spheroids/organoids?

Resulting organoids are homogeneously distributed in terms of size, with variations due to biology.

How long can organoids be cultured in the microcavities?

Organoids or spheroids can be cultured for as long as desired. For example, our embryonic stem cell-derived retinal organoids were cultured for 26 days, and we could culture primary human hepatocyte spheroids for 21 days. Check our resources for more information.

Can I establish co-cultures with multiple cell types on Gri3D®?

Yes. You can aggregate multiple cell types by mixing them before cell seeding, as we did for our blood-brain barrier organoid model. One can also add a second cell type such as immune cells for co-culture with the organoids once they are formed. This is what we did for our T-cell killing assay.

How to prevent disruption of organoids already in the microwells during media exchange?

Our uniquely designed pipetting port, adjacent to the microcavities, allows safe medium exchange or compound exposure without organoid loss. Simply remove 150 µl from the pipetting port and add new medium in the same volume back.

How often do I change the medium?

Medium change frequency depends on the cell types and specific protocols. Medium can be changed every 2-3 days or as frequently as needed.

Do I need to add extracellular matrix (ECM) to my organoid cultures on Gri3D®? If so, how often and how much?

ECM needs depend on the type of organoid. For organoids which are expanded embedded in basement membrane extract (BME), it may be necessary to mix ECM with the culture medium to allow for their 3D development and growth. In the case of Matrigel®, organoids usually need between 1.5 – 2% Matrigel®, to be added at every medium change.

What types of ECM are compatible with Gri3D®?

Gri3D is compatible with a range of ECM gels that can be diluted in media including but not limited to collagen-I, Matrigel®, Matrigel® growth factor reduced BME, Cultrex BME, laminin.

Do I need to coat Gri3D® plates?

No, PEG is naturally cell-repellent, so no coating is needed before seeding the cells.

Do I need to centrifuge Gri3D® plates after cell seeding?

No, Gri3D® technology enables cell seeding and aggregation in a single pipetting step without centrifugation. The seeding ring surrounding the hydrogel allows separation from the pipetting port and has a meniscus breaking effect which enables homogeneous cell seeding within a well.

Can the unused wells in the 96-well plate be used later?

Yes, you can use different wells of one plate for different experiments. When doing this, make sure that the hydrogel microcavities stay hydrated and that sterility is maintained. Before use, check if the unused microcavities are still hydrated.

Can I reuse Gri3D® after washing?

No, we do not recommend plate reuse after washing as we cannot guarantee the quality when reusing it.

How can I avoid damaging the hydrogel?

To avoid damaging the hydrogel, always use pipetting port for media removing and loading. When seeding the cells only touch the hard plastic seeding ring and avoid reaching the hydrogel.

What happens if I reach the hydrogel?

Our PEG hydrogel is sensitive. If the hydrogel is disturbed by means of a pipet tip, it may damage the microwells, thus risking microtissue loss.

Gri3D® Assay**Once organoids are formed in each well, how shall we proceed to assays?**

Once the organoids are formed, you can use them directly in your assay of choice. For example, if your assay is imaging, you image the cells directly on the plate after incubation with your dye of interest or proceed to immunostaining of the cells. You can also use the supernatant for your assays of interest or recover single organoids from the well for other downstream analyses.

Can I do immunostaining assays on Gri3D®?

Yes, all steps of immunofluorescence can be performed on Gri3D®, from fixation, permeabilization, blocking, antibody incubation all the way to imaging.

Can I image my samples on Gri3D®?

Gri3D® is compatible with regular transmitted light and fluorescence imaging, also in confocal mode. On Gri3D®, growing organoids are positioned in confined areas (the microcavities) and in a single focal plane, allowing the imaging of multiple organoids at the same time. This allows the establishment of efficient image-based workflows which minimize the acquisition time and maximize the data extracted.

Can I use Gri3D® for organoid screening?

Gri3D® comes in SLAS-standard 96-wellplate format and is well suited for screening programs as it generates a high volume of organoids per well in the same focal plane, allowing multiple replicates per well to be generated and thus maximizing the datapoints per well. Moreover, as there are multiple organoids in a single well, this allows for multiple assays to be performed independently of their limit of detection.

Is the plate compatible with fluorescent / colorimetric (O.D.) readings?

Fluorescence and colorimetric based assays can be performed on Gri3D®. However, Gri3D® is transparent, so we recommend sampling out in an assay plate (white-well or black-well plate) for optimal assay results.

How can I assess organoid viability on Gri3D®?

Gri3D® is compatible with various cell viability assays including fluorescence image-based assays such as LIVE/DEAD™ Kit or luminescence-based assays like CellTiter-Glo® 3D.

Does hydrogel interfere with CellTiter-Glo® 3D or other luminescence-based assays?

No, CellTiter-Glo® 3D reagents contain a lytic compartment that lyses cells and a compound that reacts to the presence of ATP coming from the lysed cells. None of these have an effect on PEG, which is a cell-inert compound. Thus, there is no interference of the hydrogel with luminescence-based assays.

Can I sample out media from Gri3D®?

Yes, you can safely sample media out by using the pipetting port for downstream assays such as mass spectrometry MS.

How can I harvest the organoids?

As cells grow in an open and solid matrix-free environment, they can be easily harvested from the microwells with a simple pipetting step on top of the growing organoids – no need for cumbersome extraction protocols. Single organoids or homogeneous organoid populations can then be processed for passaging or downstream analyses.

Is it okay that the harvested organoids/spheroids contain some of the hydrogel?

Yes, PEG is cell-inert and can be removed after organoid centrifugation.

Can I extract RNA/DNA/protein from my 3D cultures on Gri3D®?

After harvesting the organoids, you can proceed with your in-house protocol to extract DNA, RNA or protein. Depending on the amount of microtissues and their size, you may have to pool several wells to have enough starting material.

Can I pick single organoids from the microwells?

Yes, as cells grow in an open and solid matrix-free environment, one can pick single organoids from the microwells.

Can I cryosection my organoid samples?

Yes, samples in the microcavities can be fixed and cryopreserved by successive incubations in sucrose gradients. Then, the hydrogel containing the organoids can be scooped out, embedded in OCT, snap-frozen and cryo-sectioned.

Would any small molecules bind to the plate since they contain hydrogel?

In most cases there will be no binding. However, PEG can bind to molecules with a thiol group.



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the FAQs section on shop.insphero.com

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