APRICELL BIOTECHNOLOGY

# TruSphere Plate

## Information Sheet

### Dimensions

- Microwell Diameter Options: 500 µm
- Microwell volumes: 7 µL (500 µm microwells).
- Microwell Bottom Thickness (for inverted microscope imaging): 500 µm.

### Specifications

- The TruSphere Plate hydrogel inserts come pre-inserted within each well of a standard 6, 12 or 24-well plate.
- Number of spheroids per **6-well plate**:
  500 µm Microwell: 3456
- Number of spheroids per **12-well plate**:
  500 µm Microwell: 2592
- Number of spheroids per **24-well plate**:
  500 µm Microwell: 1728

### Material

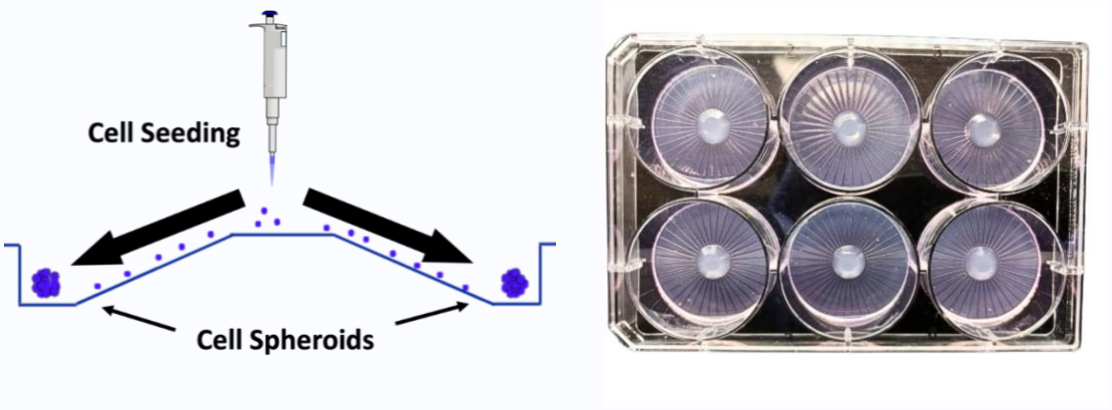
- Non-cell-adhesive hydrogel.

### Storage Conditions

- Refrigerate at 4 °C (39.2 °F).

### Shelf Life

- 12 Months.




Left image: side view schematic of one well of the TruSphere Plate.
Right image: birds-eye-view of a 6-well plate TruSphere Plate.

# TruSphere Plate Benefits

- High Throughput Culture: hundreds of spheroids from a single pipette.
- Uniform spheroids for experimental consistency.
- No risk of losing spheroids during media changes.
- Easily image spheroids in situ.
- Easy IHC analysis.

# Guidelines for Use

### Spheroid Formation

The geometry and material of the microwells in the TruSphere Plate facilitate the formation of compact and uniform spheroids/organoids. However, not all cells will readily form tight spheroids. There are some tips and techniques that can aid in spheroid formation. Specifically, optimizing the spheroid seeding density, media formulation and culture period can aid in tighter spheroid formation.

### Spheroid Size

Spheroid size is determined by the microwell size, cell type, seeding density, and culture time. Optimizing the initial seeding density will play a large role in the ability of the spheroids to initially form, as well as determine how long the spheroids can be kept in culture. Based on your experiment, larger spheroids can begin to form hypoxic cores, which may or may not be desirable.

### Media Formulation

Media formulation may affect spheroid formation. This should be tested with each cell type.

### Removing The Insert

Press a lab spatula into the gap between the insert and the plastic of the well plate. Once at the bottom of the well, flex your lab spoon towards the center. This motion should pop the insert out of the well. Securely support the insert with the lab spatula and remove it from the well.

### Extracellular Matrices Embedding

Some applications require the addition of an extracellular matrix (ECM) hydrogel. TruSphere Plate is recommended for simple spheroid formation; however, researchers who need to assess the effect of ECM hydrogel in their study can add to the models. A primary ECM can be added to the Cell seeding Zone. For co-cultures (such as fibroblasts and tumour cells) or tri-cultures, ECM can be mixed with the secondary cells and loaded into the Cell Seeding Zone of the insert.

The ECM concentration should be optimized for the specific application. Organoid cultures typically call for an undiluted Matrigel matrix in the 8 to 10 mg/mL concentration range, while other applications call for a more dilute concentration. When adding an ECM to the TruSphere Plate, users must ensure that the ECM is not excessively viscous – it must be able to flow down the microchannels leading to the microwells. The ECM can be added during spheroid formation or as an overlay to an already-formed spheroid. Media should be aspirated before adding an ECM. This can be done by aspirating media from the Media Reservoir and Cell Seeding Zone. Then, wait 3-5 minutes to ensure that any remaining media is absorbed by the hydrogel of the TruSphere Plate.

### Spheroid Handling

### Media/Buffer Exchanges

The TruSphere Plate eliminates the possibility of disrupting or aspirating the spheroids during media changes. To change the media, pipette from the Seeding Zone. Any additional media left in the microwells can be pipetted out by tilting the plate and pipetting from the edge of the insert. Complete aspiration of the media is not required when exchanging media/buffer.

### Spheroid Transfer

Spheroids can be harvested by gently flushing out the spheroids with 500 µL of culture media.

### Centrifugation

Centrifugation is not required for spheroid formation or centering within the microwells.

## Spheroid Assays

### Spheroid Assays

The transparent hydrogel of the TruSphere Plate makes it ideal for numerous fluorescent, and colorimetric assays that can be conducted directly in each insert. Users can add reagents to the well with certain volume. Depending on the size of the spheroid it may become difficult for some assay reagents to penetrate fully. Therefore, optimization of each assay is recommended using appropriate positive and negative controls.

### Viability and proliferation Assays

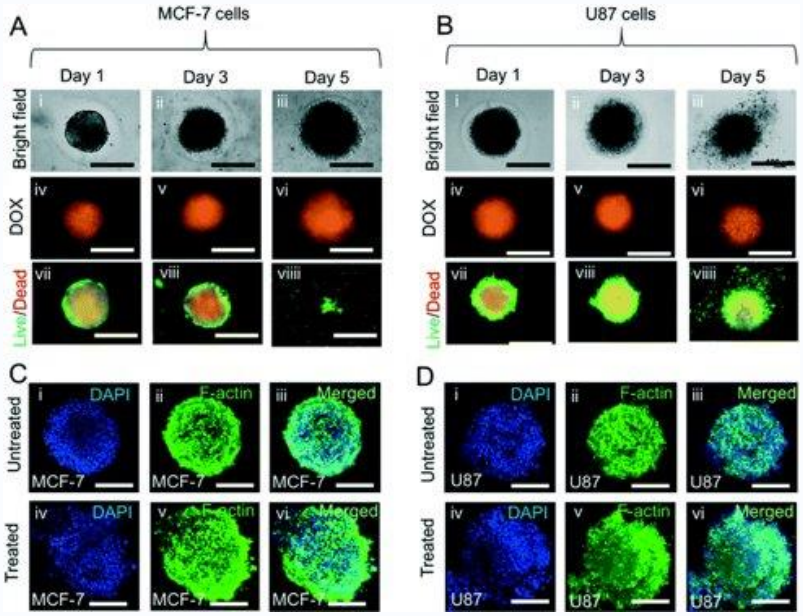
There are several commercially available 3D-specific reagents that have been optimized for use with spheroids. We recommend CellTiter-Glo® 3D cell viability assay (Promega Cat. No. G9683), PrestoBlue™ or MTT Cell Viability assay (Invitrogen™) for enumerating the total ATP content of spheroids.

### Brightfield Imaging

The transparent hydrogel of the TruSphere Plate enables clear brightfield images and is compatible with both upright and inverted microscopy. We recommend using inverted microscopy due to the nature of the microwell design. Spheroids are much closer to the bottom of the plate, as such, inverted microscopy produces clearer images with reduced background particles compared to upright microscopy.

### Fluorescent Imaging

Staining a 3D structure may require protocol optimization compared to the 2D equivalent. In general, the larger The tighter the spheroid, the longer it will be for staining. If cell permeabilization is required, the choice of reagent and length of incubation time may need to be considered. A variety of immunostaining has been successfully completed in the TruSphere Plate. These include primary and secondary conjugated antibodies with cultured cells. Users can also pre-label cells prior to seeding in the TruSphere Plate or use fluorescent protein-expressing cells to ensure that all cells are labelled as needed for the application.

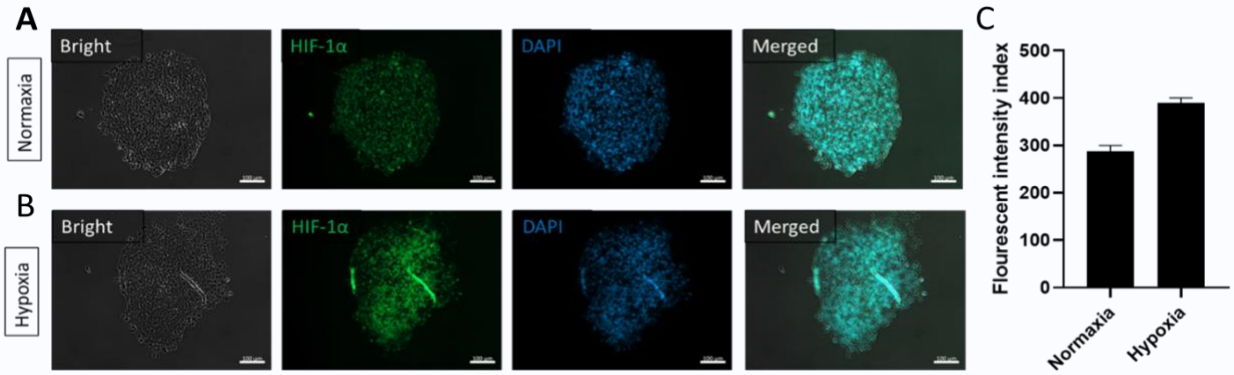



Fluorescent imaging of breast and brain tumour spheroids in the TruSphere Plate. A, B) Live-dead fluorescent imaging and C, D) Immunostaining and confocal imaging of the spheroids.

## Advanced Applications

### Immunohistochemistry Analysis

The TruSphere Plate is compatible with in situ IHC analysis. Because the TruSphere Plate is made from a soft hydrogel, users can perform all steps of IHC analysis – from dehydration to wax embedding and slicing - without having to remove the spheroids from the insert. Note: it is recommended that the dehydration step in ethanol is extended by 1.5 to 2 times the original duration to completely remove water from the insert hydrogel material. Refer to the TruSphere Plate *IHC Analysis Protocol* for specific instructions.



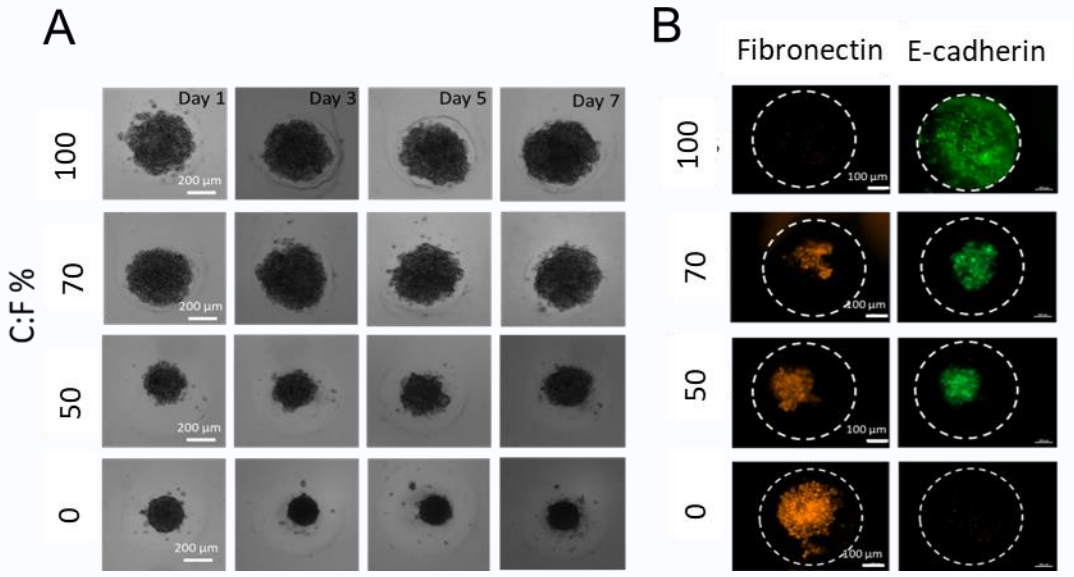

IHC analysis of the glioblastoma tumor slices in the TruSphere Plate under A) Normaxia conditions of tumor spheroids and **B)** Tumor spheroids placed in hypoxic conditions to simulate tumor conditions in the body. C) HIF-1α quantitates the expression of transcription factors under reduced $O_2$ concentrations in mammalian cells.

## Co-culture

Multiple cell types can be cultured together in a variety of ways to study cell-cell interactions, create more in-vivo like models, or add structure to a cell line that does not easily form a tight spheroid. The secondary cells can be seeded simultaneously with the tumor cells or added at different time points depending on the application. Depending on the cell types used, media formulations may require optimization to provide sufficient nutrients and growth factors to specialized cell types included in the co-culture environment.

There are three ways to do a co-culture in the TruSphere Plate:

1. By mixing the two cell types together as the initial single-cell suspension and co-seeding that mixture.
2. First, spheroids are formed, and secondary cells are mixed with an ECM hydrogel and applied to the spheroids.



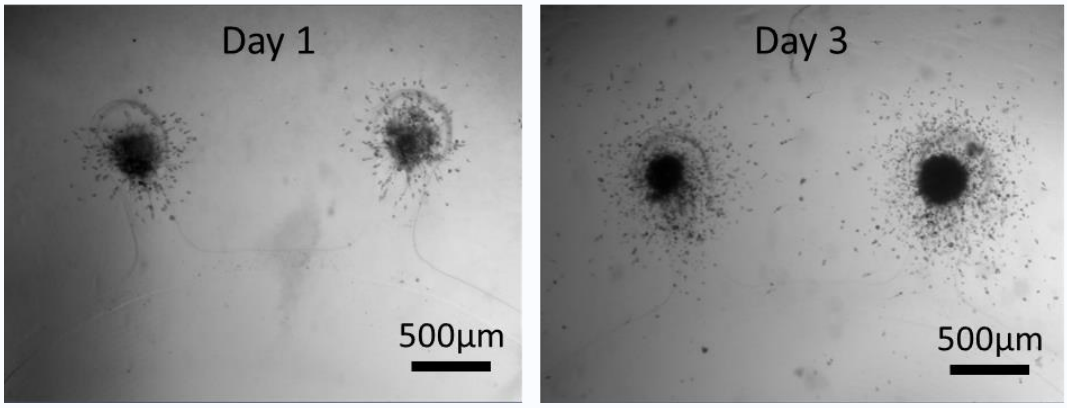

A) Bright-filed and B) fluorescent imaging of pancreatic tumour spheroids embedded under the co-seeding protocol.

## Drug Screening

The TruSphere Plate is compatible with both small-molecule and large-molecule medium-throughput drug screening. Simply mix your drug with media to the desired concentration and add it to the well.

## Invasion Assay

The TruSphere Plate offers a flexible and high-throughput format for quantitating the degree of cell invasion/migration into microchannels in response to chemoattractant and/or inhibiting compounds. Chemoattractants, inhibitors, and/or migration-regulating compounds can be added to ECM solutions when embedding spheroids or mixed with media to the desired working concentrations. The ECM / drug combination should be added to the Cell Seeding Zone to cover the microcavities in the insert.




Brightfield Images of Glioblastoma tumor spheroids array invading within an ECM over time.

# FAQ Sections

## 1. Basics

How many spheroids are in each microwell?

- There will only ever be one spheroid or organoid per microwell.

Can I transfer the inserts from one plate to another?

- Yes. Make sure the insert is completely adhered to the bottom of the new well or cover slip to ensure the best image quality.
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How do I remove the insert from the well?

- To remove the insert, press a lab spatula into the gap between it and the plastic of the well plate. Once at the bottom of the plate, flex your lab spoon towards the center. This motion should pop the insert out of the well. Securely support the insert with the lab spatula and remove it from the well.

Do I have to use all the inserts at once?

- No, you do not need to use all the inserts at once. You can use just 1 or 2 inserts at a time. To stop the un-used inserts from drying out in the incubator, add 500 µL of media or PBS to the center of each unused insert.

Do cells stick to the hydrogel?

- The hydrogel used to form the TruSphere Plate is non-cell adherent.

How big are the spheroids/organoids in this platform?

- 100 – 700 µm, depending on the size of the microwells you use. The microwell size options are: 800 µm and 500 µm and 300 µm.

Is there any media left in the microwells after aspirating from the well?

- There may be a small and non-significant amount of media left in the microwells after aspirating from the well.

Can the platform be frozen or put on ice?

- Yes. The plate can be placed on ice. We do not recommend placing it on ice for more than 20 minutes at a time. Freezing our platform is not recommended as it can distort the delicate microwell structure and reduce spheroid consistency.

How can I control the size of the spheroids/organoids?

- The size of the spheroids/organoids is determined by various factors, including the quantity of cells seeded, the type of cells, the size of the microwells, and the duration of culture.

Is adding an ECM hydrogel necessary?

- Adding an ECM hydrogel is not always necessary, depending on the type of model you wish to create. Spheroids can be formed without a hydrogel. However, culturing primary cells to form organoids typically requires an ECM hydrogel.

## 2. Imaging

What types of imaging is it compatible with?

- The TruSphere Plate is compatible with all standard forms of microscopy: inverted, upright, bright field, fluorescent, confocal, etc.

Is it compatible with an inverted microscope?

- Yes. In fact, an inverted microscope is ideal. For improved imaging the inserts can be removed from the well and placed on a coverslip.

Is there any visual distortion or reflection when imaging through the hydrogel platform?

- No. Users have not reported any distortion/reflection for imaging.

Can you do real-time imaging with staining?

- Yes, as long as it doesn't affect cell proliferation.

Can you do 3D staining/imaging?

- Yes. The TruSphere Plate is compatible with confocal microscopy, and all staining can be done within the insert. For higher-resolution images in confocal imaging, we recommend removing the insert from its well using a lab spatula and placing it on the coverslip. To remove the insert, press the lab spatula into the gap between the insert and the plastic of the well plate. Once at the bottom of the 12-well plate, flex your lab spoon towards the center; this motion should pop the insert out of the well. Securely support the insert with the lab spatula and remove from the 12-well plate.

## 3. Technical Questions

Can you modify the matrix stiffness in this model?

- The hydrogel type, cross-linking and concentration and time are all factors that can impact the matrix stiffness. What ECM hydrogel you choose for your experiment is ultimately up to you. The TruSphere Plate should be compatible with whatever you end up choosing. Ensure that the viscosity of the hydrogel is not excessively viscous when pipetting into the Cell Loading Zone.

Can you stain the spheroids?

- All staining can be done on the plate. There is no need to remove the spheroids or even to digest the extracellular matrix.