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 a microcavity 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 to 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 homogenously 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 microcavities 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 microwells stay hydrated and that sterility is maintained. Before use, check if the unused microwells 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 microcavities, thus risking organoid 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 essays 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 number of organoids and their size, you may have to pool several wells to have enough starting material.

Can I pick single organoids from the microcavities?

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

Can I cryosection my organoid samples?

Yes, samples in the microcavites 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.