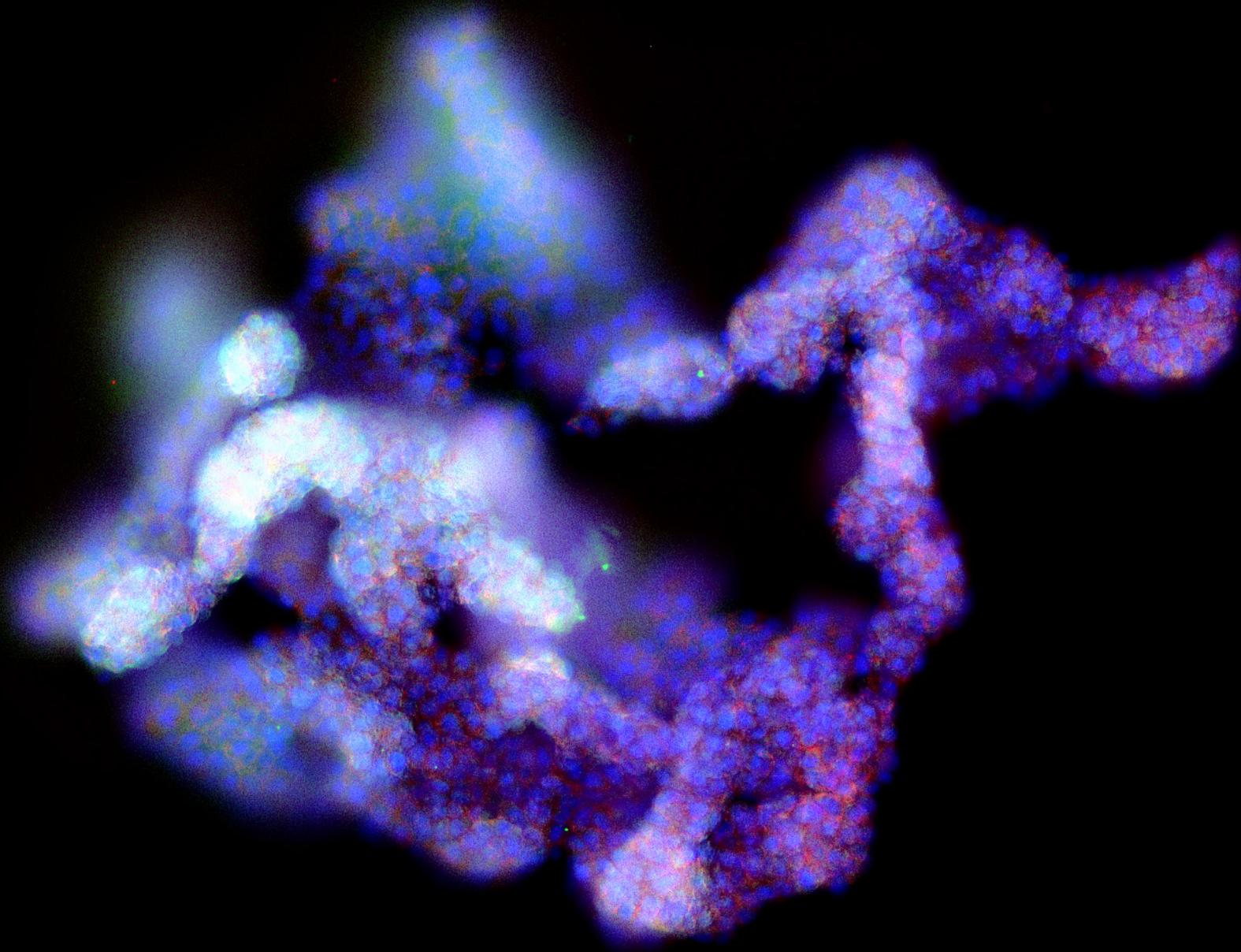


# XENO-FREE HYDROGELS for 3D Cell Culture Research

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Advancing personalized medicine, cell therapy, and drug discovery  
with an innovative 3D cell platform



PRODUCT CATALOG

866-3D-CELLS | +1 973.855.4945



[www.thewellbio.com](http://www.thewellbio.com)

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For research use only. Not for use in diagnostic procedures.

# VITROGEL - A XENO-FREE FUNCTIONAL HYDROGEL SYSTEM



Closely mimicking the natural extracellular matrix (ECM) environment, our state-of-the-art hydrogel system gives an outstanding balance of biological functions and ease of operation to establish a robust 3D cell culture platform or an injectable delivery system for drug discovery, tissue engineering, cell therapy, and personalized medicine.

A xeno-free system is key for 3D cell culture in clinical applications. Our cutting-edge hydrogel enables high-throughput performance to establish 3D organotypic models from both cell lines and patient-derived cells. Using our hydrogel system as an injectable delivery system, scientists can achieve better cell retention and higher cell viability for cell therapy. This groundbreaking technology makes cell recovery from the 3D hydrogel matrix easier than ever before.



Easy-to-use



Xeno-free



Room Temp  
Stable



Transparent



Reproducible



Tunable  
Hydrogel



Easy Cell  
Harvesting



Biocompatible



Bifunctional  
Modifiable



Injectable



Automation  
Friendly



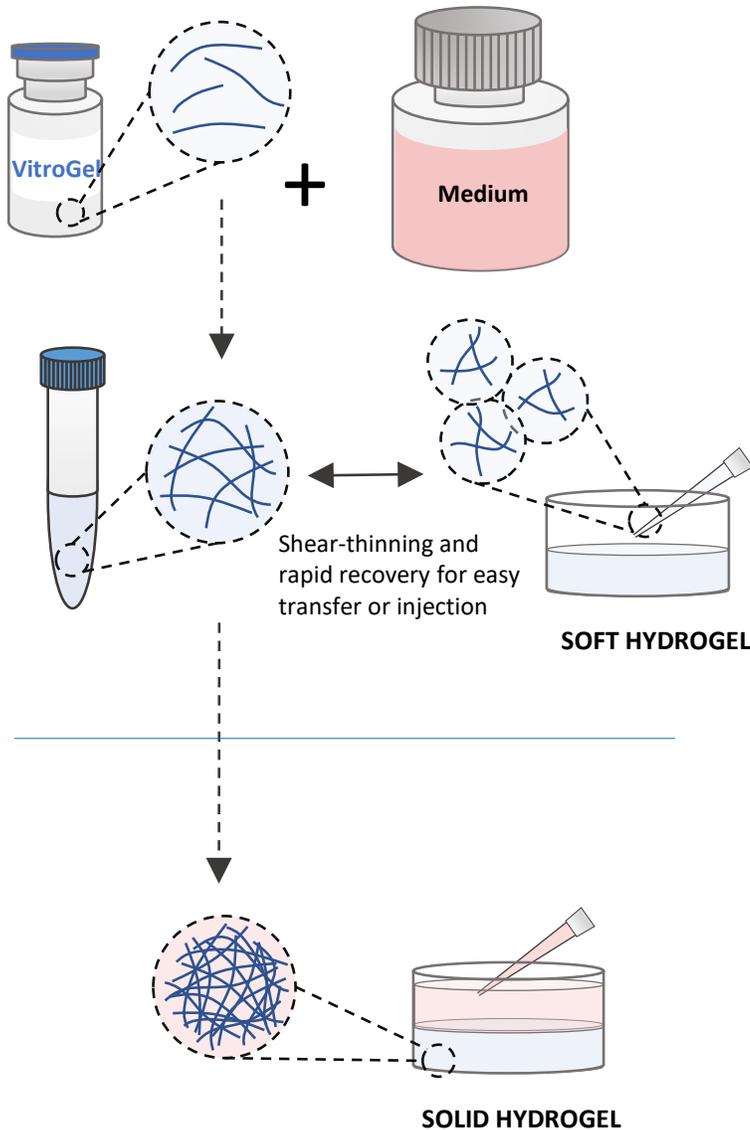
Mix & Match

|                              | VitroGel | Basement Membrane Matrix | Polymer Matrix | Hanging Drop Plate | Low Adhesion Plate | Magnetic Levitation Plate |
|------------------------------|----------|--------------------------|----------------|--------------------|--------------------|---------------------------|
| Easy-to-use                  | ●        |                          | ●              | ●                  | ●                  | ●                         |
| Mimic natural ECM            | ●        | ●                        |                |                    |                    |                           |
| Xeno-free                    | ●        |                          | ●              | ●                  | ●                  | ●                         |
| Room temperature stable      | ●        |                          | ●              | ●                  | ●                  | ●                         |
| Neutral pH                   | ●        |                          | N/A            | N/A                | N/A                | N/A                       |
| Easy Cell harvesting         | ●        |                          |                | ●                  | ●                  | ●                         |
| Transparent                  | ●        | ●                        |                | ●                  | ●                  | ●                         |
| Multi-functional ligands     | ●        | ●                        |                |                    |                    |                           |
| Wide range hydrogel strength | ●        |                          |                |                    |                    |                           |
| Injectable                   | ●        | ●                        |                |                    |                    |                           |
| Automation friendly          | ●        |                          | ●              | ●                  | ●                  | ●                         |

## Performance Comparison To Other 3D Cell Culture Methods

VitroGel provides an outstanding balance of biological functions and operating feasibility. The system can closely mimic the natural extracellular matrix and allow the high-throughput performance to build robust 3D cell models.

# HOW DOES GELATION WORK IN VITROGEL?



## STAGE ONE:

VitroGel solution is room temperature stable and free-flowing. The hydrogel gelation/formation starts by mixing the VitroGel solution with the cell culture medium. Hydrogel molecules interact with ionic molecules such as  $\text{Ca}^{2+}$  and  $\text{Na}^{+}$  from the cell culture medium and form the matrix structure (hydrogel).

The process of hydrogel formation is slow when a small amount of ionic molecules are used. At this stage, the hydrogel is soft and possesses a shear-thinning and rapid recovering mechanical property, which makes the hydrogel easy to transfer to culture plate or use for injection.

## STAGE TWO:

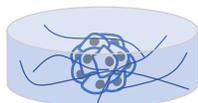
After soft hydrogel formation, adding an additional cell culture medium on top of the hydrogel would allow more ionic molecules to penetrate the hydrogel matrix and further saturate the hydrogel cross-linking. A solid hydrogel would form during this process.

# MULTIPLE CELL CULTURE METHODS WITH VITROGEL HYDROGELS

The XENO-FREE bio-functional VitroGel hydrogel system is versatile for many applications. Choose either the “ready-to-use” hydrogel system for optimized formulation and simple operating process or the high-concentration hydrogel system to create a customized microenvironment by “Mix & Match” and tuning of the hydrogels. There are multiple ways to use our hydrogel system to fulfill many research needs. To show the flexibility of our hydrogels, we list five of the most popular cell culture methods that can be performed with our hydrogel: 3D cell culture, 2D hydrogel coating, static suspension culture, hydrogel-cell bead, and as an injectable carrier. These five culture methods apply to all of our ready-to-use VitroGel and high concentration VitroGel systems. Cells cultured in these methods can be easily harvested with the VitroGel® Cell Recovery Solution for downstream analysis or subculture.

## 3D CULTURE

Encapsulate cells in the hydrogel matrix to promote cell-matrix and cell-cell interactions.



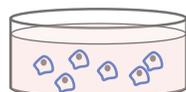
## 2D HYDROGEL COATING

Control the substance properties for culturing cells on top of the hydrogel. Great for cell submergence or invasion studies.



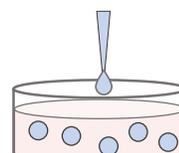
## STATIC SUSPENSION CULTURE

Change the viscosity of the cell medium to create a cell suspension environment for large-scale cell expansion.



## HYDROGEL-CELL BEAD

Encapsulate cells in the hydrogel matrix by creating hydrogel-cell droplets.



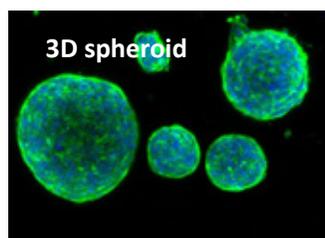
## ANIMAL INJECTION

Mix compounds or cells with the injectable hydrogel to increase cell retention and viability for *in vivo* studies.



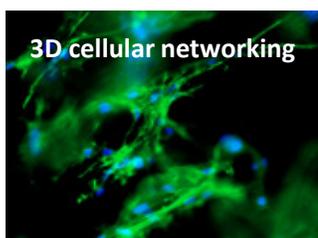
## Multiple Applications

From simple 3D cell spheroids to comprehensive organoid and co-culture model to functional assay and *in vivo* studies, this powerful hydrogel system can help accomplish these research and more. Some application examples are listed below.



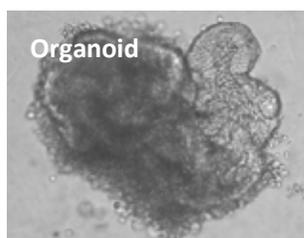
3D spheroid

Human colon cancer cultured on VitroGel 3D



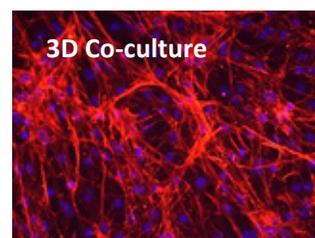
3D cellular networking

Fibroblast-like bone marrow 3D cultured on VitroGel Hydrogel Matrix



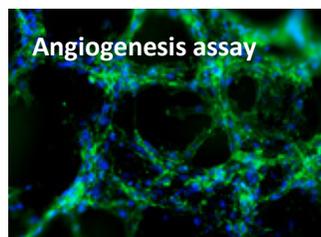
Organoid

Mouse intestinal organoid culture on VitroGel ORGANOID



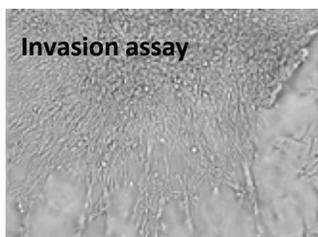
3D Co-culture

Co-culture of alveolar epithelial cells on VitroGel RGD



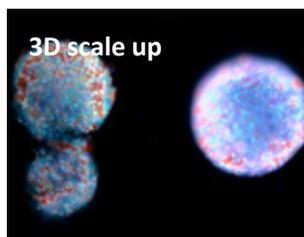
Angiogenesis assay

Tube formation of endothelial cells on top of VitroGel AAK hydrogel



Invasion assay

Invasion of glioblastoma U87-MG cells from the top of VitroGel RGD hydrogel



3D scale up

Human pluripotent stem cells static suspension culture in VitroGel STEM



*In vivo* study

VitroGel is biocompatible without toxicity for animal study

**ROBUST 3D CELL PLATFORM  
WITH AN OUTSTANDING BALANCE OF  
SIMPLICITY AND VERSATILITY**



# VITROGEL® READY-TO-USE HYDROGEL

A series of user-friendly functional hydrogel offering an excellent balance of simplicity and versatility.

These ready-to-use VitroGel hydrogels have optimized formulations of multi-functional ligands and concentration to support a wide range of cell types for different applications, including various 3D cell models, stem cell spheroid, and organoids.

**JUST ADD CELLS:** Each ready-to-use VitroGel can mix with cell suspension directly. There is no additional adjustment required. For many users, this is the preferred hydrogel for 3D cell culture and 2D coating.



## THE READY-TO-USE VITROGEL INCLUDES:

**VitroGel® Hydrogel Matrix**  
**VitroGel® ORGANOID**

**VitroGel® STEM**  
**VitroGel® HEK293**

**VitroGel® MSC**  
**VitroGel® Angiogenesis Assay**

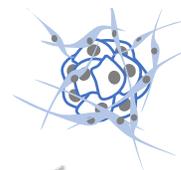
VitroGel Ready-to-use hydrogel is good for multiple applications:



**3D cell model**



**2D hydrogel coating**



**Co-culture**



**Invasion assay**



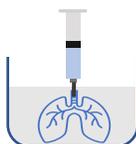
**3D migration**



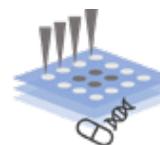
**Animal injection**



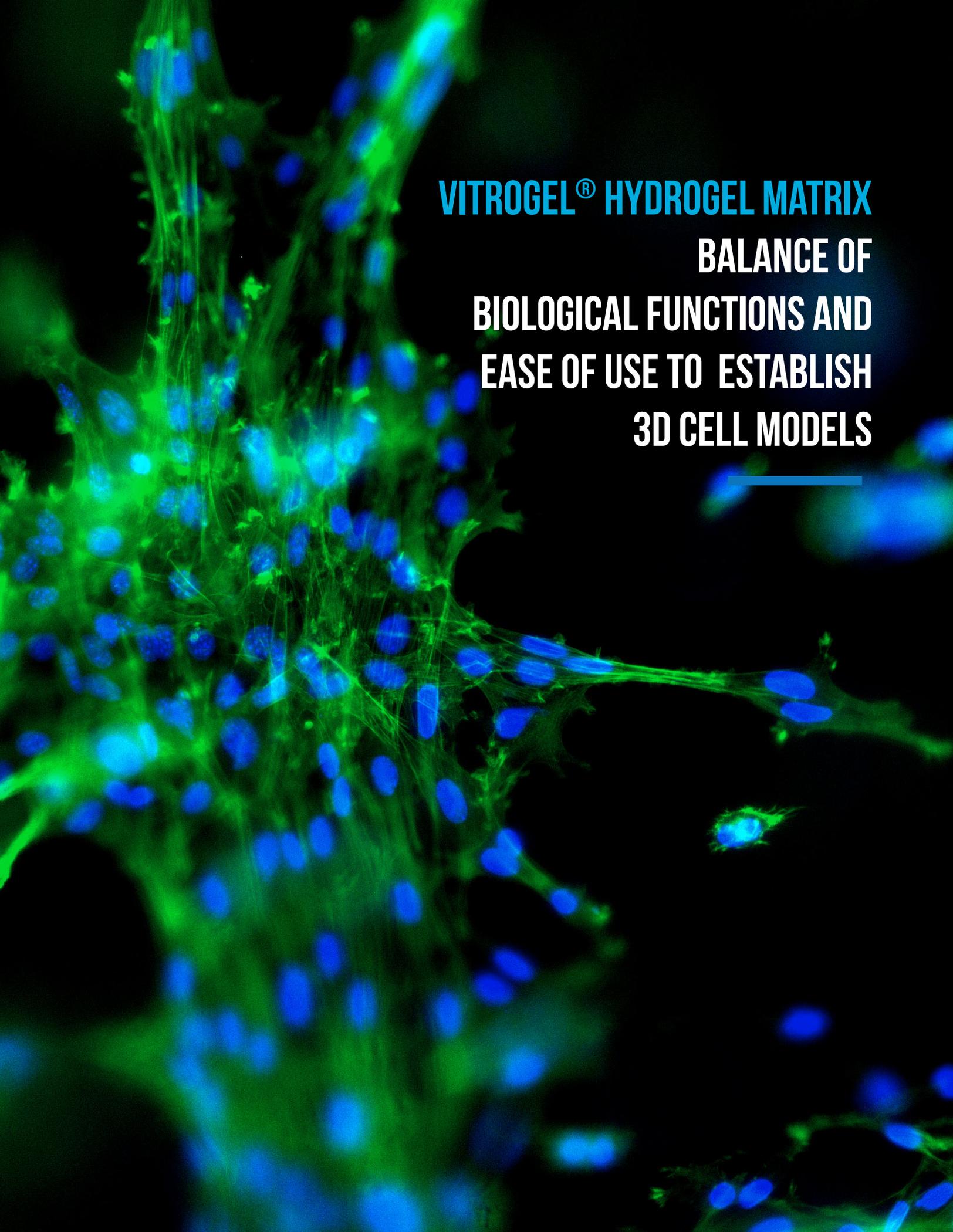
**Control release**



**FRESH bioprinting**



**High-throughput screening**

A fluorescence microscopy image showing a dense network of cells within a hydrogel matrix. The cells are stained with a blue dye, likely DAPI, to highlight their nuclei. The surrounding matrix is stained with a green dye, showing a complex, interconnected network of fibers. The overall appearance is that of a highly porous and interconnected 3D cellular structure.

**VITROGEL<sup>®</sup> HYDROGEL MATRIX**  
**BALANCE OF**  
**BIOLOGICAL FUNCTIONS AND**  
**EASE OF USE TO ESTABLISH**  
**3D CELL MODELS**

---

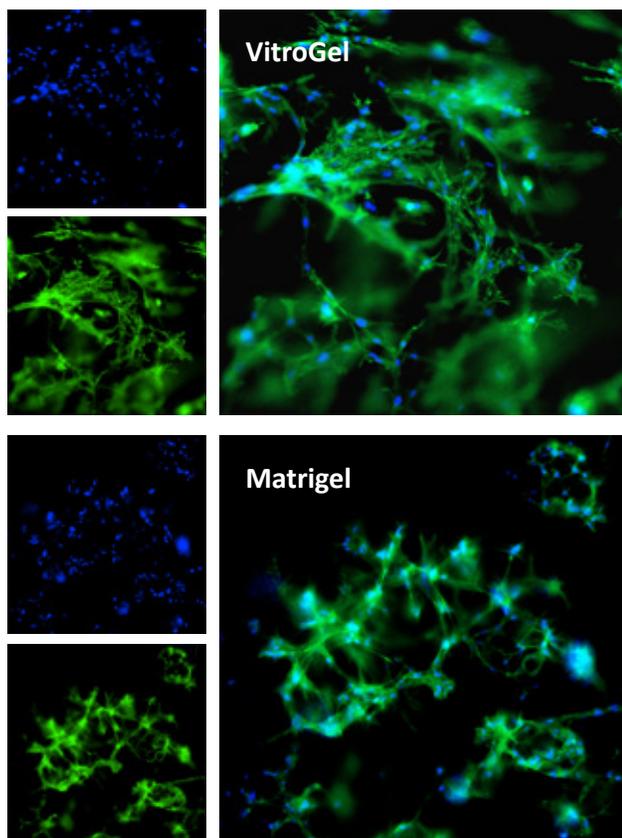
# VITROGEL® HYDROGEL MATRIX

Xeno-free (animal origin-free) multi-functional hydrogel system.



VitroGel® Hydrogel Matrix is a ready-to-use, xeno-free (animal origin-free) functional hydrogel for 3D cell culture research. VitroGel Hydrogel Matrix is an optimized formulation of multi-functional ligands and concentration to support a wide range of cell types for different applications.

VitroGel Hydrogel Matrix closely mimics the natural extracellular matrix (ECM) environment to make cells feel more like at home. The hydrogel is room temperature stable, has a neutral pH, is transparent, permeable, and compatible with different imaging systems. The solution transforms into a hydrogel matrix by simply mixing with the cell culture medium. Cells cultured in this system can be easily harvested with our [VitroGel Cell Recovery Solution](#).



## Why VitroGel Hydrogel Matrix?

**XENO-FREE:** Without undesired proteins for pre-clinical/clinical applications

**MULTIFUNCTIONAL LIGANDS:** Supports strong cell-matrix interactions for many cell types

**READ-TO-USE & EASY CELL RECOVERY**

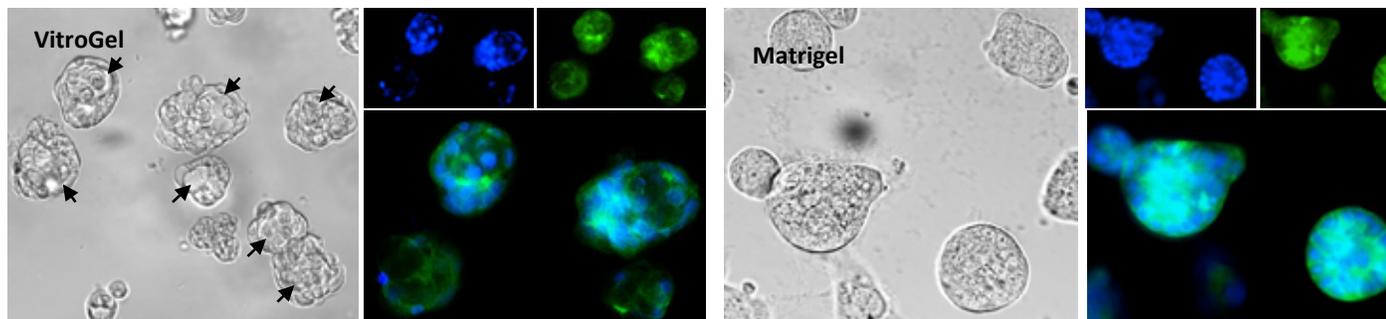
**BATCH-TO-BATCH CONSISTENT**

| Features                | Specifications   |
|-------------------------|--|
| <b>Formulation</b>      | Xeno-free, functional hydrogel   |
| <b>Use</b>              | 3D and 2D cell culture   |
| <b>Operation</b>        | Ready-to-use at room temperature   |
| <b>Biocompatibility</b> | Biocompatible, safe for animal studies                                     |
| <b>Injection</b>        | Injectable hydrogel for <i>in vivo</i> studies and lab automation          |
| <b>Cell Harvesting</b>  | 20 min cell recovery using <a href="#">VitroGel Cell Recovery Solution</a> |
| <b>pH</b>               | Neutral  |
| <b>Storage</b>          | Store at 2-8°C. Ships at ambient temperature                               |
| <b>Sizes</b>            | 10 mL and 2 mL   |
| <b>Number of Uses</b>   | (10 mL) 300 uses (2 mL) 60 uses  |

| Product                  | Size  | SKU #  |
|--------------------------|-------|--------|
| VitroGel Hydrogel Matrix | 10 mL | VHM01  |
|                          | 2 mL  | VHM01S |

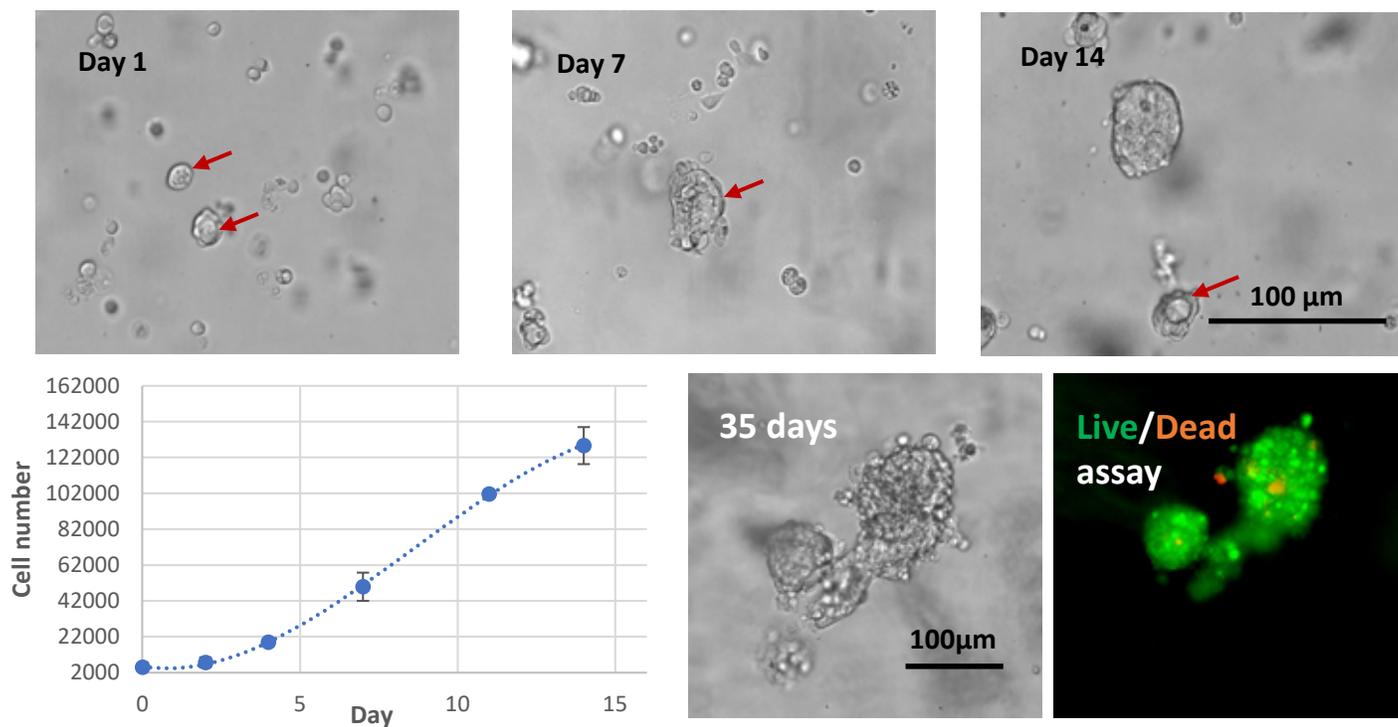
### Bone marrow cells 3D cultured in VitroGel Hydrogel Matrix and Matrigel

The fibroblast-like mouse bone marrow stromal cells (OP9-GFP) were 3D cultured in VitroGel Hydrogel Matrix and Matrigel. Single cells were homogeneously suspended within each hydrogel, with each forming stretched fibroblast-like structure on day 1. The images above shows a clear 3D cellular networking structure formed in both hydrogels on day 7. Compared to Matrigel, the multiple functional ligands in the VitroGel Hydrogel Matrix promote a stronger cell-matrix interaction, which helps accelerate the cell proliferation and cell-cell communication during the 3D cell culture.



### Human mammary breast cancer cells cultured in VitroGel Hydrogel Matrix and Matrigel

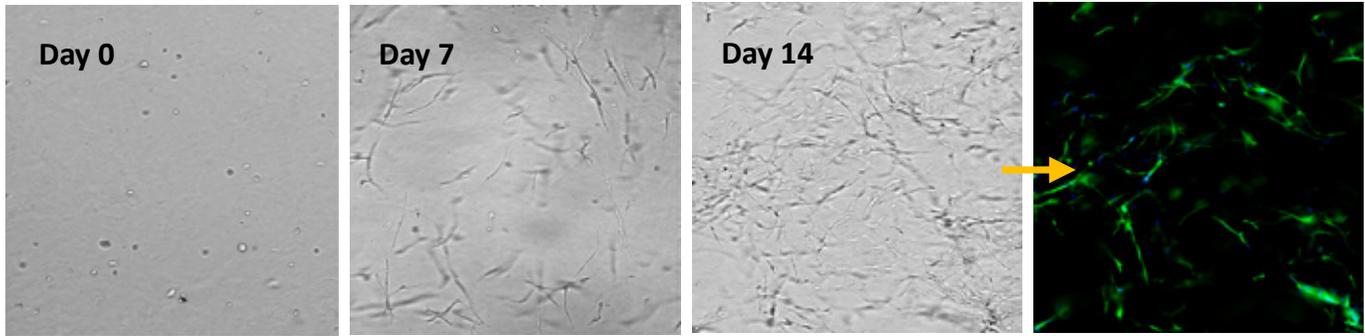
3D cell culture of human mammary breast cancer cells (MCF-7) at day 7 in VitroGel Hydrogel Matrix and Matrigel. The cells were prepared as single cell suspensions and encapsulated within each hydrogel respectively. The grape-shaped like cell colonies appeared on day 1 for both hydrogels. However, cells displayed 3D luminal structures (see arrows) only within the VitroGel Hydrogel Matrix. (Z-stack imaging system with 2D image projection was used. Blue: DAPI; Green: ActinGreen™).



### Long term 3D culture of MCF7 breast cancer cells in VitroGel Hydrogel Matrix (SKU: VHM01)

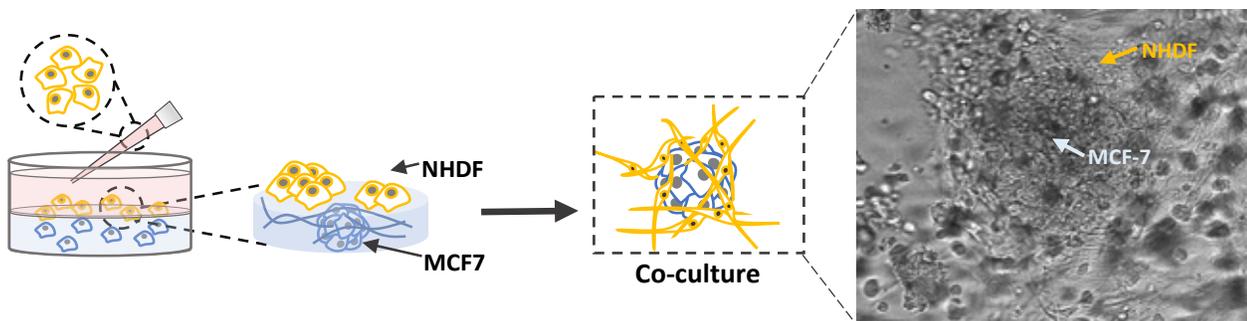
The images above show the cells at different stages of the long-term culture on days 1, 7, 14, and 35. The formation of the lumen structures were observed by day 7. By day 14, cellular polarity loss gave rise to spheroid structures. The malignant stage of the spheroid continued to produce the heterogenous mass of cells observed on day 35. The live/dead assay shows disorganized metastatic organization of cells within the tumor while the viability image shows a small zone of dead cell (red) at the center of the spheroid as well as surrounding the sphere.

Matrigel is a trademark of Corning Incorporated



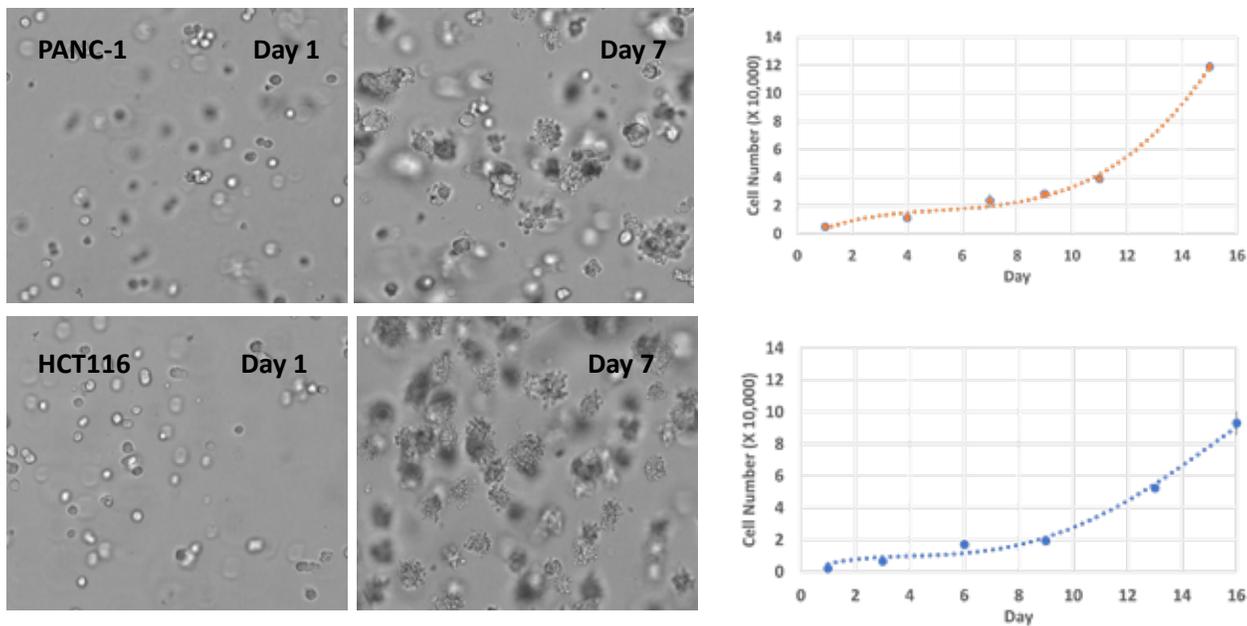
**3D cell culture of normal Human Dermal Fibroblast (NHDF) cells in VitroGel Hydrogel Matrix**

Human Dermal Fibroblast (NHDF) cells were encapsulated within the VitroGel Hydrogel Matrix and cultured for 14 days. The images above show the 3D networking of the fibroblast structures, indicating a strong matrix-cell interactions.



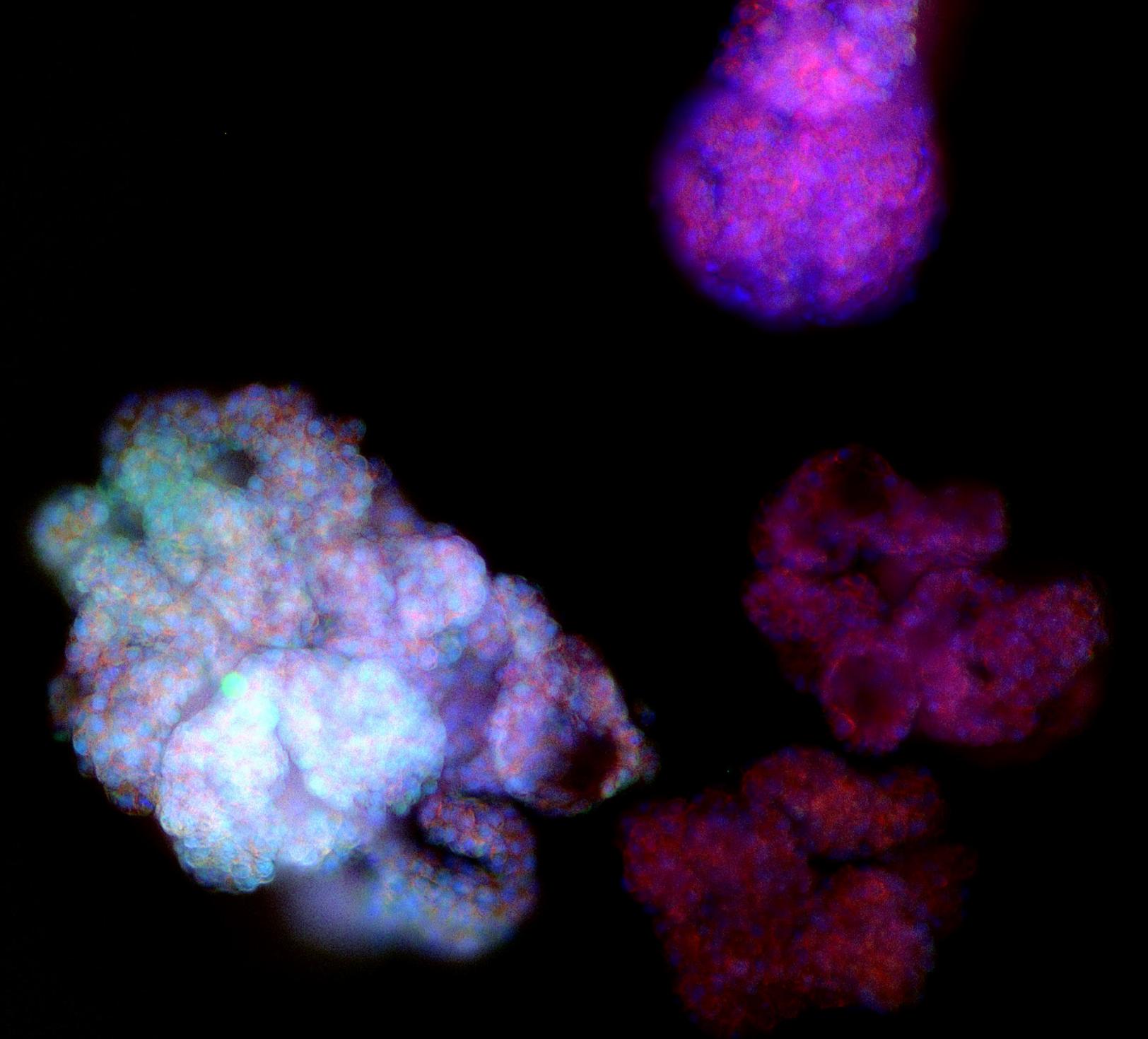
**Co-culture of human mammary breast cancer cells (MCF-7) and normal Human Dermal Fibroblast (NHDF) cells in VitroGel Hydrogel Matrix**

MCF-7 cells were encapsulated in a hydrogel matrix by mixing the cell suspension with VitroGel Hydrogel Matrix. After allowing the mixture to stabilize at room temperature for 10 min, NHDF cells were added on top of the hydrogel for 2D hydrogel coating culture. The NHDF cells attached and moved inside the hydrogel matrix after 48 hours culturing. The NHDF cells grew surrounding the MCF-7 spheroids and supported the fast growth of MCF-7 for large tumor structure formation.



**3D cell culture of Pancreatic Cancer cells (PANC-1) and colon cancer cells (HCT116) in VitroGel Hydrogel Matrix**

PANC-1 and HCT116 cells were seeded within VitroGel Hydrogel Matrix as single cells. The images above show both cell type's rapid growth and tumor spheroid formations in the VitroGel Hydrogel Matrix. The hydrogel system is suitable for long-term cell culture for more than 21 days.



**VITROGEL<sup>®</sup> ORGANOID**

**XENO-FREE  
ORGANOID CULTURE**

---

# VITROGEL® ORGANOID

Xeno-free (animal origin-free) hydrogel for organoid culture.

VitroGel® ORGANOID are xeno-free (animal origin-free) hydrogels that support the growth of patient-derived organoids or organoids developed from pluripotent stem cells (PSCs), co-culture, and PDX model.

VitroGel ORGANOID hydrogels are ready-to-use at room temperature and have a neutral pH, transparent, permeable, and compatible with different imaging systems. The solution transforms into a hydrogel matrix by simply mixing with the cell culture medium. VitroGel ORGANOID hydrogels are good for both 3D cell culture and 2D hydrogel coating applications

The organoid hydrogels can work in conjunction with VitroGel STEM (a xeno-free hydrogel for 3D static suspension cultures and scale-up of human pluripotent stem cells) by transferring stem cell spheroids cultured in VitroGel STEM to VitroGel ORGANOID hydrogels for organoid differentiation. Key growth factors and molecules can be mixed directly with the hydrogel matrix or by adding on the top of the hydrogel. Organoids cultured in this system can be easily harvested out with our VitroGel Cell Recovery Solution. VitroGel ORGANOID hydrogels provide a well-defined 3D microenvironment for the future of personalized medicine.

The VitroGel ORGANOID Discovery Kit (Cat# VHM04-K) includes all four organoid hydrogels (VitroGel ORGANOID-1, VitroGel ORGANOID-2, VitroGel ORGANOID-3, VitroGel ORGANOID-4), which were formulated with various bio-functional ligands, mechanical strengths, and degradability to fulfill the needs of different organoid culture conditions. Use the Discovery Kit to screen which organoid hydrogel version works best for your organoid culture.

| Product                         | Size     | SKU #   |
|---------------------------------|----------|---------|
| VitroGel ORGANOID Discovery Kit | 4 X 2 mL | VHM04-K |
| VitroGel ORGANOID-1             | 10 mL    | VHM04-1 |
| VitroGel ORGANOID-2             | 10 mL    | VHM04-2 |
| VitroGel ORGANOID-3             | 10 mL    | VHM04-3 |
| VitroGel ORGANOID-4             | 10 mL    | VHM04-4 |



## Why VitroGel ORGANOID?

**XENO-FREE:** Without undesired proteins for pre-clinical/clinical applications

**MULTIPLE APPLICATIONS:** Supports organoid formation from a wide range of resource including patient-derived samples and hPSCs

**READY-TO-USE & EASY CELL RECOVERY:** Room temperature stable to mix with cells medium directly; harvest cells from the hydrogel without enzyme solution

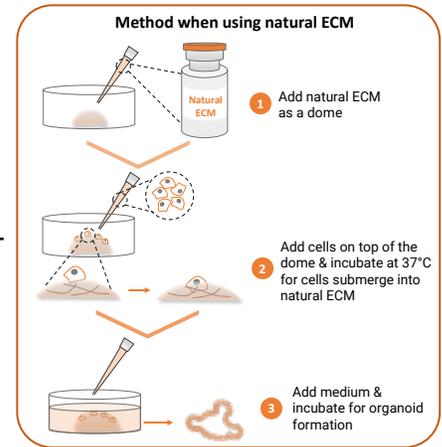
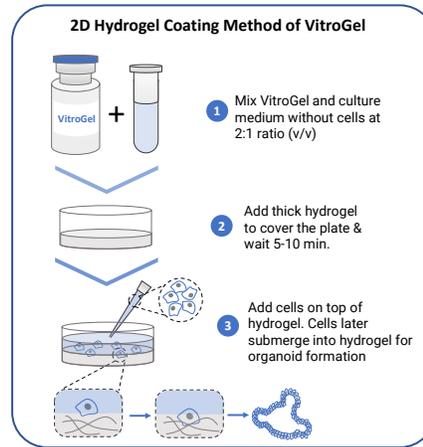
**BATCH-TO-BATCH CONSISTENT**

| Features                | Specifications   |
|-------------------------|--|
| <b>Formulation</b>      | Xeno-free, functional hydrogel   |
| <b>Use</b>              | Organoid culture   |
| <b>Operation</b>        | Ready-to-use at room temperature   |
| <b>Biocompatibility</b> | Biocompatible, safe for animal studies   |
| <b>Injection</b>        | Injectable hydrogel for <i>in vivo</i> studies and lab automation                                  |
| <b>Cell Harvesting</b>  | Easy cell recovery using <a href="#">VitroGel Cell Recovery Solution</a>                           |
| <b>pH</b>               | Neutral  |
| <b>Storage</b>          | Store at 2-8°C. Ships at ambient temperature   |
| <b>Sizes</b>            | Single Vial: 10 mL<br>Discovery Kit: (2 mL) each of ORGANOID-1, ORGANOID-2, ORGANOID-3, ORGANOID-4 |
| <b>Number of Uses</b>   | (10 mL) 300 uses at 50 µL/test<br>( 2 mL) 60 uses at 50 µL/test                                    |

# VitroGel Organoid Methods vs Natural ECM Methods

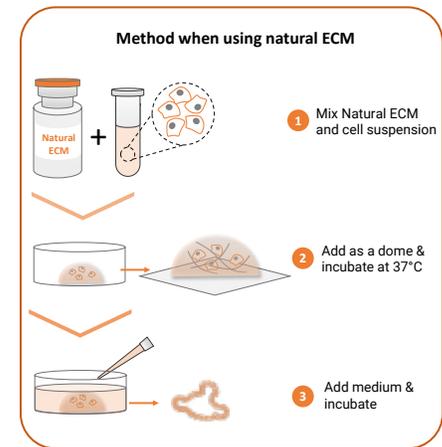
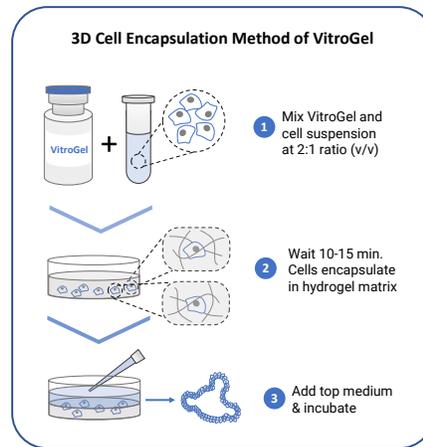
## Method 1 2D Hydrogel Coating

Add cells on top of hydrogel for optimal interactions among cells, hydrogel, and medium.



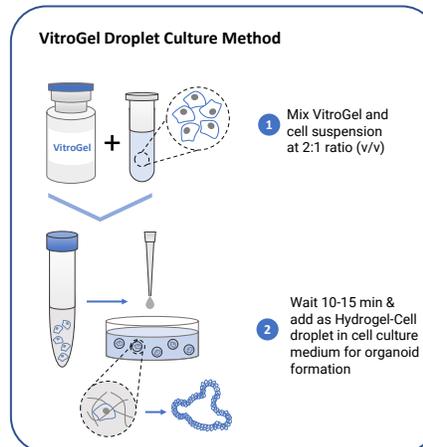
## Method 2 3D Cell Culture Encapsulation

Encapsulate cells within the hydrogel for excellent mechanical support and the cell-matrix interactions.



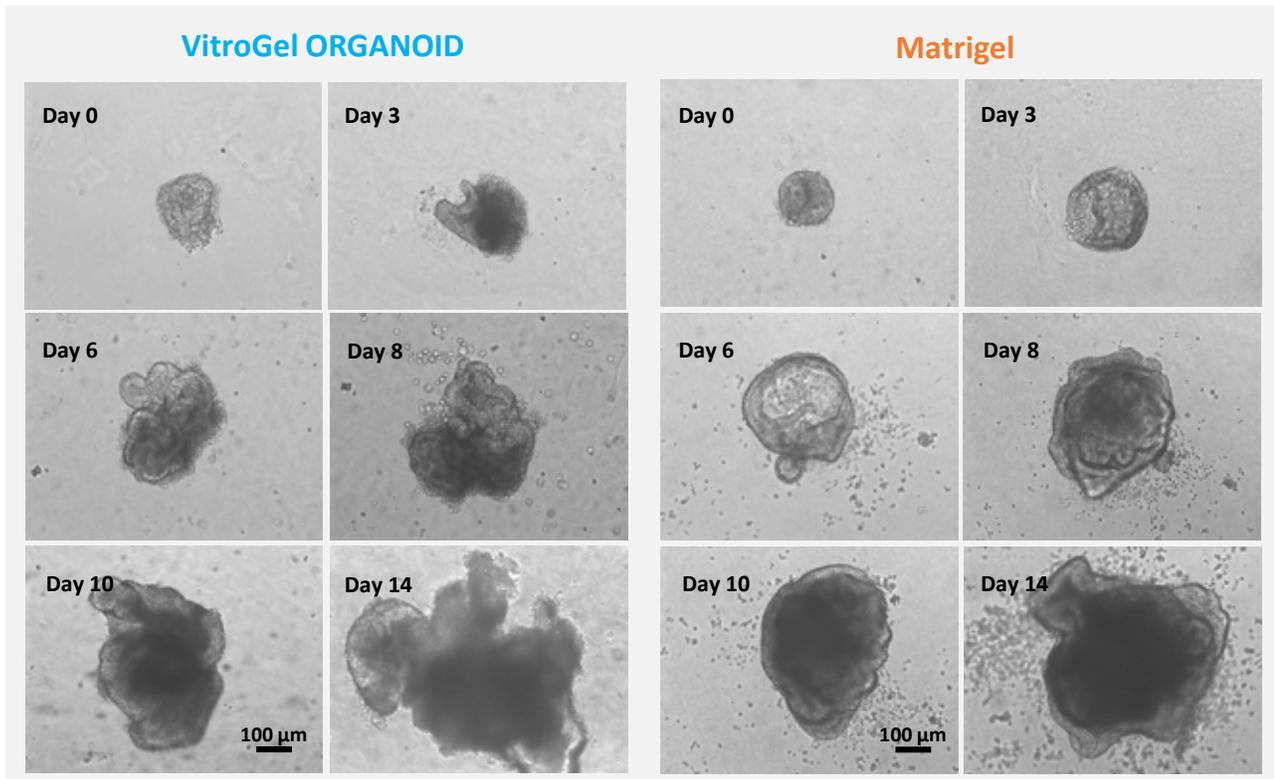
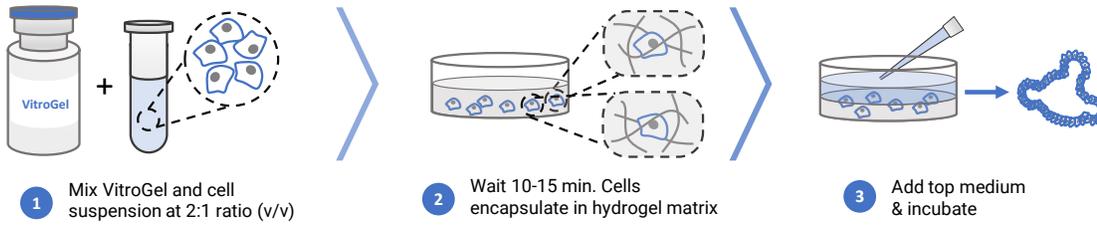
## Method 3 2D Hydrogel Coating

Create a VitroGel-Cell droplet. This is a unique culture method only to the VitroGel system for generating great cell-matrix interactions and excellent medium penetration for organoid formation and scale-up.



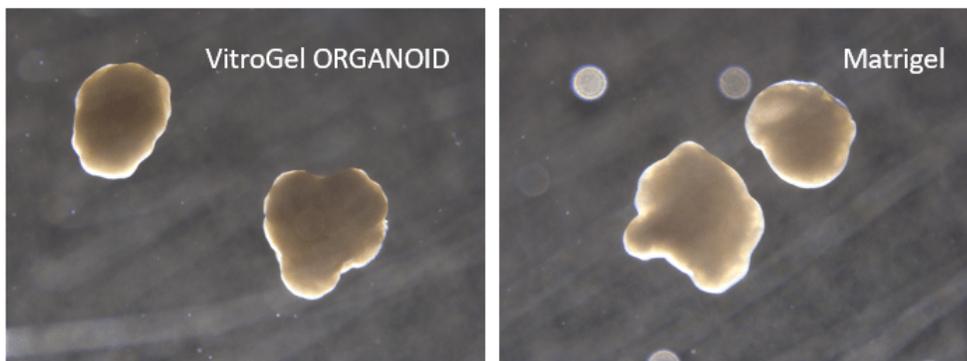
## Xeno-free 3D Organoid Workflow

Use VitroGel ORGANOID for organoid culture and maturation.



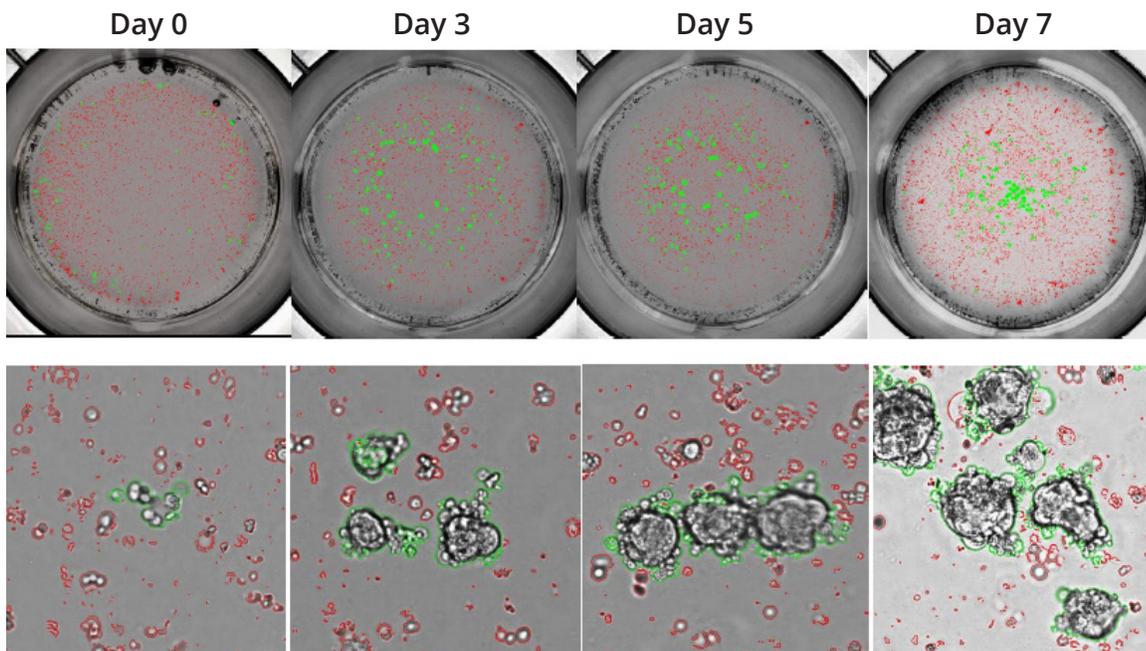
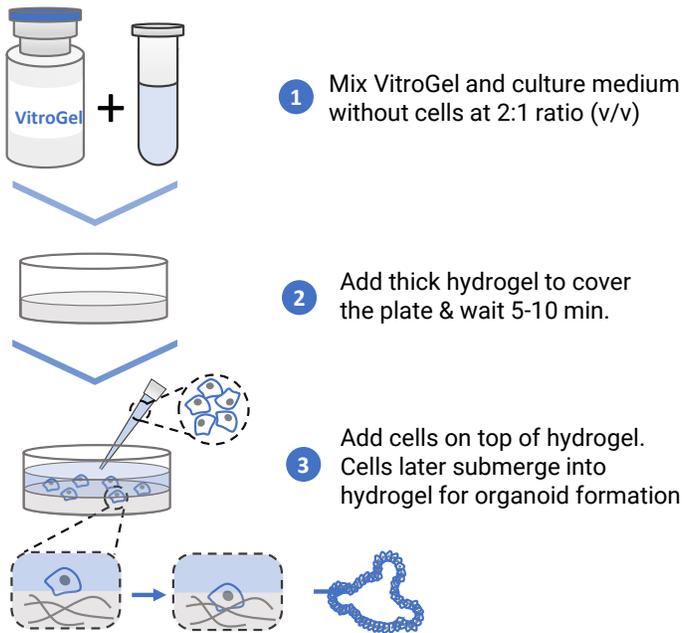
### Mouse intestinal organoid culture on VitroGel ORGANOID and Matrigel

Small organoids recovered from liquid nitrogen were directly seeded with VitroGel and Matrigel, respectively. Images show the growth of mouse intestinal organoid from day 0 to day 14.



### Cerebral organoid culture with VitroGel ORGANOID and Matrigel comparison

## Xeno-free Organoid 2D Hydrogel Coating Method



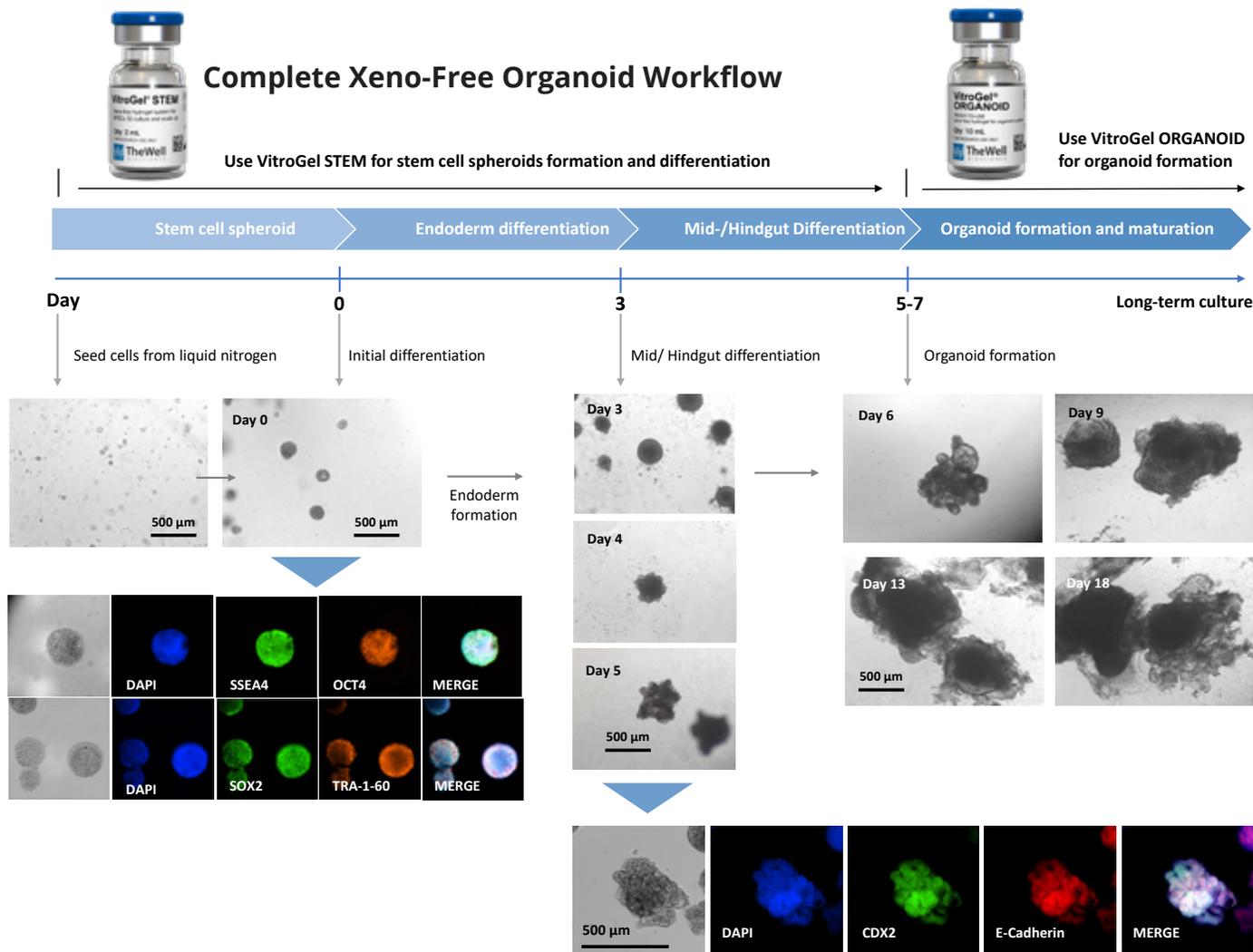
- Lower limit of object size, 50  $\mu\text{m}$
- Green, live organoids; Red, others

### Organoids from breast cancer PDX cultured on VitroGel ORGANOID

Cells were cultured on top of 2D hydrogel coating surface for 7 days.

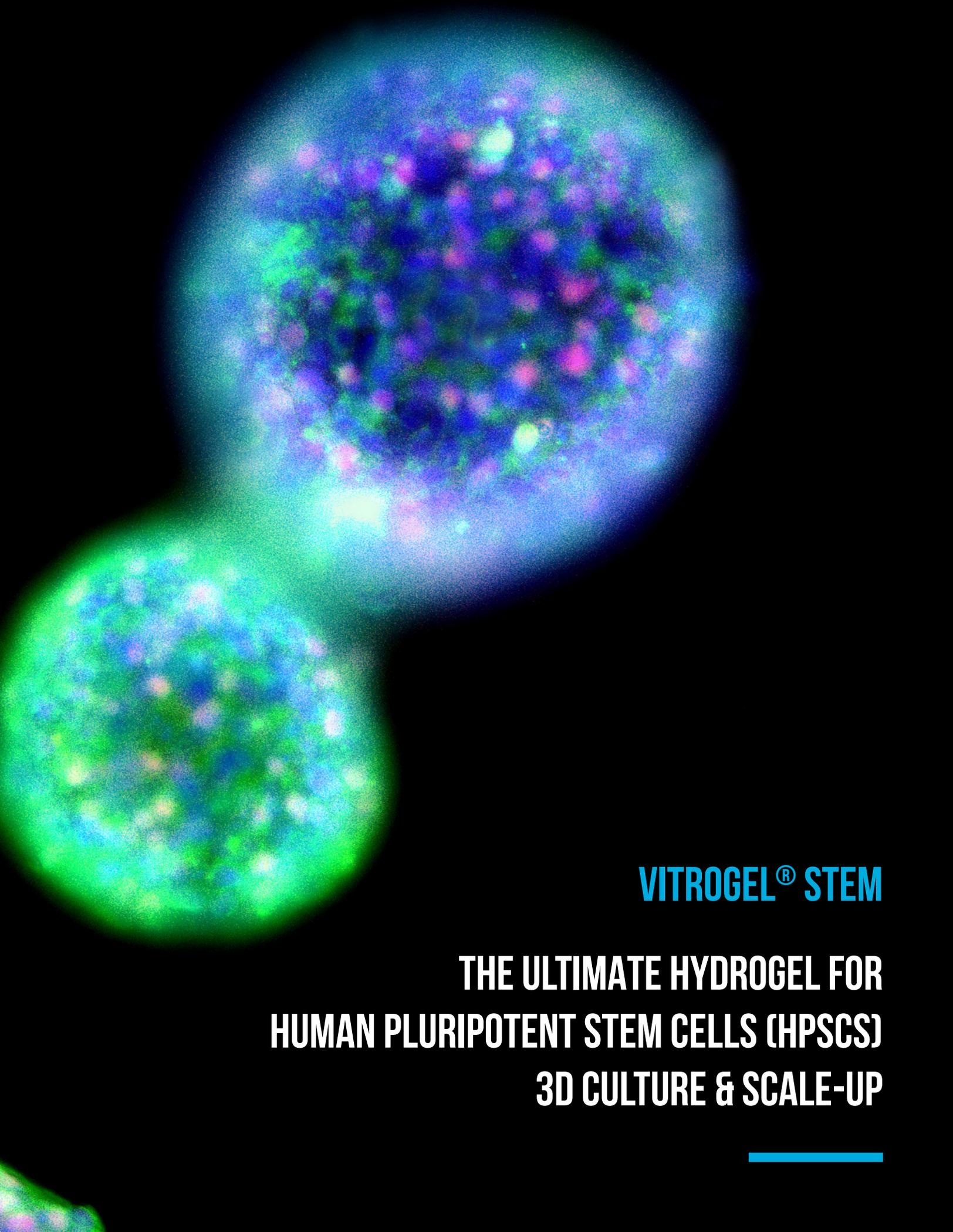
# Start from iPSC spheroids for stem cell differentiation and organoid formation

Diagram below shows culturing human intestinal organoid from stem cell spheroids.



### Culture human intestinal organoid from stem cell spheroids.

Human iPSCs recovered from liquid nitrogen were seeded with VitroGel STEM for static suspension culture (Refer to protocol of VitroGel STEM, Cat# VHM02). The high-quality stem cell spheroids formed within 3-5 days with full pluripotent properties (showing the positive markers of SSEA4, OCT4, SOX2, and TRA-1-60). The spheroids were harvested by centrifuging (100 x g, 3 min), and resuspended with VitroGel STEM in endoderm differentiation medium for 3 days. The endoderm cell spheroids were then harvested by centrifuging (100 x g, 3 min), and resuspended with VitroGel STEM in mid/hindgut differentiation medium for 3-4 days. The mid/hindguts were collected by centrifuging (100 x g, 3 min), and characterized with CDX2 and E-Cadherin. Resuspended the mid/hindgut with organoid formation medium and mixed with VitroGel ORGANOID, following the protocol of VitroGel ORGANOID, for organoid formation and long-term maturing culture.



**VITROGEL® STEM**

**THE ULTIMATE HYDROGEL FOR  
HUMAN PLURIPOTENT STEM CELLS (HPSCS)  
3D CULTURE & SCALE-UP**

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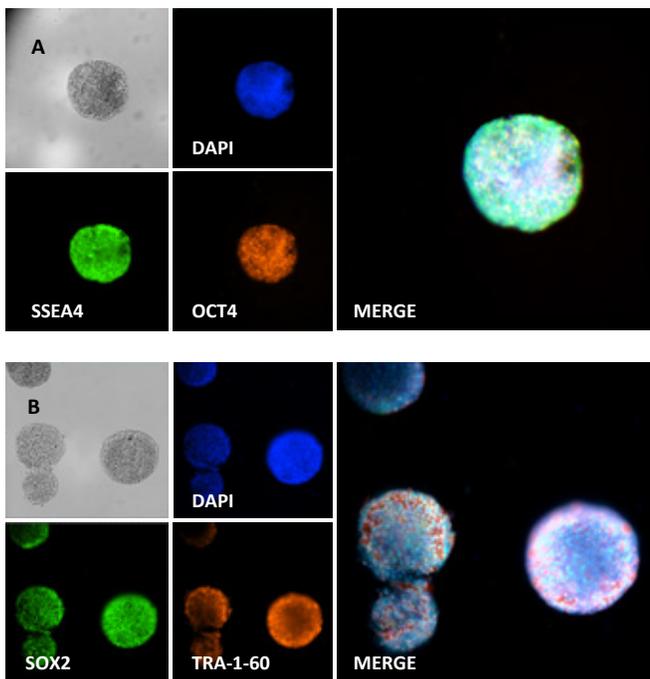
# VITROGEL® STEM

Xeno-free (animal origin-free) hydrogel for hPSCs 3D culture and scale-up.



VitroGel® STEM is a xeno-free (animal origin-free) hydrogel system developed to improve the performance of three-dimensional (3D) static suspension cultures and scale-up of human pluripotent stem cells (hPSCs) to create a high-throughput system to model various tissue and disease states.

This hydrogel system is ready to use with an optimized formulation that fully supports the rapid expansion of high-quality 3D stem cell spheroids with pluripotent properties. hPSCs directly thawed from liquid nitrogen or passaged from 2D matrix coated culture vessels can be immediately mixed with the hydrogel solution for static suspension cultures. Moreover, the optimization protocol is ideal for time-sensitive experiments, as it does not require excessive medium exchanges, which can ultimately save on time and materials. This hydrogel system is compatible with most hPSC culture media and tissue culture vessels. Due to the unique static suspension culture procedure, cell harvesting is simple and effective. The 3D stem cell spheroids that are developed using this system can be used for further sub-cultures, patterned differentiations, organoid development, or re-establishing 2D culture morphologies.



## Immunofluorescence images of hPSC spheroids with key pluripotent stem cell markers.

A) images of hPSC spheroids with the SSEA4 and OCT4 expression,  
B) images of hPSC spheroids with the SOX2 and TRA-1-60 expression.

## Why VitroGel STEM?

**XENO-FREE:** Without undesired proteins for pre-clinical/clinical applications

**UNIQUE:** Ability to seed stem cells directly from liquid nitrogen (LN<sub>2</sub>) or 2D culture systems and efficient cell harvesting or sub culturing

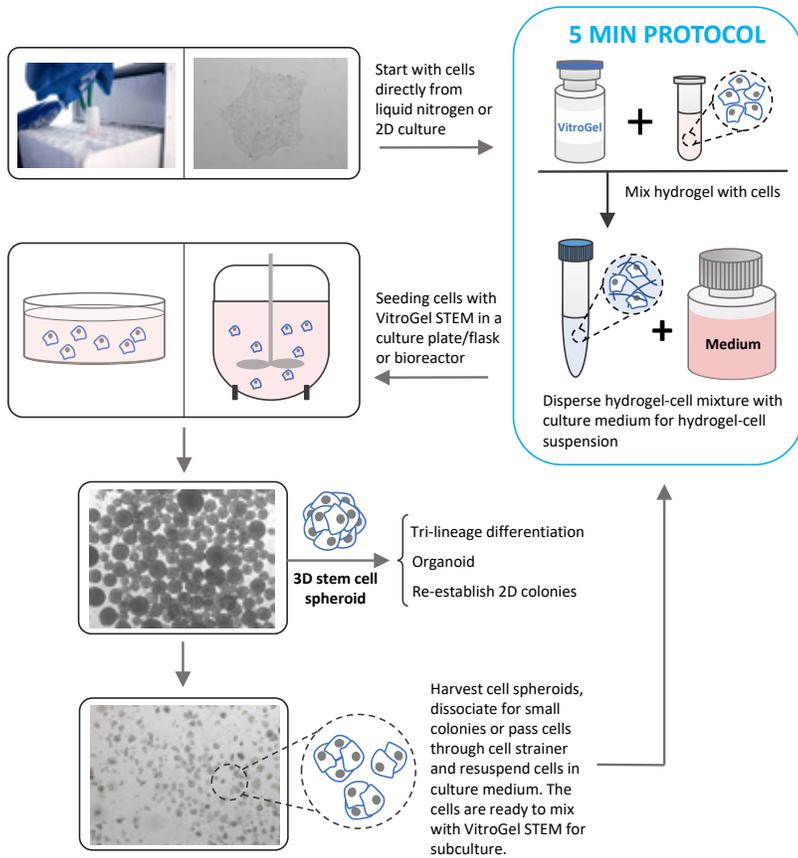
**HIGH PERFORMANCE:** Yields high-quality 3D stem cells with high cell viability and excellent cell growth rates

**FLEXIBLE:** Compatible with most culture vessels and stem cell culture media

**COST-EFFECTIVE:** Eliminates the need for matrix coating and reduce the medium change

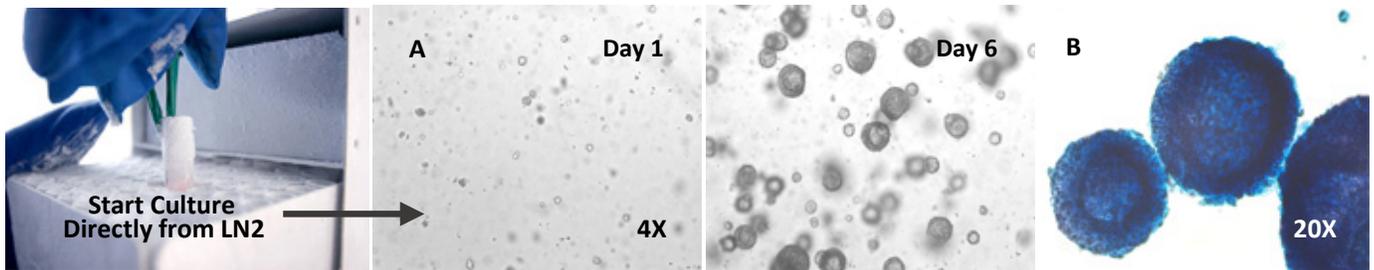
| Features                | Specifications   |
|-------------------------|--|
| <b>Formulation</b>      | Xeno-free, functional hydrogel   |
| <b>Use</b>              | 3D static suspension culture for hPSCs                                     |
| <b>Operation</b>        | Ready-to-use at room temperature   |
| <b>Biocompatibility</b> | Biocompatible, safe for animal studies                                     |
| <b>Injection</b>        | Injectable hydrogel for <i>in vivo</i> studies and lab automation          |
| <b>Cell Harvesting</b>  | 20 min cell recovery using <a href="#">VitroGel Cell Recovery Solution</a> |
| <b>pH</b>               | Neutral  |
| <b>Storage</b>          | Store at 2-8°C. Ships at ambient temperature                               |
| <b>Sizes</b>            | 10 mL and 2 mL   |
| <b>Number of Uses</b>   | (10 mL) 90-180 mL suspension culture<br>(2 mL) 15-30 mL suspension culture |

| Product       | Size  | SKU #  |
|---------------|-------|--------|
| VitroGel STEM | 10 mL | VHM02  |
|               | 2 mL  | VHM02S |



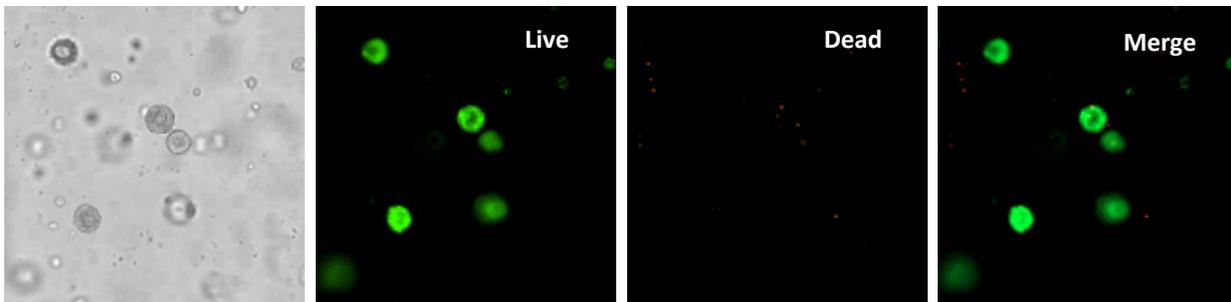
Benefits Comparison

|                                  | VitroGel STEM | Natural ECM       |
|----------------------------------|---------------|-------------------|
| Culture Method                   | 3D Suspension | 2D Matrix Coating |
| Xeno-free                        | Yes           | No                |
| 3D Spheroid Formation            | Yes           | No                |
| 3D Differentiation (Directly)    | Yes           | No                |
| Easy to Scale-up                 | Yes           | No                |
| Preparation Time                 | < 10 min      | 2 hr              |
| Cell Viability after Seeding     | ●●●●●         | ●●○○○             |
| Cell Proliferation               | ●●●●●         | ●●○○○             |
| Easy Cell Harvesting             | ●●●●●         | ●●○○○             |
| Cell Quality for Differentiation | ●●●●●         | ●●○○○             |
| Easy for Sub-culture             | ●●●●●         | ●●○○○             |



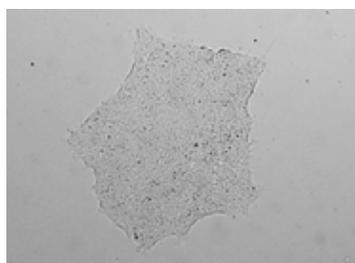
3D static suspension culture of hPSC directly from Liquid Nitrogen (LN2)

Start the suspension culture by using the healthy and high-quality cells directly from LN2. hPSC-hydrogel aggregates successfully to form healthy spheroids after 1 day in culture. The hPSC spheroids continue to expand from day 1 to 6 (Figure A). The resulting hPSC spheroids also show hallmark features of healthy and high-quality stem cell spheroids, i.e., shallow craters or pockmarks. Figure B shows that hPSC static suspension cultures from liquid nitrogen are positive for Alkaline Phosphatase, indicating successful expansion of healthy stem cell populations.

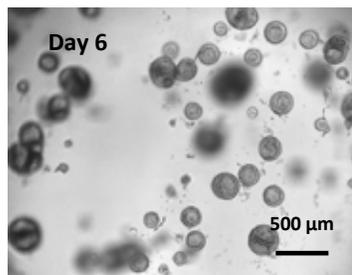
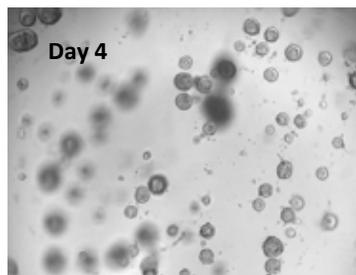
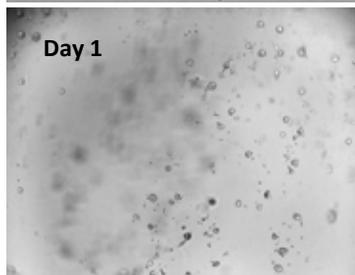
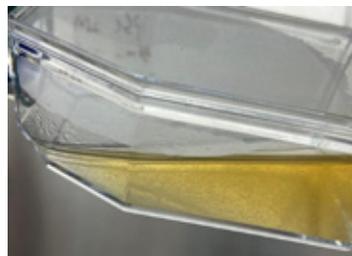


Live/Dead assay of hPSC spheroids.

The health and live cell spheroids show in the green color. The dead cells shows as small clumps or individual cells surrounding the big cell spheroids.

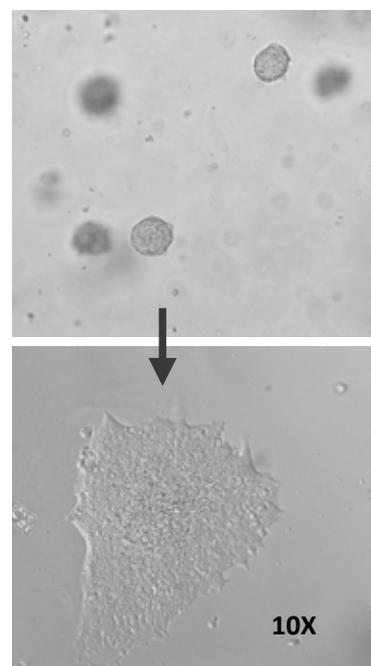
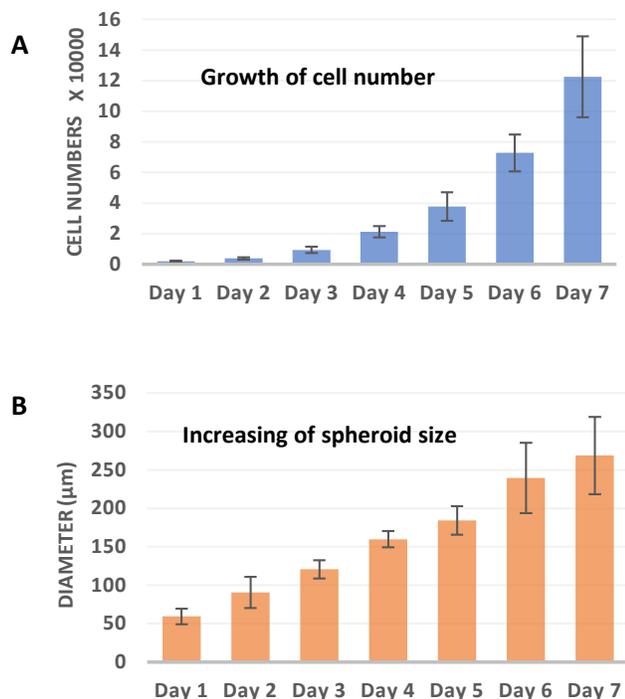


Start with healthy and high-quality 2D cultured stem cells



### 3D static suspension culture of hPSC from 2D matrix culture

After 24 hours, small hPSC spheroids start to form. From day 1 to 6, cells in the suspension cultures quickly grew, leading to the generation of healthy and high-quality stem cell spheroids. After day 3, cell number grew exponentially (Figure A below) and spheroid size steadily increases (Figure B below). The hPSC spheroids display characteristics of shallow craters or pockmarks, indicating expression of hPSC markers and successful expansion of healthy and high-quality stem cell spheroids. The resulting spheroids provide researchers with large numbers of healthy hPSCs for further experiments.



### The growth of cell number and spheroid size of hPSC in VitroGel static suspension culture

A) The chart shows the growth of the cell number from day 1 to day 7 after seeded for 3D static suspension culture (The cell proliferation assay was tested by CCK8 assay). B) Increasing hPSC spheroid sizes from around 60 μm at day 1 to over 250 μm at day 7.

### Re-establish 2D colonies from 3D hPSC spheroids

The hPSC spheroids cultured in 3D static suspension can be harvested and seeded to 2D matrix coating surface. The 2D cell colonies formed within 24 hours after attached back to the matrix coating surface.

A fluorescence microscopy image showing a dense network of green fluorescent cells and fibers, likely representing a mesenchymal stem cell culture on a vitroGel MSC scaffold. The cells are interconnected by a complex web of fibers, creating a porous, interconnected structure. The background is dark, making the bright green fluorescence stand out.

**VITROGEL<sup>®</sup> MSC**

**MESENCHYMAL STEM CELL  
SCALE-UP  
NO MORE MICRO-CARRIERS**

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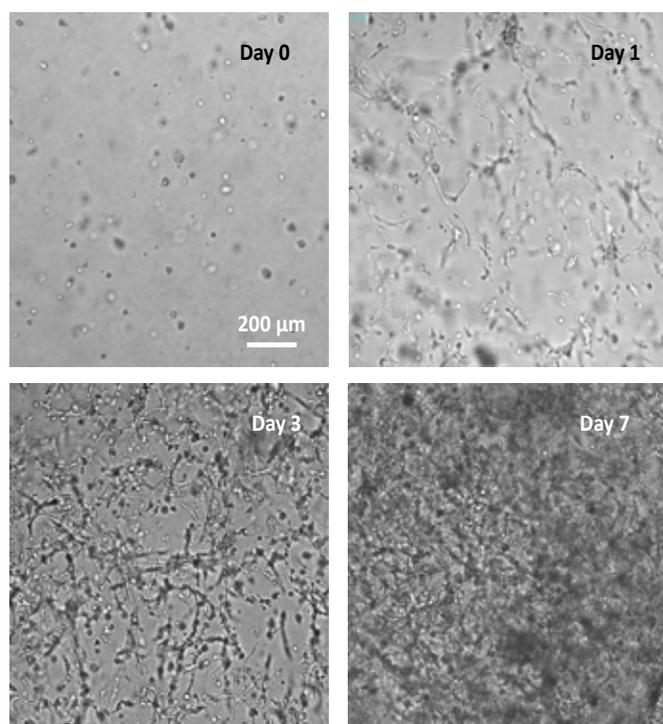
# VITROGEL® MSC

Xeno-free hydrogel for mesenchymal stem cell 3D culture and scale-up.



VitroGel® MSC is a xeno-free (animal origin-free) hydrogel system developed to support three-dimensional (3D) cultures of mesenchymal stem cells (MSCs). This hydrogel system can be used to make hydrogel cell beads for MSC scale-up. Microcarriers are not required for MSC scale-up.

VitroGel MSC is ready-to-use with an optimized formulation that fully supports the rapid expansion of MSCs. Cells directly thawed from liquid nitrogen or passaged from 2D culture vessels can be immediately mixed with the hydrogel solution for 3D culture or hydrogel-cell bead generation. This hydrogel system is compatible with most MSC culture media and tissue culture vessels. Cell harvesting after 3D culture is simple and efficient using the VitroGel Cell Recovery Solution.



### 3D culture of MSC in VitroGel MSC

MSC cells were suspended in cell medium at  $8 \times 10^5$  cells/mL and mixed with VitroGel MSC for 3D culture (according to the 3D cell culture protocols of VHM03). The images show the growth and expansion of cells inside of 3D hydrogel from day 0 to day 7.

## Why VitroGel MSC?

**NO MORE MICROCARRIERS:** Innovative hydrogel allows 3D MSC scale-up without using microcarriers

**HIGH PERFORMANCE:** Yields high-quality 3D MSCs with high cell viability and excellent cell growth rates

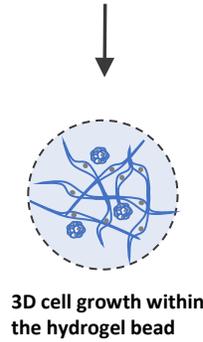
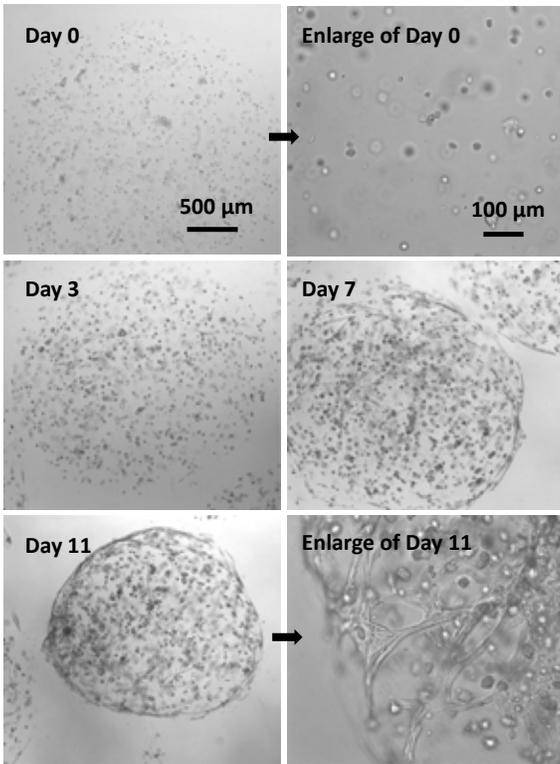
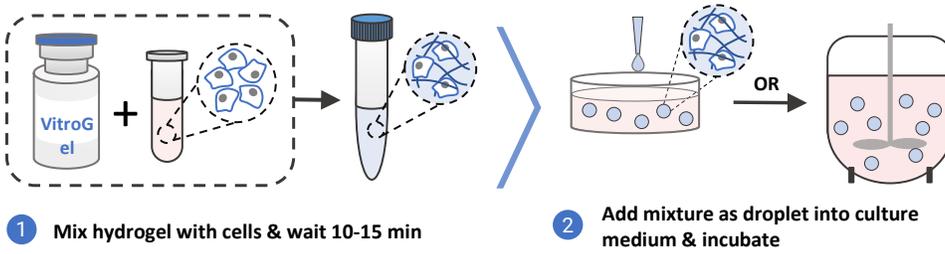
**EASY CELL HARVESTING:** Harvest cells from the hydrogel without enzyme solution

**LONG TERM CULTURE:** Supports MSC 3D culture for more than 30 days, maintaining great cell performance at low passage number

**INJECTABLE HYDROGEL:** Supports MSC injection as an injectable carrier for excellent cell retention and viability

| Features                | Specifications   |
|-------------------------|--|
| <b>Formulation</b>      | Xeno-free, functional hydrogel   |
| <b>Use</b>              | 3D cell culture, 2D hydrogel coating, hydrogel-cell bead formation       |
| <b>Operation</b>        | Ready-to-use at room temperature   |
| <b>Biocompatibility</b> | Biocompatible, safe for animal studies                                   |
| <b>Injection</b>        | Injectable hydrogel for <i>in vivo</i> studies and lab automation        |
| <b>Cell Harvesting</b>  | Easy cell recovery using <a href="#">VitroGel Cell Recovery Solution</a> |
| <b>pH</b>               | Neutral  |
| <b>Storage</b>          | Store at 2-8°C. Ships at ambient temperature                             |
| <b>Sizes</b>            | 10 mL and 2 mL   |
| <b>Number of Uses</b>   | (10 mL) 300 uses & (2 mL) 60 uses  |

| Product      | Size  | SKU #  |
|--------------|-------|--------|
| VitroGel MSC | 10 mL | VHM03  |
|              | 2 mL  | VHM03S |

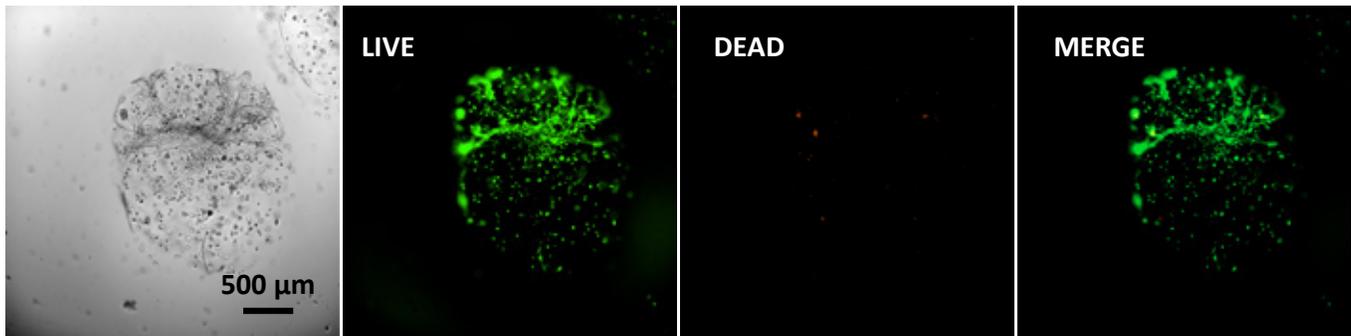


Benefits Comparison

|                                      | VitroGel MSC                | Multi-layer Flask | Microcarriers   |
|--------------------------------------|-----------------------------|-------------------|-----------------|
| Preparation Time                     | <30 min                     | <30 min           | >4 hr           |
| Cell Proliferation                   | 5-100X                      | 5-30X             | 5-30X           |
| Culture Scale                        | Small to Large              | Small to Medium   | Medium to Large |
| Culture Vessel                       | Well/Plate/Flask Bioreactor | Flask             | Bioreactor      |
| Scale-up with Low Passage Cycle      | Yes                         | No                | No              |
| Long-term Culture Capable (>30 days) | Yes                         | No                | No              |
| Easy Cell Harvesting without Trypsin | Yes                         | No                | No              |
| Cell Viability                       | ●●●●●                       | ●●●○○             | ●●●○○           |
| Cell Quality                         | ●●●●●                       | ●●●○○             | ●●●○○           |
| Easy for Sub-culture                 | ●●●●●                       | ●●●○○             | ●●●○○           |

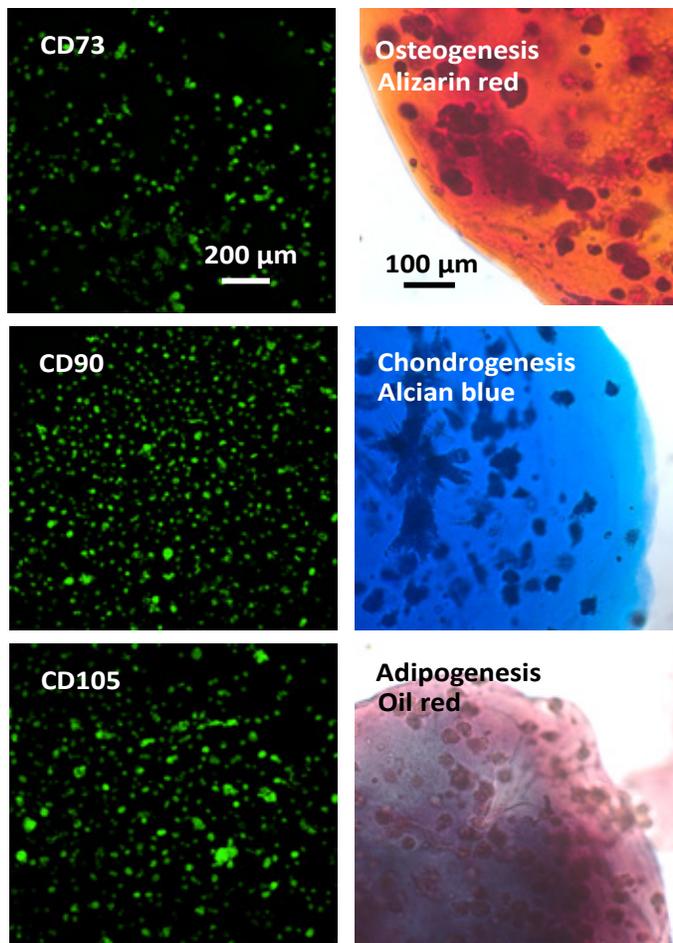
3D culture of MSC in hydrogel beads.

MSCs were mixed with VitroGel MSC and add to the cell culture medium as droplet for hydrogel-cell bead formation (according to the hydrogel-cell bead protocols of VHM03). The size of the hydrogel beads can be controlled by the volume of the droplets. MSC cells can grow within the hydrogel beads for long term-culture (>3 weeks). The images show the growth and expansion of cells inside of hydrogel beads from day 0 to day 11. The enlarged images show the cells grew from single cells to cell spheroid and matrix structure from day 0 to day 11.



**Live/dead assay of MSCs cultured in VitroGel MSC hydrogel beads.**

MSCs cultured in hydrogel beads for 11 days were stained with Cyto3DTM live/dead assay kit (Cat# BM01). The live cells are shown in green color and dead cells are shown in orange color. The images show that cells cultured in VitroGel MSC hydrogel beads have high-cell viability performance.

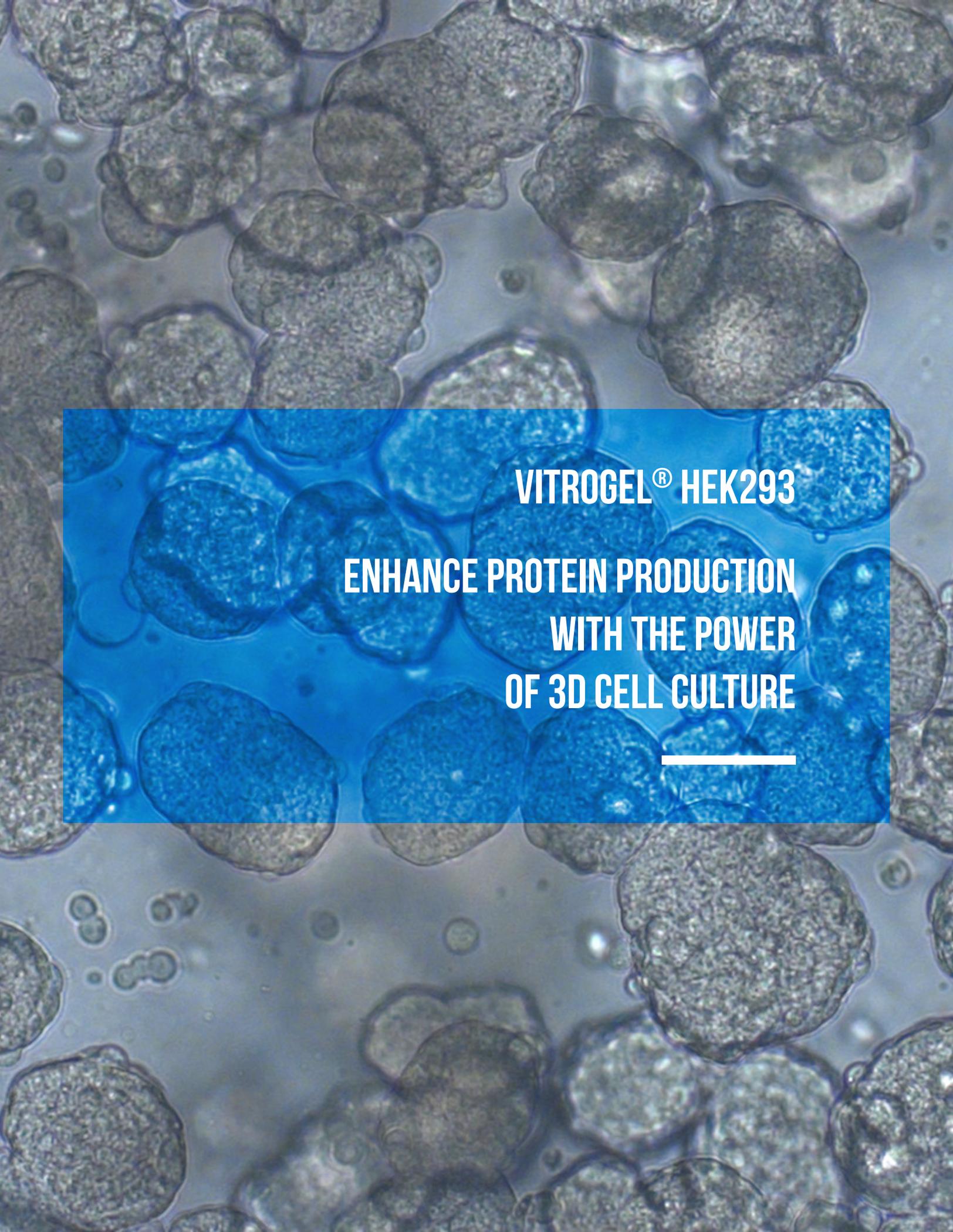


**Immunofluorescent images of MSCs after harvesting from VitroGel MSC hydrogel beads (Left).**

The images show that cells recovered from VitroGel MSC hydrogel beads present all three biomarkers (CD73, CD90 and CD105), which indicate the full characters of mesenchymal stem cells in 3D hydrogel-MSC beads.

**Differentiations of MSCs cultured in VitroGel MSC hydrogel beads (Right).**

MSCs were cultured in hydrogel beads for 7 days and then differentiate in osteogenesis, chondrogenesis and adipogenesis differentiation media for three week respectively. The images show that cells cultured in VitroGel MSC hydrogel beads can be successfully differentiated into osteocytes, chondrocytes, and adipocytes.

A microscopic view of HEK293 cells in a 3D culture matrix. The cells are arranged in a dense, interconnected network, appearing as a complex, porous structure. The cells are stained, giving them a dark, granular appearance. The background is a light, translucent blue. A semi-transparent blue rectangular box is overlaid on the center of the image, containing white text.

**VITROGEL® HEK293**

**ENHANCE PROTEIN PRODUCTION  
WITH THE POWER  
OF 3D CELL CULTURE**

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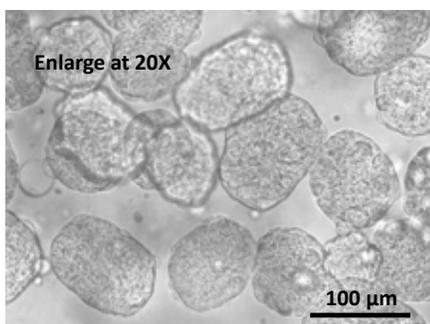
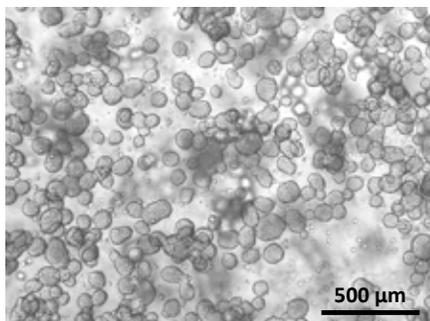
# VITROGEL® HEK293

Xeno-free (animal origin-free) hydrogel for HEK293 cells 3D culture and scale-up.



VitroGel® HEK293 is a xeno-free (animal origin-free) functional hydrogel system developed to support three-dimensional (3D) cultures of human embryonic kidney 293 (HEK293) cells.

VitroGel HEK293 is a unique system to support the rapid 3D growth of HEK293 cells easily. Cells directly thawed from liquid nitrogen or passaged from 2D culture vessels can be immediately mixed with the hydrogel solution for 3D static suspension cultures. Both our standard 3D cell culture protocol and the 3D static suspension culture protocols can be used for rapid cell expansion. The high-quality 3D spheroids enhance the cell performance for protein expression and downstream applications. HEK293 cells cultured in VitroGel HEK293 can easily be scale-up for large-scale bioreactors or spin flasks. By using the VitroGel Cell Recovery Solution, the cell harvesting after 3D culture is simple and efficient.



## 3D static suspension culture of HEK293 cells with high cell density seeding (24 hours).

For protein production purpose, cell suspension can be prepared at high cell density (107 cells/mL) to reach the peak of protein expression within 48 to 72 hours. The images show the fast formation of cell spheroids with over 100 μm size in diameter at 24 hours after cell seeded in VitroGel HEK293 hydrogel for static suspension culture.

## Why VitroGel STEM?

**XENO-FREE:** Without undesired proteins for pre-clinical/clinical applications

**HIGH PERFORMANCE:** Yields high-quality 3D cell spheroids for high yield protein production

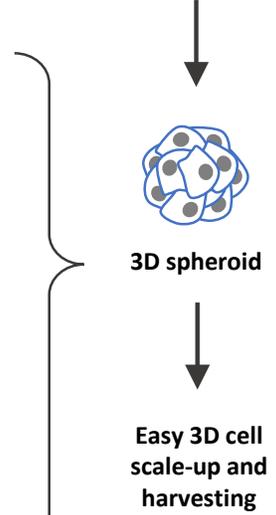
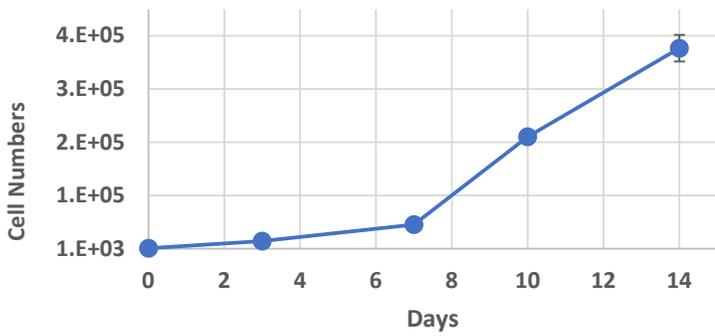
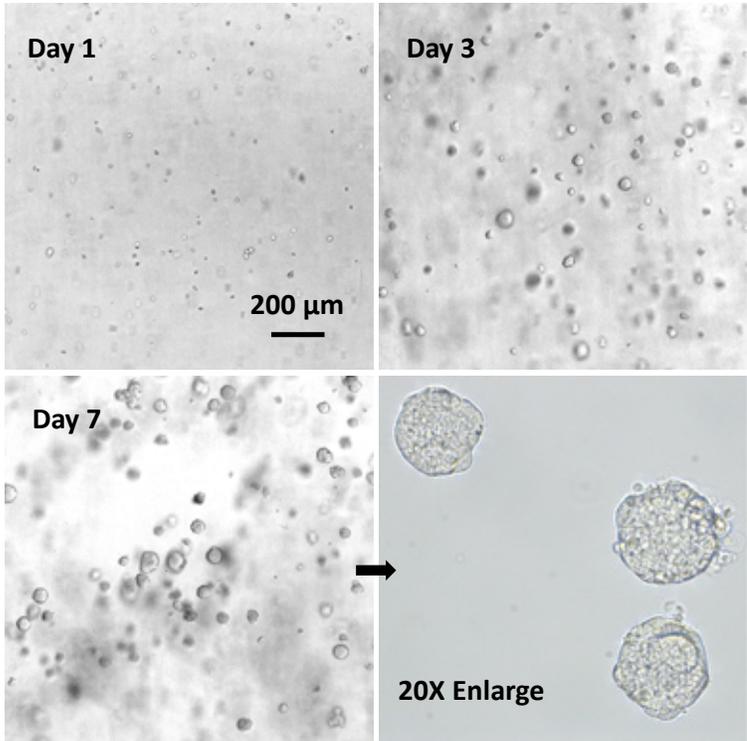
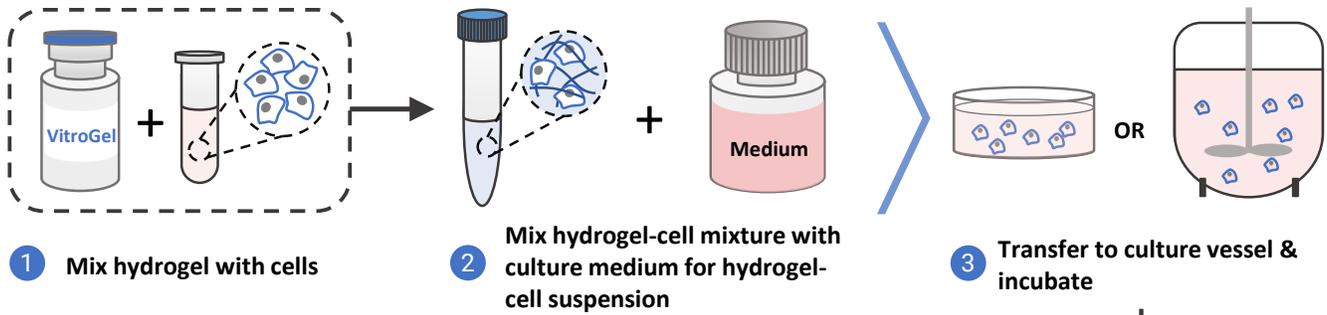
**FLEXIBLE & EASY SCALE-UP:** Compatible with most culture vessels and cell culture media; Easy scale-up for a spin flask or a bioreactor.

### EASY-TO-USE & EASY CELL RECOVERY:

Seed cells directly from liquid nitrogen (LN<sub>2</sub>) or 2D culture systems and efficient cell harvesting without using enzyme solution

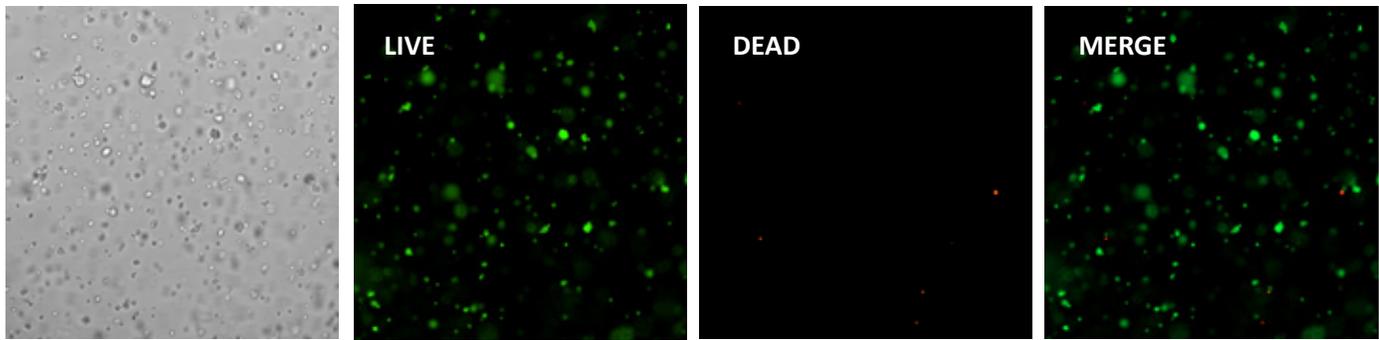
| Features                | Specifications   |
|-------------------------|--|
| <b>Formulation</b>      | Xeno-free, functional hydrogel   |
| <b>Use</b>              | 3D cell culture, 2D hydrogel coating, hydrogel-cell bead formation       |
| <b>Operation</b>        | Ready-to-use at room temperature   |
| <b>Biocompatibility</b> | Biocompatible, safe for animal studies                                   |
| <b>Injection</b>        | Injectable hydrogel for <i>in vivo</i> studies and lab automation        |
| <b>Cell Harvesting</b>  | Easy cell recovery using <a href="#">VitroGel Cell Recovery Solution</a> |
| <b>pH</b>               | Neutral  |
| <b>Storage</b>          | Store at 2-8°C. Ships at ambient temperature                             |
| <b>Sizes</b>            | 10 mL and 2 mL   |
| <b>Number of Uses</b>   | (10 mL) 300 uses & (2 mL) 60 uses  |

| Product         | Size  | SKU #  |
|-----------------|-------|--------|
| VitroGel HEK293 | 10 mL | VHM05  |
|                 | 2 mL  | VHM05S |



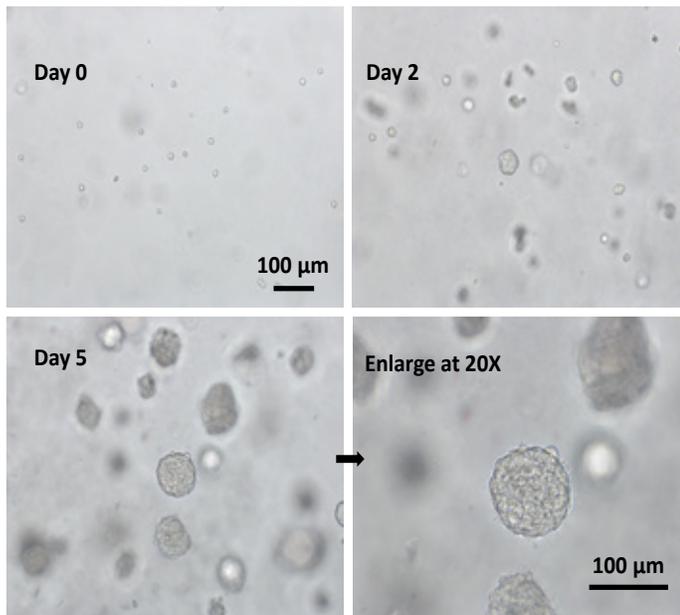
**3D static suspension culture of HEK293 cells in VitroGel HEK293**

Cells were mixed with VitroGel HEK293 at (2:1 gel/cells v/v ratio) and then mixed with cell culture medium at 1:3 ratio (cell-gel mixture: culture medium at 1:3 v/v). HEK293 cells form cell spheroids in the hydrogel. The images show the growth and expansion from day 0 to day 7. The enlarged images show the cell spheroids after 7 days of culture. The cell proliferation was tested by CellCounting Kit-8 (CCK-8) assay. The curve shows the increase of cell number in 14 days culture.



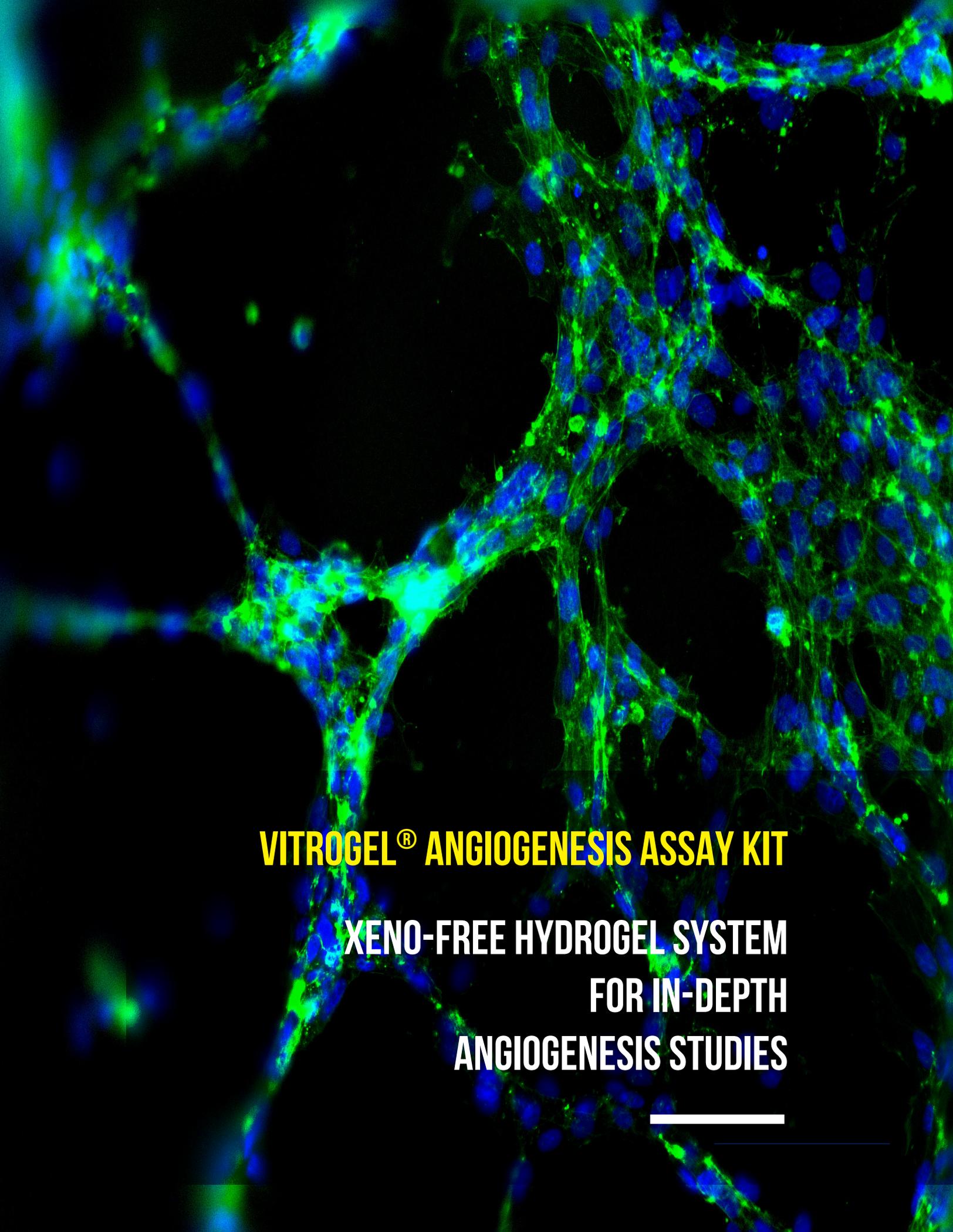
#### Cell viability of HEK293 cells in 3D suspension culture in VibroGel HEK293.

3D static suspension cultures of HEK293 cells in VibroGel HEK293 were stained with Cyto3D™ Live/Dead Assay Kit (Cat# BM01). The live cells are shown in green color and dead cells are shown in orange color. The images show cells cultured in VibroGel HEK293 hydrogel perform with high cell viability.



#### Subculture of HEK293 cell spheroids with VibroGel.

The cell spheroid culture with VibroGel can be collected by centrifuging (100 x g, 3 min). (Refer to cell harvesting protocol of VibroGel Cell Recovery Solution, CAT# MS03-100, for details). The collected cell spheroid can be dissociated into single cells by trypsin. After that, the cells can be resuspended with culture medium and mixed with VibroGel HEK293 for subculture. The images show the formation of cell spheroids from day 0 to day 5 after subculture.

A fluorescence microscopy image showing a complex, interconnected network of cells within a hydrogel. The cells are stained with a green fluorescent marker, likely highlighting the cytoskeleton or specific cell components, and a blue fluorescent marker, likely DAPI, which stains the nuclei. The network is dense and multi-layered, with many small gaps and channels. The background is dark, making the glowing cells stand out.

**VITROGEL<sup>®</sup> ANGIOGENESIS ASSAY KIT**

**XENO-FREE HYDROGEL SYSTEM  
FOR IN-DEPTH  
ANGIOGENESIS STUDIES**

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# VITROGEL® ANGIOGENESIS ASSAY KIT

Xeno-free hydrogel system for 2D gel coating and 3D culture of angiogenesis tube formation, invasion, and animal injection.

VitroGel Angiogenesis Assay Kit is a revolutionary tool for researchers to study the effect of both hydrogel properties and culture medium on angiogenesis process. The kit can be used to study the angiogenesis tube formation and invasion on both 2D hydrogel coating method and 3D cell culture method. The VitroGel system is also good for animal injection for *in vivo* study.



There are two versions of VitroGel Angiogenesis Assay Kits:

- 1) VitroGel Angiogenesis Assay Kit (Ready-To-Use): Assay kit with fixed hydrogel mechanical strength to support the angiogenesis assay with adjustable supplements.
- 2) VitroGel Angiogenesis Assay HC Kit (Cat No. TWG011-K): Assay kit with a tunable high concentration hydrogel to allow full control of the hydrogel's mechanical strength with adjustable supplements.

The ready-to-use VitroGel Angiogenesis Assay Kit contains:

- **VitroGel AAK**, a xeno-free ready-to-use hydrogel.
- **AAK Supplement 1**, a hydrogel growth supplement without vascular endothelial growth factors (VEGFs) for cell attachment and growth.
- **AAK Supplement 2**, a hydrogel tube formation supplement with VEGFs as a positive control for tube formation.

The VitroGel AAK hydrogel is room temperature stable and can be directly mixed with each supplement at 2:1 (v/v) ratio for hydrogel formation. Researchers can adjust the hydrogel's molecular cues by adding the growth factors/inhibitors directly to the supplements before mixing with the VitroGel AAK hydrogel. Cells cultured in this system can be harvested easily with the VitroGel Cell Recovery Solution.

| PRODUCT                         | TYPE/SIZE | SKU #    |
|---------------------------------|-----------|----------|
| VitroGel Angiogenesis Assay Kit | TYPE 1    | VHM06-K1 |
|                                 | TYPE 2    | VHM06-K2 |
|                                 | TYPE 3    | VHM06-K3 |
| VitroGel AAK Hydrogel           | 2 mL      | VHM06S   |
| AAK Supplement 1                | 500 µL    | AAKS-1   |
| AAK Supplement 2                | 500 µL    | AAKS-2   |

## Why VitroGel Angiogenesis Assay Kit?

**Xeno-free functional hydrogel supporting angiogenesis process**

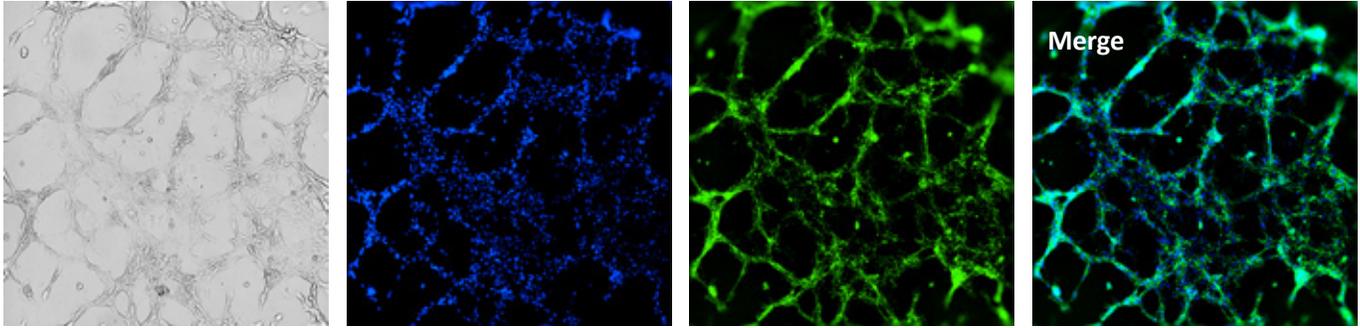
### MULTIPLE APPLICATIONS IN ONE KIT:

Tube formation, invasion, and animal injection

**CONTROL HYDROGEL PROPERTIES:** Add your own growth factors and compare with positive control

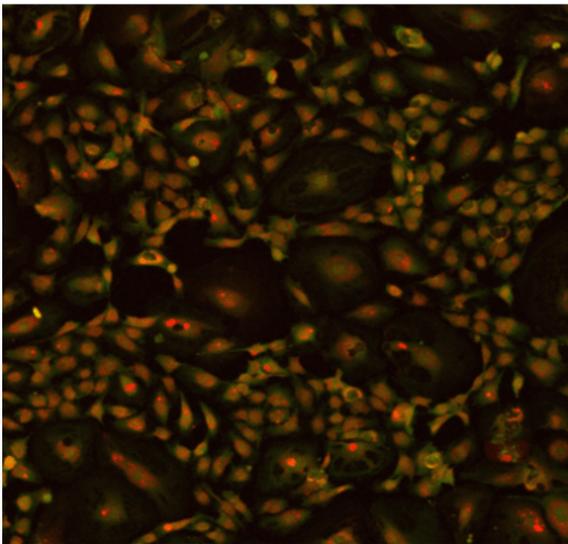
**EASY CELL HARVESTING:** Harvest cells from the hydrogel within 20 min at 37 °C without enzyme solution

| Features                   | Specifications   |
|----------------------------|--|
| <b>Type 1 Kit Contents</b> | VitroGel AAK (2 mL)<br>AAK Supplement 1 (1 x 500 µL)<br>AAK Supplement 2 (1 x 500 µL)                          |
| <b>Type 2 Kit Contents</b> | VitroGel AAK (2 mL)<br>AAK Supplement 1 (2 x 500 µL)   |
| <b>Type 3 Kit Contents</b> | VitroGel AAK (2 mL)<br>AAK Supplement 2 (2 x 500 µL)   |
| <b>Formulation</b>         | Ready-to-use, xeno-free functional hydrogel<br>AAK Supplement 1: Without VEGFs<br>AAK Supplement 2: With VEGFs |
| <b>Use</b>                 | Angiogenesis Assay, tube formation, invasion, animal injection   |
| <b>Biocompatibility</b>    | Biocompatible, safe for animal studies   |
| <b>Injection</b>           | Injectable hydrogel for <i>in vivo</i> studies and lab automation  |
| <b>Cell Harvesting</b>     | Easy cell recovery using <a href="#">VitroGel Cell Recovery Solution</a>                                       |
| <b>pH</b>                  | Neutral  |
| <b>Shipping</b>            | Supplements require dry ice shipment   |
| <b>Storage</b>             | VitroGel AAK hydrogel: 2-8°C<br>AAK Supplement 1: -20°C<br>AAK Supplement 2: -20°C                             |
| <b>Number of Uses</b>      | 60 tests per kit   |



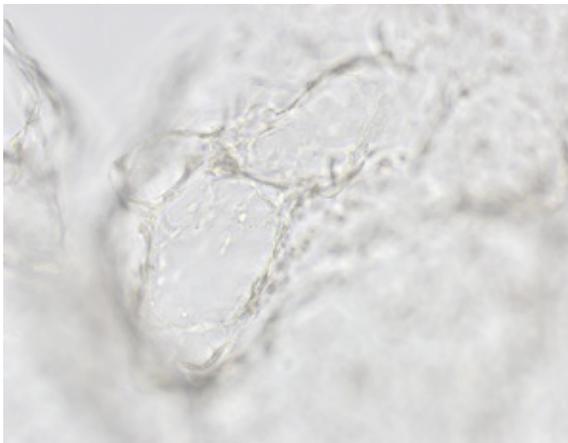
**Tube formation of endothelial cells on top of VitroGel AAK hydrogel with tube formation supplement, AAK Supplement 2.**

The image above shows the tube morphology of HUVEC cells on top of VitroGel AAK hydrogel. The cells were fixed and stained with DAPI (blue) and ActinGreen™ (green).



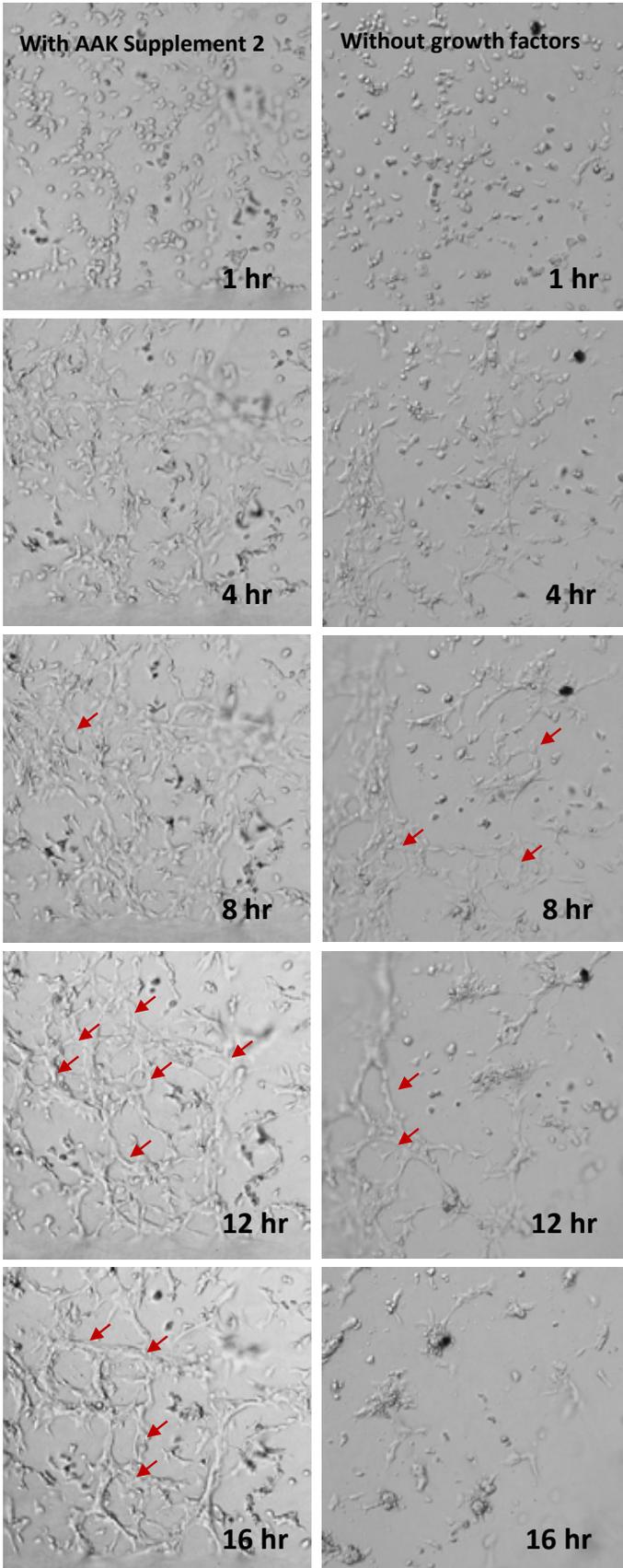
**HUVEC cell growth on top of VitroGel AAK hydrogel with cell growth supplement, AAK Supplement 1.**

The image show HUVEC cells attached and growing on the surface of VitroGel AAK hydrogel with cell growth supplement, AAK Supplement 1. The cells were fixed and stained with DRAQ5™ (red) and ActinGreen™ (green).



**3D tube structure of HUVEC cells cultured within VitroGel Angiogenesis Assay Kit.**

The image shows HUVEC cells mixed with VitroGel AAK hydrogel supplement with tube formation growth factors (AAK Supplement 2) and cultured for 7 days.



**Comparison of endothelial cells growth on top of VitroGel AAK hydrogel with and without growth factor supplement.**  
 The images on the left column show cells attached on the surface of the hydrogel and forming luminal structures after 8 hours, which further developed to a tube structures (red arrows). The images on the right column show cells attached on the surface of the hydrogel and form less tube structure (red arrows) compared to the hydrogel with full growth factor supplement.



**POWERFUL  
TUNABLE HYDROGELS  
FOR BUILDING  
COMPLEX ECMS**

# VITROGEL® HIGH CONCENTRATION HYDROGEL

## 3D CELL CULTURE YOUR WAY!

Building blocks for unlimited creativity and maximum flexibility

VitroGel High Concentration Hydrogels offer a set of building blocks to create a functional micro-environment that closely mimics the natural extracellular matrix (ECM) for cells to feel at home.

These high concentration formulations allow the maximum flexibility to manipulate the mechanical strength of hydrogel from 10 to 4000 Pa. The hydrogels come with several types of functional ligand modifications such as RGD (VitroGel RGD), collagen (VitroGel COL), laminin (VitroGel IKVAV & VitroGel YIGSR), and matrix metalloproteinase (VitroGel MMP). This system allows scientists to investigate the cell behaviors in response to an individual functional ligand or create multi-functional hydrogel combinations by blending different versions of VitroGel.



| Features          | Specifications   |
|-------------------|--|
| Operation         | Room temperature   |
| Hydrogel Strength | 10 to 4,000 Pa of G' depending on dilution ratio. Dilute with VitroGel Dilution Solution (TYPE 1 or TYPE 2) for different concentrations |
| Injection         | Injectable hydrogel  |
| Cell Harvesting   | 20 min cell recovery using <a href="#">VitroGel Cell Recovery Solution</a>   |
| pH                | Neutral  |
| Storage           | Store at 2-8°C. Ships at ambient temperature   |
| Number of Uses    | Dilution ratio:<br>1:2 = 225 uses at 50 µL per well<br>1:3 = 300 uses at 50 µL per well<br>1:5 = 450 uses at 50 µL per well              |

## EASY MANIPULATION OF HYDROGEL PROPERTIES FOR DIFFERENT APPLICATIONS



Mechanical Strength



Functional Ligands



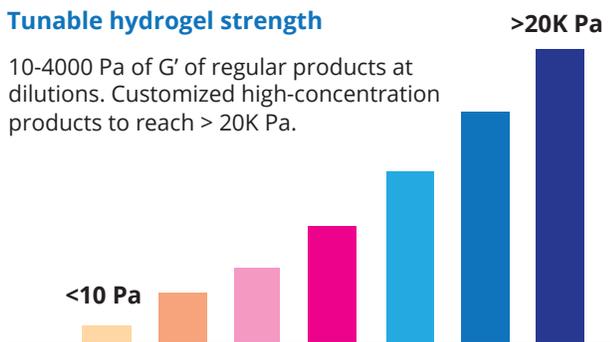
Degradation



Serum/Growth Factors/  
Cytokine/Chemokine

### Tunable hydrogel strength

10-4000 Pa of G' of regular products at dilutions. Customized high-concentration products to reach > 20K Pa.



Simply diluting the hydrogel controls the strength. The hydrogel strength above is Elastic Modulus (G') which is up to 60K Pa when converted to stiffness (Young's Modulus).



The VitroGel system can be "mixed and matched" with each other. Scientists can create tailor-made customized 3D culture micro-environment by blending different VitroGel system for a wide variety of functional ligands and degradability. This feature permits examination of cellular responses in a more physiologically relevant context while still giving experimenters full control.

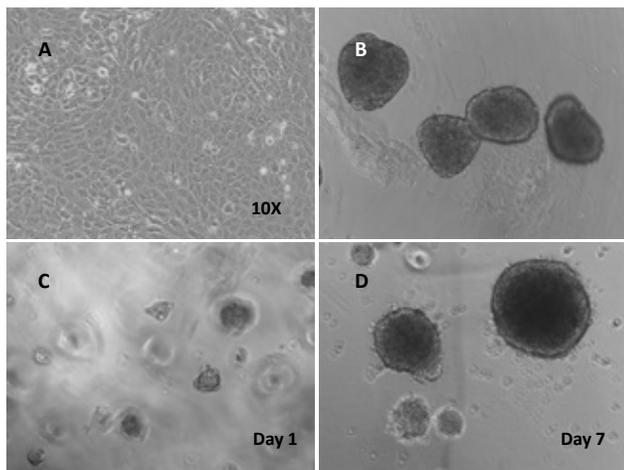
# VITROGEL® 3D HIGH CONCENTRATION

Tunable, xeno-free hydrogel matrix.

VitroGel® 3D High Concentration is a tunable, xeno-free (animal origin-free) hydrogel system. This pure hydrogel matrix allows the maximum flexibility to manipulate the 3D cell culture environment for different needs.



VitroGel 3D High Concentration comes with VitroGel Dilution Solution to adjust the final hydrogel strength from 10 to 4000 Pa (G'). The tunability of the hydrogel gives researchers the ability to create an optimized environment for cell growth. The VitroGel 3D High Concentration hydrogel matrix structure is good for cell spheroid formation, suspension cells, or cells requiring low cell-matrix interactions.



### Beta Lox 5 (BL5) cells 3D culture in VitroGel 3D system.

A. BL5 cells culture on the surface of regular tissue culture treated well plate (control); B. Normal human islets grew in suspension culture (comparison); C. 3D culture of BL5 cells in VitroGel 3D at Day 1; D. 3D culture of BL5 cells in VitroGel 3D at Day 7. Under 3D culture of VitroGel 3D, BL5 cells form islet-like structures very similar to normal human islets. The hydrogel is prepared at 1:3 dilution. The images were taken at 10X magnification.

## Why VitroGel 3D High Concentration?

### PURE XENO-FREE HYDROGEL MATRIX:

Provides excellent 3D matrix support for 3D cell spheroid formation

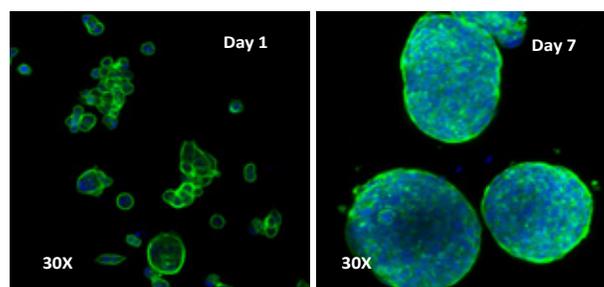
**TUNABLE HYDROGEL STRENGTH:** Covers a wide range of applications with great flexibility

**MIX & MATCH:** Adjust the functional ligand concentrations of other hydrogels without sacrificing the hydrogel strength

**EASY CELL HARVESTING:** Efficient cell harvesting with the enzyme-free VitroGel Cell Recovery Solution

| Features           | Specifications   |
|--------------------|--|
| <b>Contents</b>    | VitroGel 3D High Concentration, 3mL<br>VitroGel Dilution Solution, 50 mL                         |
| <b>Formulation</b> | Xeno-free tunable hydrogel, pure and unmodified.<br>HIGH CONCENTRATION                           |
| <b>Use</b>         | Good for cell spheroid formation, suspension cells or cells require low cell-matrix interactions |

| Product   | Size | SKU #  |
|---|------|--------|
| VitroGel 3D High Concentration Kit (Dilution Solution TYPE 1 or TYPE 2) | 3 mL | TWG001 |



### Human colon cancer cells (HCT 116) cells cultured on top of VitroGel 3D hydrogel.

Cells were added on top of a thick hydrogel coating plate. Cell spheroids formed within 24 hours and continued to increase in size out to 7 days.

# VITROGEL® RGD HIGH CONCENTRATION

RGD Modified. Tunable, xeno-free hydrogel matrix.

VitroGel® RGD High Concentration is a tunable, xeno-free (animal origin-free) hydrogel system modified with cell adhesive peptide RGD to promote cell attachment and cell-matrix interactions during the 3D cell culture. VitroGel RGD High Concentration comes with VitroGel Dilution Solution to adjust the final hydrogel strength from 10 to 4000 Pa (G').



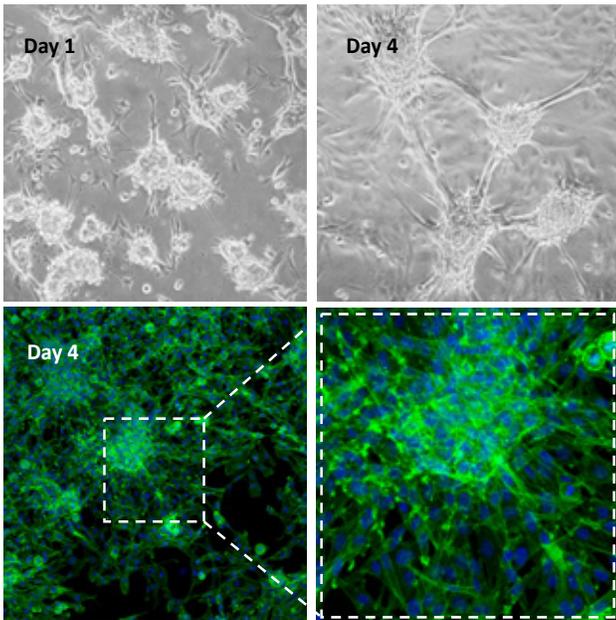
## Why VitroGel RGD High Concentration?

**POPULAR FOR MANY APPLICATIONS:** A widely used RGD modified hydrogel that is versatile for many different uses

**ROBUST AND FLEXIBLE:** Excellent cell-matrix interaction in all different ranges of hydrogel strengths

**MIX & MATCH:** Create a multiple-functional hydrogel by mixing with other versions of VitroGel

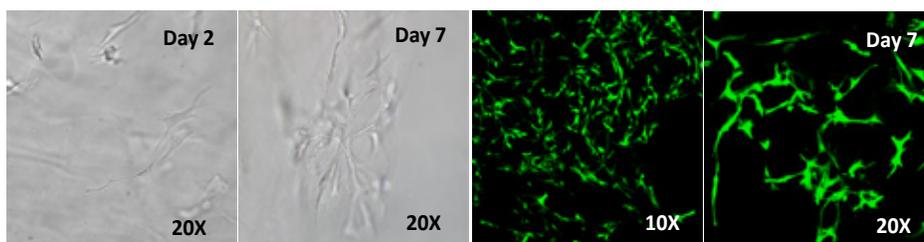
**EASY CELL HARVESTING:** Efficient cell harvesting with the enzyme-free VitroGel Cell Recovery Solution



**Glioblastoma cells (SNB 75) cultured on top of VitroGel RGD**  
The hydrogel coating surface was prepared by mixing VitroGel RGD with cell culture medium at 4:1 (v/v) ratio. The gelation process was stable at room temperature for 20 min before adding cells on top. Cells attached and formed the colony structure on the surface of the hydrogel (day 1). The attached cells expanded and connected colonies for a cell-networking structure (day 4).

| Features               | Specifications   |
|------------------------|--|
| <b>Contents</b>        | VitroGel RGD High Concentration, 3mL<br>VitroGel Dilution Solution, 50 mL            |
| <b>Formulation</b>     | Xeno-free tunable hydrogel modified with RGD peptide HIGH CONCENTRATION              |
| <b>Use</b>             | Good for adhesion cells or cells requiring stronger cell-matrix interactions.        |
| <b>Mix &amp; Match</b> | Can be blended with other versions of VitroGel to create a multi-functional hydrogel |

| Product  | Size | SKU #  |
|--|------|--------|
| VitroGel RGD High Concentration Kit (Dilution Solution TYPE 1 or TYPE 2) | 3 mL | TWG003 |



### 3D culture of Mouse bone marrow cells (OP9) in VitroGel RGD

Hydrogel was prepared at 1:3 dilution with VitroGel Dilution Solution (Type 1). VitroGel RGD shows support for OP9 cell proliferation and cell-cell communication. The stronger cell-matrix interactions help the cells to form the cell-networking structure.

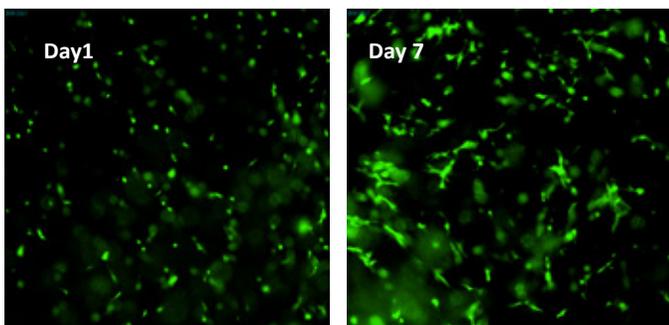
# VITROGEL® COL HIGH CONCENTRATION

Collagen-mimetic peptide modified. Tunable, xeno-free hydrogel matrix.

VitroGel® COL High Concentration is a tunable, xeno-free hydrogel system that mimics the functions of native collagen. The hydrogel can promote osteoblastic differentiation in vitro and enhancing osteoblastic activity *in vivo*, which shows great potential for tissue engineering and regeneration medicine applications.



VitroGel COL High Concentration comes with VitroGel Dilution Solution to adjust the final hydrogel strength from 10 to 4000 Pa (G').



### 3D culture of mouse bone marrow stromal cells (OP9) in the mixture of VitroGel COL.

Cells were cultured with 1:3 diluted VitroGel COL. The single cells were suspended in hydrogel matrix (Day 1) and extended to form fibroblast-like cell matrix structure (Day 7).

## Why VitroGel COL High Concentration?

**ROOM TEMPERATURE STABLE HYDROGEL THAT CAN MIMIC FUNCTIONS OF COLLAGEN**

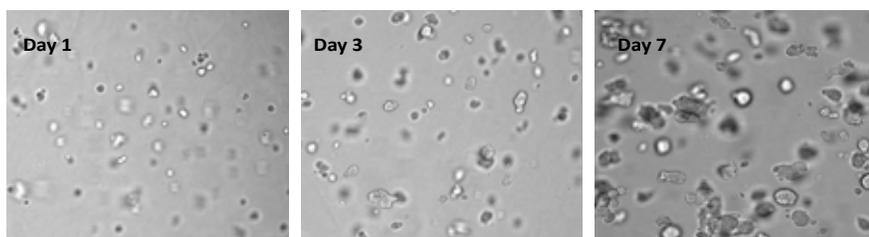
**TUNABLE HYDROGEL STRENGTH:** Cover a wide range of applications with great flexibility

**MIX & MATCH:** Create a multi-functional hydrogel by mixing with other versions of VitroGel

**EASY CELL HARVESTING:** Efficient cell harvesting with the enzyme-free VitroGel Cell Recovery Solution

| Features               | Specifications   |
|------------------------|--|
| <b>Contents</b>        | VitroGel COL High Concentration, 3mL<br>VitroGel Dilution Solution, 50 mL  |
| <b>Formulation</b>     | Xeno-free tunable collagen mimetic functional hydrogel. HIGH CONCENTRATION   |
| <b>Use</b>             | Support integrin binding to promote osteoblastic differentiation in vitro and enhancing osteoblastic activity <i>in vivo</i> |
| <b>Mix &amp; Match</b> | Can be blended with other versions of VitroGel to create a multi-functional hydrogel   |

| Product  | Size | SKU #  |
|--|------|--------|
| VitroGel COL High Concentration Kit (Dilution Solution TYPE 1 or TYPE 2) | 3 mL | TWG009 |



### 3D culture of glioblastoma cells (SNB-75) in VitroGel COL.

Cells were cultured with 1:5 diluted VitroGel COL. Single cells were suspended in the hydrogel matrix on Day 1 and grew into 3D colonies within 7 days.

# VITROGEL® MMP HIGH CONCENTRATION

Matrix metalloproteinases (MMP) sensitive biodegradable hydrogel.  
Tunable, xeno-free hydrogel matrix.

VitroGel® MMP High Concentration is a tunable, xeno-free (animal origin-free) hydrogel system modified with matrix metalloproteinase-sensitive peptides. The enzyme sensitive hydrogel can be degraded by several MMPs (MMP1, MMP2, MMP3, MMP9 and MMP13) and can support different biological activities such as cell proliferation, migration (adhesion/dispersion), differentiation, angiogenesis, apoptosis, etc.



VitroGel MMP High Concentration comes with VitroGel Dilution Solution to adjust the final hydrogel strength from 10 to 4000 Pa.

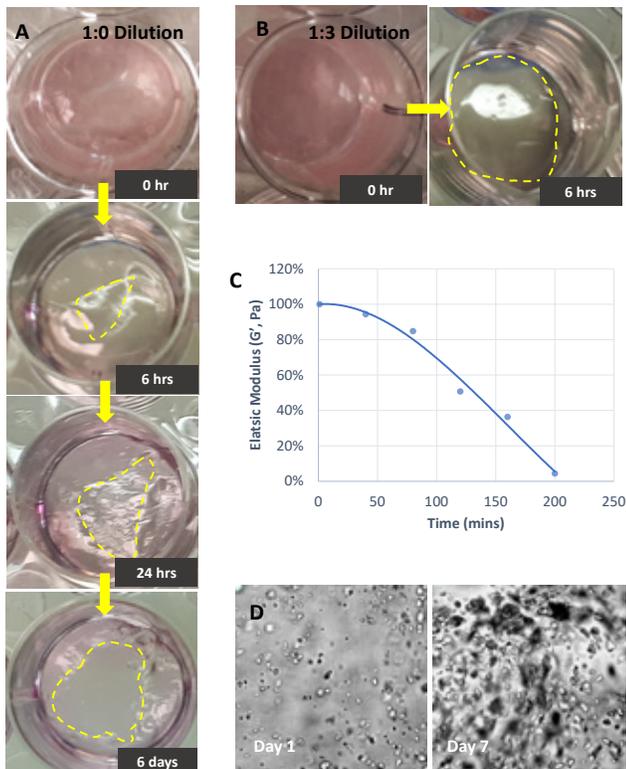
## Why VitroGel MMP High Concentration?

**RAPID DEGRADATION:** Enzyme sensitive biodegradable hydrogel

**TUNABLE HYDROGEL STRENGTH:** Cover a wide range of applications with great flexibility

**MIX & MATCH FOR ADJUSTABLE DEGRADATION:** Create a biodegradable hydrogel by mixing with other versions of VitroGel

**EASY CELL HARVESTING:** Efficient cell harvesting with the enzyme-free VitroGel Cell Recovery Solution



| Features               | Specifications  |
|------------------------|---|
| <b>Contents</b>        | VitroGel MMP High Concentration, 3mL<br>VitroGel Dilution Solution, 50 mL                                   |
| <b>Formulation</b>     | Xeno-free tunable hydrogel modified with matrix metalloproteinases sensitive peptide.<br>HIGH CONCENTRATION |
| <b>Use</b>             | MMP sensitive biodegradable hydrogel.   |
| <b>Mix &amp; Match</b> | Can be blended with other versions of VitroGel to create a multi-functional hydrogel                        |

| Product  | Size | SKU #  |
|--|------|--------|
| VitroGel MMP High Concentration Kit (Dilution Solution TYPE 1 or TYPE 2) | 3 mL | TWG010 |

### Degradation of VitroGel MMP gel with MMP2 enzyme.

A) Hydrogel was prepared without dilution (1:0 dilution) and incubated for 24 hours before adding MMP2 (1  $\mu\text{g}/\text{mL}$ ) on the top of the hydrogel. The plate was incubated in a 5%  $\text{CO}_2$  incubator at 37°C and was shaken using an orbital shaker at 40 rpm. The hydrogel obtains a heterogenous consistency after 24 hours and by day 6, the entire middle section appears degraded. B) The 1:3 diluted hydrogel was prepared with VitroGel Dilution Solution and incubated as 1:0 diluted hydrogel. The hydrogel appears to have completely dissolved after 6 hours after exposed to MMP2. C) The rheological data shows a rapid decline in the elastic modulus of the 1:3 diluted hydrogel over time after adding MMP2. D) 3D culture of glioblastoma cells (SNB 75) in VitroGel MMP for day 1 and day 7.

# VITROGEL® YIGSR HIGH CONCENTRATION

Laminin-derived functional peptide (YIGSR) modified. Tunable, xeno-free hydrogel matrix.

VitroGel® YIGSR High Concentration is a tunable, xeno-free (animal origin-free) hydrogel system modified with laminin-derived functional peptide (YIGSR). YIGSR sequence resides on the laminin  $\beta$ 1 chain, which involves endothelial cell adhesion, cell proliferation and motility/migration.



VitroGel YIGSR High Concentration comes with VitroGel Dilution Solution to adjust the final hydrogel strength from 10 to 4000 Pa.

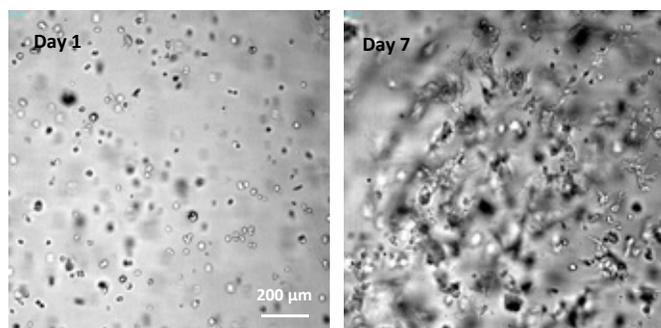
## Why VitroGel YIGSR High Concentration?

**LAMININ FUNCTIONAL HYDROGEL**

**TUNABLE HYDROGEL STRENGTH:** Cover a wide range of applications with great flexibility

**MIX & MATCH:** Create a multi-functional hydrogel by mixing with other versions of VitroGel

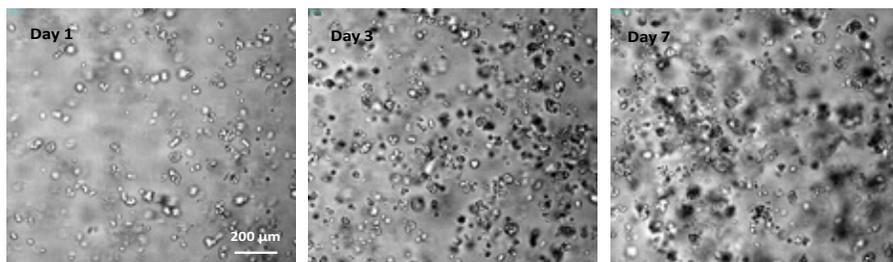
**EASY CELL HARVESTING:** Efficient cell harvesting with the enzyme-free VitroGel Cell Recovery Solution



**3D culture of glioblastoma cells (U-87 MG) in VitroGel YIGSR.** Cells were cultured with 1:3 diluted VitroGel YIGSR according to the user handbook (50% FBS was used to prepare cell suspension to achieve hydrogel with a final 10% FBS concentration).

| Features               | Specifications   |
|------------------------|--|
| <b>Contents</b>        | VitroGel YIGSR High Concentration, 3mL<br>VitroGel Dilution Solution, 50 mL          |
| <b>Formulation</b>     | Xeno-free tunable hydrogel modified with YIGSR peptide.<br>HIGH CONCENTRATION        |
| <b>Use</b>             | Support endothelial cell adhesion, cell proliferation and motility/migration         |
| <b>Mix &amp; Match</b> | Can be blended with other versions of VitroGel to create a multi-functional hydrogel |

| Product  | Size | SKU #  |
|--|------|--------|
| VitroGel YIGSR High Concentration Kit (Dilution Solution TYPE 1 or TYPE 2) | 3 mL | TWG008 |



**3D culture of breast cancer cells (MCF-7) in VitroGel YIGSR** Cells were cultured with 1:3 diluted VitroGel YIGSR according to the user handbook (50% FBS was used to prepare cell suspension to achieve hydrogel with a final 10% FBS concentration).

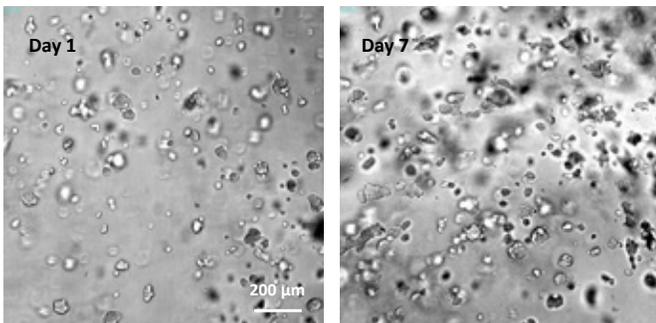
# VITROGEL® IKVAV HIGH CONCENTRATION

Laminin-derived functional peptide (IKVAV) modified.  
Tunable, xeno-free hydrogel matrix.

VitroGel® IKVAV High Concentration is a tunable, xeno-free (animal origin-free) hydrogel system modified with laminin-derived functional peptide (IKVAV). IKVAV is the bioactive sequence located on the C-terminal end of the long arm of the  $\alpha$ -1 chain of laminin, which is actively involved in different biological activities such as neuronal progenitor cell differentiation, promoting cell adhesion, neurite outgrowth, angiogenesis, and tumor growth.



VitroGel IKVAV High Concentration comes with VitroGel Dilution Solution to adjust the final hydrogel strength from 10 to 4000 Pa.



**3D culture of glioblastoma cells (SNB 75) in VitroGel IKVAV.** Cells were cultured with 1:3 diluted VitroGel IKVAV according to the user handbook (50% FBS was used to prepare cell suspension to achieve hydrogel with a final 10% FBS concentration).

## Why VitroGel IKVAV High Concentration?

### LAMININ FUNCTIONAL HYDROGEL

**TUNABLE HYDROGEL STRENGTH:** Cover a wide range of applications with great flexibility

**MIX & MATCH:** Create a multi-functional hydrogel by mixing with other versions of VitroGel

**EASY CELL HARVESTING:** Efficient cell harvesting with the enzyme-free VitroGel Cell Recovery Solution

| Features               | Specifications  |
|------------------------|---|
| <b>Contents</b>        | VitroGel IKVAV High Concentration, 3mL<br>VitroGel Dilution Solution, 50 mL   |
| <b>Formulation</b>     | Xeno-free tunable hydrogel modified with IKVAV peptide.<br>HIGH CONCENTRATION   |
| <b>Use</b>             | Support neuronal progenitor cells differentiation, promoting cell adhesion, neurite outgrowth, angiogenesis, and tumor growth |
| <b>Mix &amp; Match</b> | Can be blended with other versions of VitroGel to create a multi-functional hydrogel  |

| Product  | Size | SKU #  |
|--|------|--------|
| VitroGel IKVAV High Concentration Kit (Dilution Solution TYPE 1 or TYPE 2) | 3 mL | TWG007 |

## VITROGEL® ANGIOGENESIS ASSAY HC KIT

Xeno-free, tunable hydrogel system for 2D gel coating and 3D culture of angiogenesis tube formation, invasion, and animal injection.

VitroGel Angiogenesis Assay Kit is a revolutionary tool for researchers to study the effect of both hydrogel properties and culture medium on angiogenesis process. The kit can be used to study the angiogenesis tube formation and invasion on both 2D hydrogel coating and 3D cell culture methods. The VitroGel system is also good for animal injection for *in vivo* studies.

There are two versions of VitroGel Angiogenesis Assay Kits:  
 1) VitroGel Angiogenesis Assay Kit (Ready-To-Use): Assay kit with a fixed hydrogel mechanical strength to support the angiogenesis assay with adjustable supplements.  
 2) VitroGel Angiogenesis Assay HC Kit (Cat No. TWG011-K): Assay kit with a tunable high concentration hydrogel to allow full control of the hydrogel's mechanical strength with adjustable supplements.

Besides molecular cues, the VitroGel Angiogenesis Assay Kit allows researchers to explore the effects of hydrogel mechanical properties on angiogenesis. The high concentration VitroGel AAK-HC hydrogel is room temperature stable and can be adjusted by simply mixing the hydrogel solution and dilution solution at different ratios (recommend 1:1 to 1:5 v/v) to achieve different mechanical strengths. The diluted hydrogel solution can be directly mixed with supplement at 2:1 (v/v) ratio for hydrogel formation. Researchers can adjust the molecular cues of the hydrogel by adding the growth factors/inhibitors directly to the supplement before mixing with VitroGel AAK-HC. Cells cultured in this system can be harvested easily with the VitroGel Cell Recovery Solution.

| PRODUCT   | TYPE/SIZE | SKU #     |
|---|-----------|-----------|
| VitroGel Angiogenesis Assay HC Kit                              | TYPE 1    | TWG011-K1 |
|   | TYPE 2    | TWG011-K2 |
|   | TYPE 3    | TWG011-K3 |
| VitroGel AAK-HC Hydrogel (includes AAK Dilution Solution 10 mL) | 1 mL      | TWG011S   |
| AAK Supplement 1  | 500 µL    | AAKS-1    |
| AAK Supplement 2  | 500 µL    | AAKS-2    |

### Why VitroGel Angiogenesis Assay HC Kit?

**XENO-FREE FUNCTIONAL HYDROGEL SUPPORTING ANGIOGENESIS**

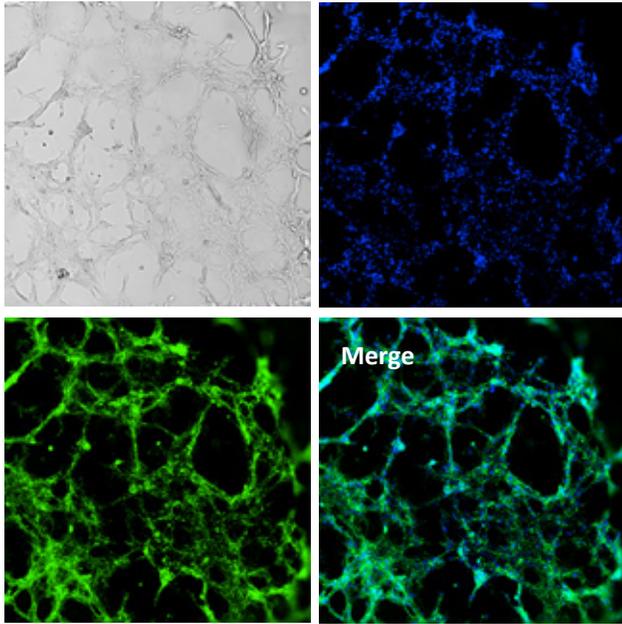
**CONTROL AND STUDY THE EFFECT OF THE HYDROGEL MECHANICAL PROPERTIES ON ANGIOGENESIS**

**MULTIPLE APPLICATIONS IN ONE KIT:**

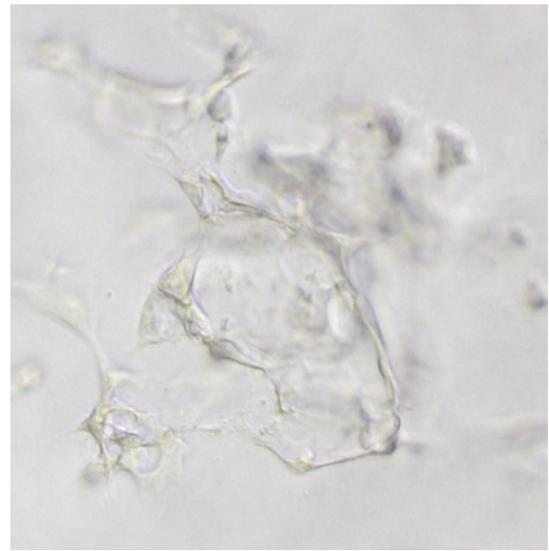
Tube formation, invasion, and animal injection

**EASY CELL HARVESTING:** Efficient cell harvesting with the enzyme-free VitroGel Cell Recovery Solution

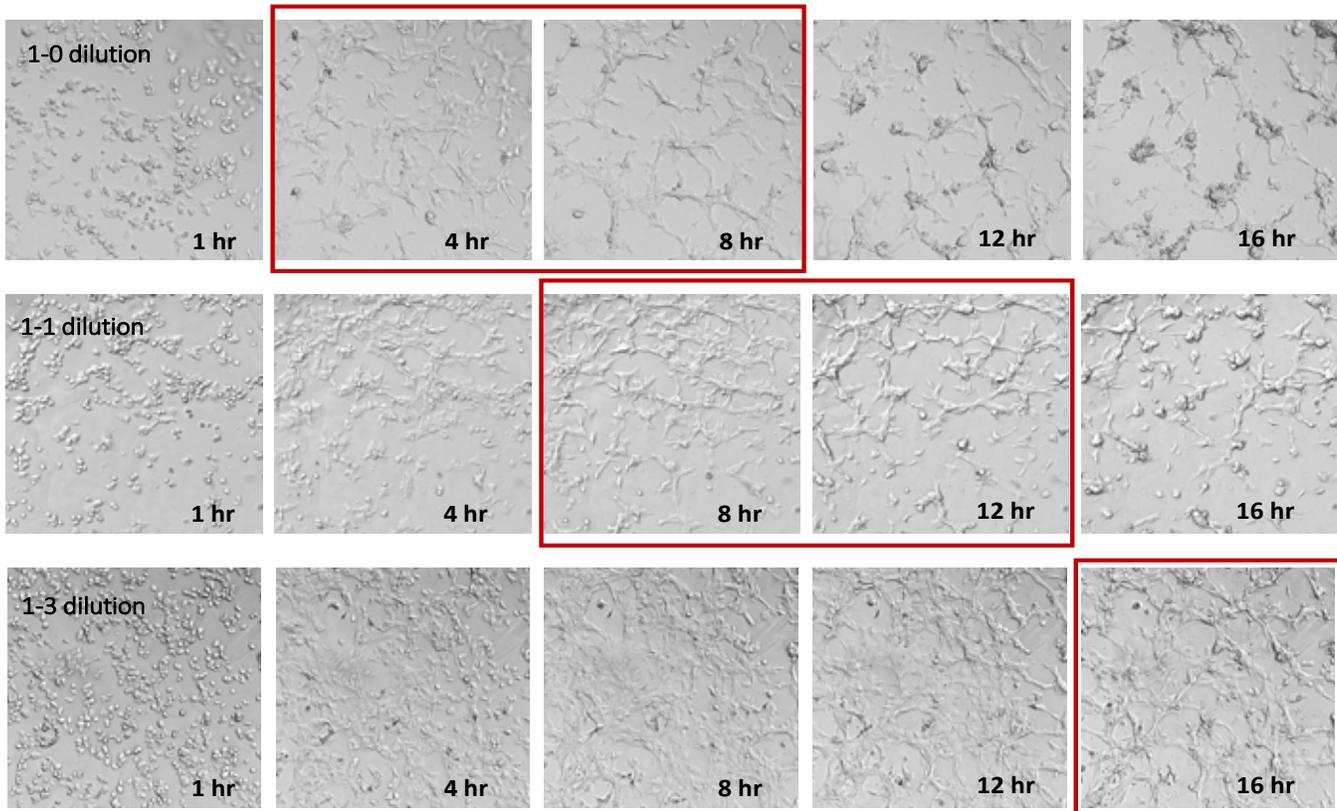
| Features                   | Specifications  |
|----------------------------|---|
| <b>TYPE 1 Kit Contents</b> | VitroGel AAK-HC (1 mL)<br>AAK Dilution Solution (10 mL)<br>AAK Supplement 1 (3 x 500 µL)<br>AAK Supplement 2 (3 x 500 µL) |
| <b>TYPE 2 Kit Contents</b> | VitroGel AAK-HC (1 mL)<br>AAK Dilution Solution (10 mL)<br>AAK Supplement 1 (6 x 500 µL)                                  |
| <b>TYPE 3 Kit Contents</b> | VitroGel AAK-HC (1 mL)<br>AAK Dilution Solution (10 mL)<br>AAK Supplement 2 (6 x 500 µL)                                  |
| <b>Formulation</b>         | Tunable, xeno-free functional hydrogel<br>AAK Supplement 1: Without VEGFs<br>AAK Supplement 2: With VEGFs                 |
| <b>Use</b>                 | Angiogenesis Assay, tube formation, invasion, animal injection  |
| <b>Biocompatibility</b>    | Biocompatible, safe for animal studies  |
| <b>Injection</b>           | Injectable hydrogel for <i>in vivo</i> studies and lab automation   |
| <b>Cell Harvesting</b>     | 20 min cell harvesting using <a href="#">VitroGel Cell Recovery Solution</a>  |
| <b>pH</b>                  | Neutral   |
| <b>Shipping</b>            | Supplements require dry ice shipment  |
| <b>Storage</b>             | VitroGel AAK-HC hydrogel: 2-8°C<br>AAK Supplement 1: -20°C<br>AAK Supplement 2: -20°C                                     |
| <b>Number of Uses</b>      | 30-180 tests per kit  |



**Tube formation of endothelial cells on top of VitroGel AAK-HC hydrogel with tube formation supplement, AAK Supplement 2**  
 The image above shows the tube morphology of HUVEC cells on top of VitroGel AAK-HC hydrogel. The cells were fixed and stained with DAPI (blue) and ActinGreen™ (green).

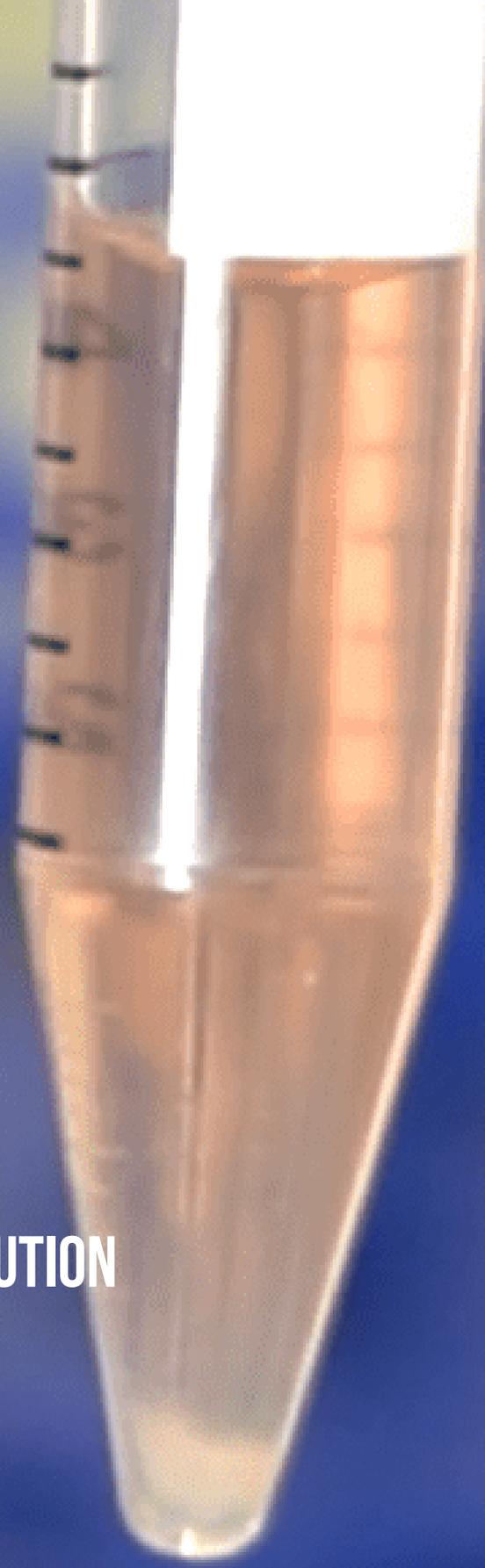


**3D tube structure of HUVEC cells cultured with the VitroGel Angiogenesis Assay HC Kit**  
 The concentration of VitroGel AAK-HC hydrogel was adjusted by diluting with the AAK-HC Dilution Solution. HUVEC cells were prepared in hydrogel supplement before mixing with diluted hydrogel solution for 3D culture. The image above shows the tube networking structure inside of VitroGel AAK-HC hydrogel.



**Effects of hydrogel mechanical strength on HUVEC tube formation.**

Different concentrations of VitroGel AAK-HC hydrogels were prepared by diluting the high concentration VitroGel AAK-HC with AAK-HC Dilution Solution at 1:0, 1:1, and 1:3 (v/v) ratios. The hydrogels were then mixed with AAK Supplement 2 (with VEGFs for tube formation) and incubated in the 96-well plate for 15 minutes. HUVECs cell were added on top of the hydrogel and observed under a live cell imaging system. The images indicate stronger hydrogel mechanical strength provided earlier cell tube structure formation (4-8 hours) than softer hydrogel (8-12 hours for 1:1 dilution and around 16 hours for 1:3 dilution). On the other hand, after tube formation, cells on the surface of 1:0 dilution hydrogel started to aggregate and form cell spheroids (around 12 hours). A similar aggregation happened to the cells on the surface of 1:1 diluted hydrogel (around 16 hours). For the soft hydrogel (1:3 dilution), the cells can attach well on the surface of the hydrogel and gradually form the tube structure within 18 hours. The results show the strong effect of the hydrogel mechanical strength on endothelial cell tube formation.



# VITROGEL<sup>®</sup> CELL RECOVERY SOLUTION

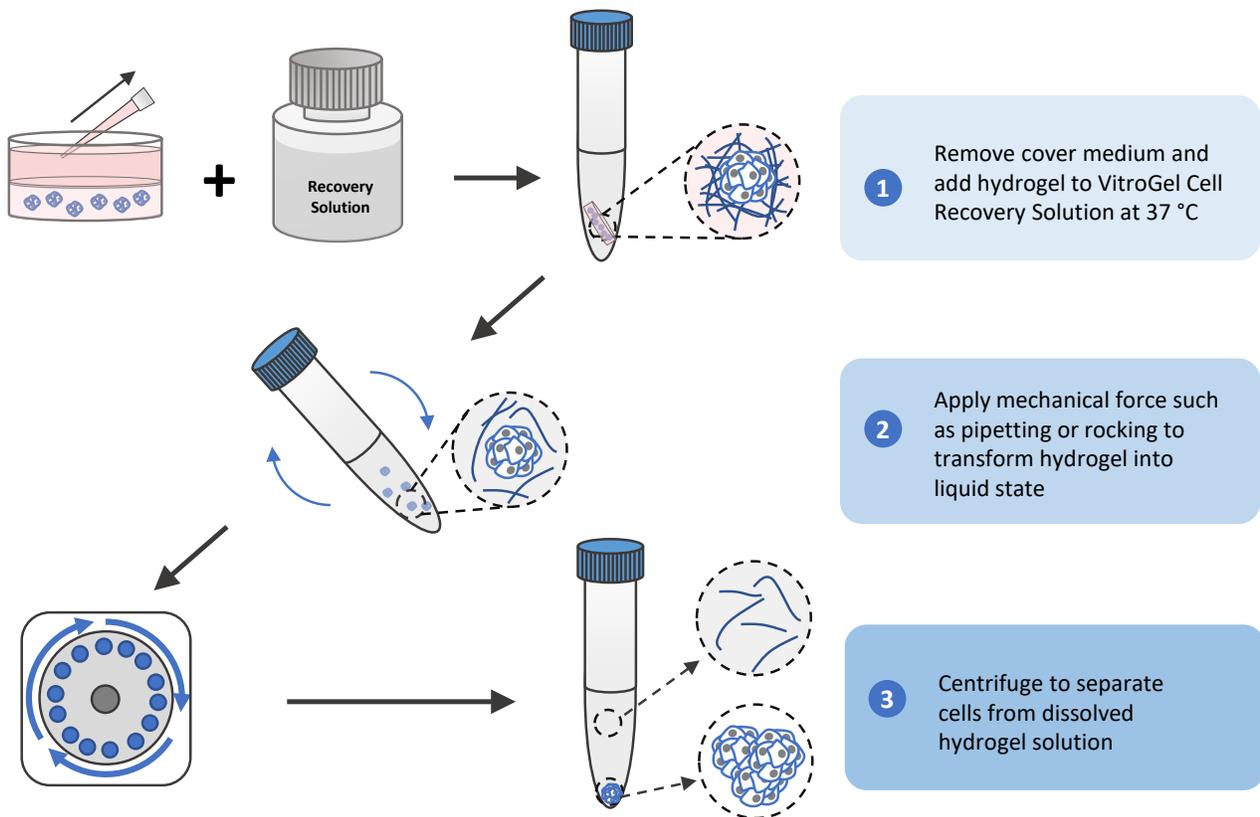
ENZYME-FREE FORMULATION  
FOR 3D CELL HARVESTING  
IN 20 MINUTES



# HOW DOES CELL HARVESTING WORKS IN VITROGEL?

Harvesting cells from most hydrogel matrices is not an easy task. Using harsh chemical solutions, strong enzymes, or changing the temperature may damage your cells. The yield rate of cell recovery is low with these methods. With the VitroGel system, harvesting cells from the hydrogel matrix is as easy as 3D cell culture. Using our enzyme-free VitroGel® Cell Recovery Solution, scientists can recover cells at neutral pH and 37°C for the operating temperature. The solution can maintain high cell viability during the recovery process. Cells can be sub-culture in both 2D and 3D cultures after recovery.

The VitroGel Cell Recovery Solution helps to release the ionic molecules from the hydrogel matrix, converting the solid hydrogel back to the soft hydrogel state. At this state, the hydrogel maintains the unique shear-thinning properties; it can further transform into a liquid state with a little mechanical disruption (such as rocking or shaking the tube) and dilution. Once the hydrogel dissolves as a liquid form, cells can be harvested by centrifuging.



# VITROGEL® CELL RECOVERY SOLUTION

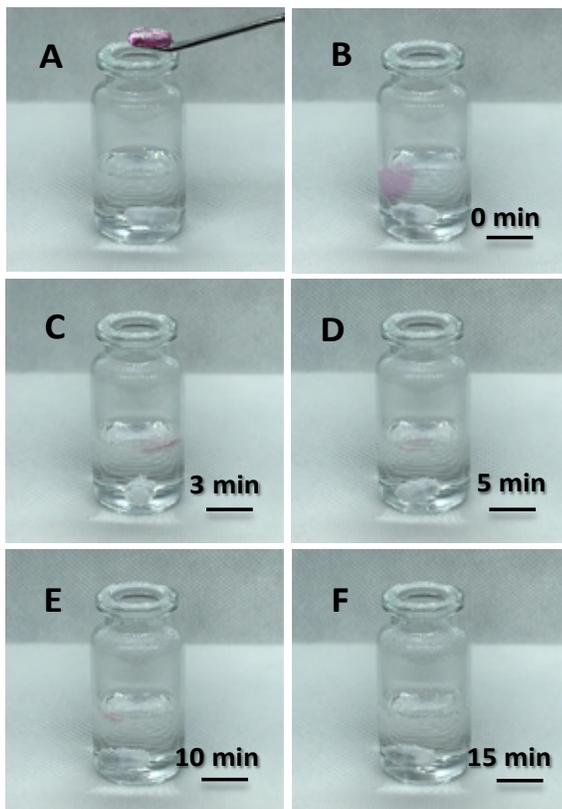
Enzyme-free formulation for recovering cells from hydrogel system in 20 minutes.

VitroGel® Cell Recovery Solution is an enzyme-free, ready-to-use solution to harvest 2D or 3D cultured cells from VitroGel hydrogel fast and safely. The solution can recover cells from VitroGel hydrogels in 20 minutes.



VitroGel Cell Recovery Solution is room temperature stable, has a neutral pH and works at 37°C operating temperature. The solution can maintain high cell viability during the recovery process. Harvested cells can be sub-culture in both 2D and 3D culture.

The VitroGel Cell Recovery Solution can be used before or after fixation/staining preparation for further high-quality downstream analysis.



### Hydrogel dissolved in cell recovery solution

A) hydrogel before adding to recovery solution, B-F) Time 0 to 15 min after adding hydrogel to recovery solution (at 37 °C, 20 rpm).

## Why VitroGel Cell Recovery Solution?

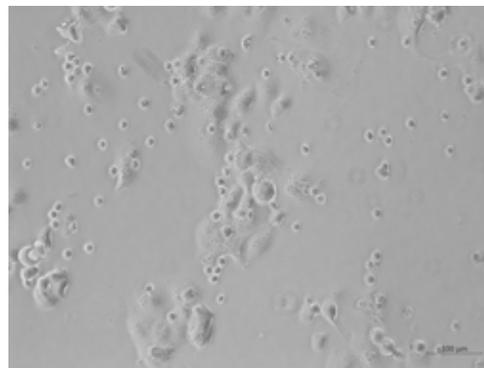
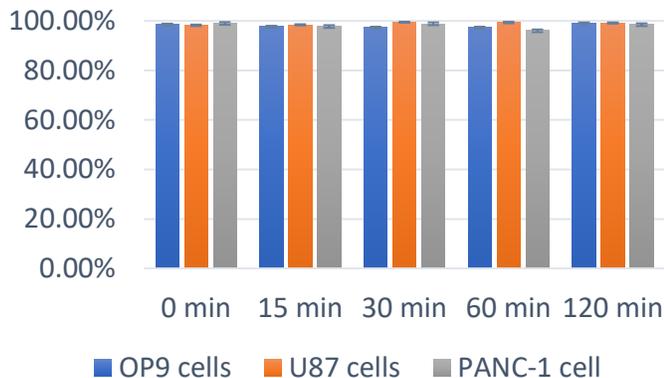
**ENZYME-FREE:** Maintain the 3D cell structure after cell harvesting

**WORKS AT 37 °C:** Work at physiological temperature to keep high cell viability

**20 MIN PROCESS:** Rapid cell harvesting process with simple mixing steps

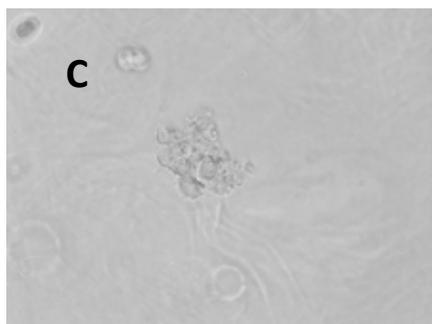
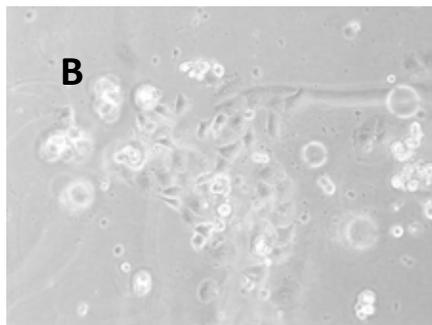
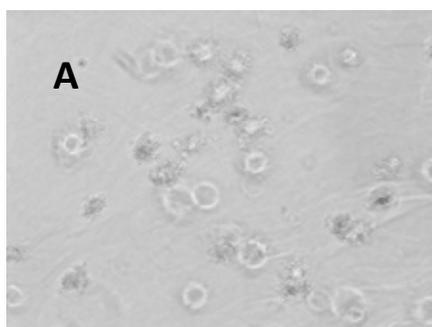
| Features               | Specifications   |
|------------------------|--|
| <b>Formulation</b>     | Enzyme-free  |
| <b>Use</b>             | Harvest cells from VitroGel hydrogel while maintaining high cell viability.<br>Use before or after sample fixation and stained preparation for imaging or downstream data analysis |
| <b>Processing Time</b> | 15-20 min  |
| <b>Downstream</b>      | Recovered cells can be further sub-culture for both 2D and 3D  |
| <b>pH</b>              | Neutral  |
| <b>Number of Uses</b>  | 30-180 tests per kit   |

| Product                         | Size   | SKU #    |
|---------------------------------|--------|----------|
| VitroGel Cell Recovery Solution | 100 mL | MS03-100 |



**The VitroGel Cell Recovery Solution maintains high cell viability**

Cell viability of OP9, U87-MG and PANC-1 cells after adding to the cell harvesting solution at time 0, 15, 30, 60 and 120 min. Cells maintain over 95% cell viability after being suspended in VitroGel Cell Recovery Solution for 2 hours. PANC-1 cells grown on 2D well plate before transfer to the cell harvesting solution.



**Cells can be sub-culture in both 2D and 3D culture after harvesting**

A) PANC-1 cells growth on 3D hydrogel before being harvested with VitroGel Cell Recovery Solution; B) PANC-1 cells harvested from 3D hydrogel with VitroGel Cell Recovery Solution, and then subcultured on the surface of hydrogel (day 2); C) PANC-1 cells harvested from 3D hydrogel using the cell harvesting solution and 3D subcultured in the hydrogel system again (day 2).

A fluorescence microscopy image showing numerous 3D cell spheroids of varying sizes. The spheroids are primarily green, indicating live cells, with some showing small red spots, indicating dead cells. The background is black.

**CYTO3D™ LIVE-DEAD ASSAY KIT**

**3D CELL VIABILITY ANALYSIS IN MINUTES**

# CYTO3D™ LIVE-DEAD ASSAY KIT

Live dead cell viability analysis for 3D and 2D cell culture.

The Cyto3D™ Live-Dead Assay Kit is used to determine the live/dead nucleated cells by using a fast one-step staining procedure for analysis on a dual-fluorescence system. This kit is recommended for viability analysis of cells cultured in 3D, 2D coating, and on monolayer.



Acridine orange (AO) and propidium iodide (PI), both nuclear staining (nucleic acid binding) dyes, are used in this kit. AO is permeable to both live and dead cells and stains all nucleated cells to generate green fluorescence. PI only penetrates the membranes of nucleated cells with compromised membranes and stains the dead cells to generate red fluorescence. Due to the quenching, when cells are stained with both AO and PI, all live nucleated cells fluoresce green, and all dead nucleated cells fluoresce red (the PI reduces the fluorescence intensity of the AO by fluorescence resonance energy transfer (FRET)). Non-nucleated materials such as red blood cells, platelets, and debris have no fluorescence and are ignored by fluorescence microscopes.

Dual-Fluorescence Viability, using AO and PI, is the recommended viability analysis method for cell lines, primary cells, and stem cells.

## Why Cyto3D Live-Dead Assay Kit?

**READY-TO-USE:** Single tube system for directly adding to cells without additional preparation

**FAST AND SENSITIVE:** 5-10 minutes incubation time and obtain accurate results

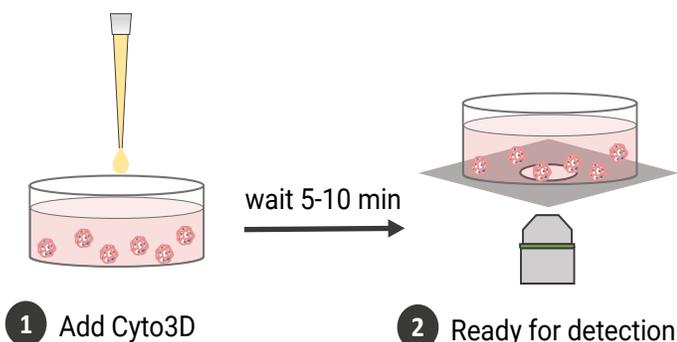
**EXCELLENT FOR 3D CULTURE:** Easy penetration for 3D cultured cells

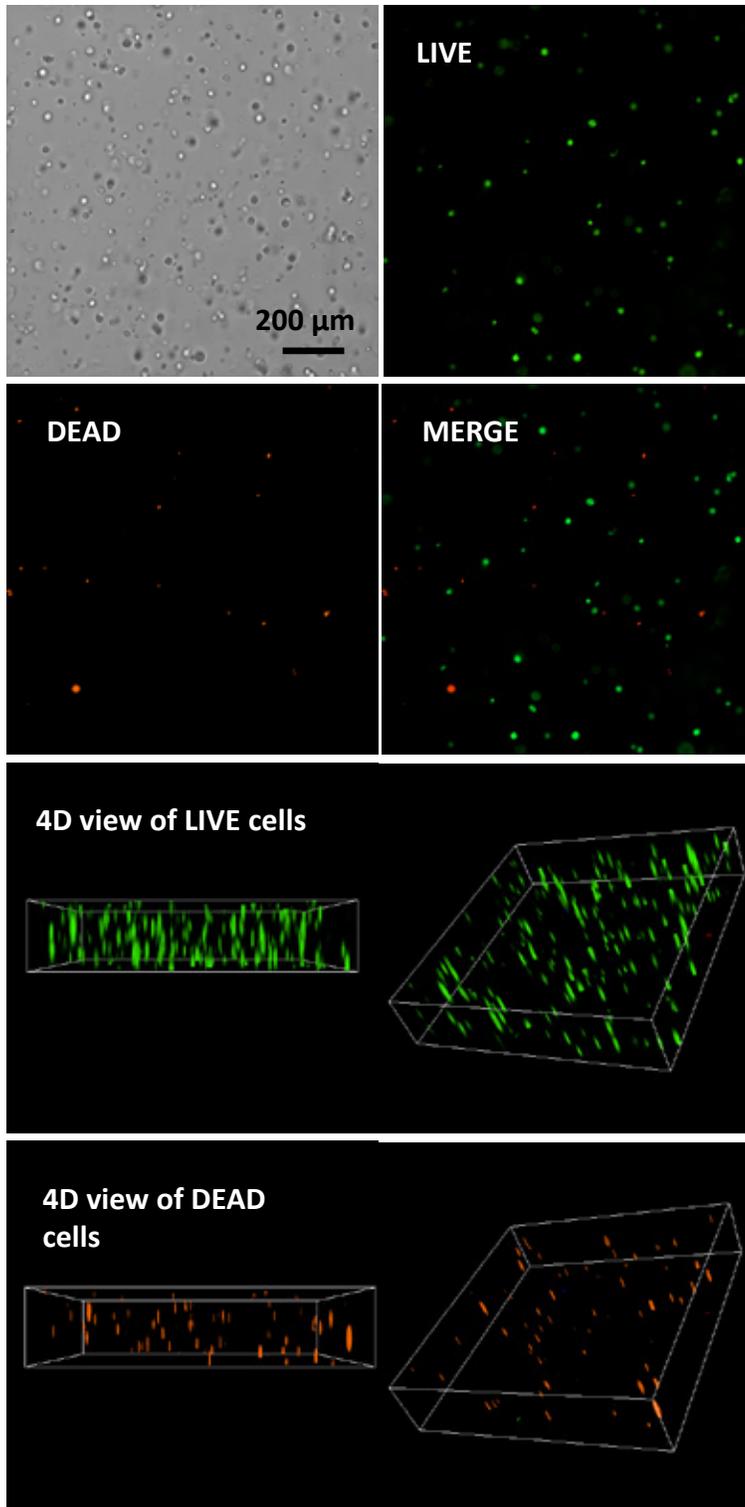
**COST-EFFICIENT:** Use only 2  $\mu$ L for 100  $\mu$ L sample

| Features                         | Specifications   |
|----------------------------------|--|
| <b>Formulation</b>               | Premixed acridine orange (AO) and propidium iodide (PI), nuclear staining dyes         |
| <b>Use</b>                       | Live dead cell viability analysis for 3D and 2D cell culture                           |
| <b>Detection Method</b>          | Fluorescent  |
| <b>Excitation/ Emission:</b>     | AO (494/517nm),<br>PI (535/617nm)  |
| <b>For use with (Equipment):</b> | Fluorescence microscope, flow cytometer, microplate reader, fluorescence cell counter. |
| <b>Number of Uses</b>            | 1 mL: 500 (at 2 $\mu$ L per 100 $\mu$ L)   |

| Product                    | Size | SKU # |
|----------------------------|------|-------|
| Cyto3D Live-Dead Assay Kit | 1 mL | BM01  |

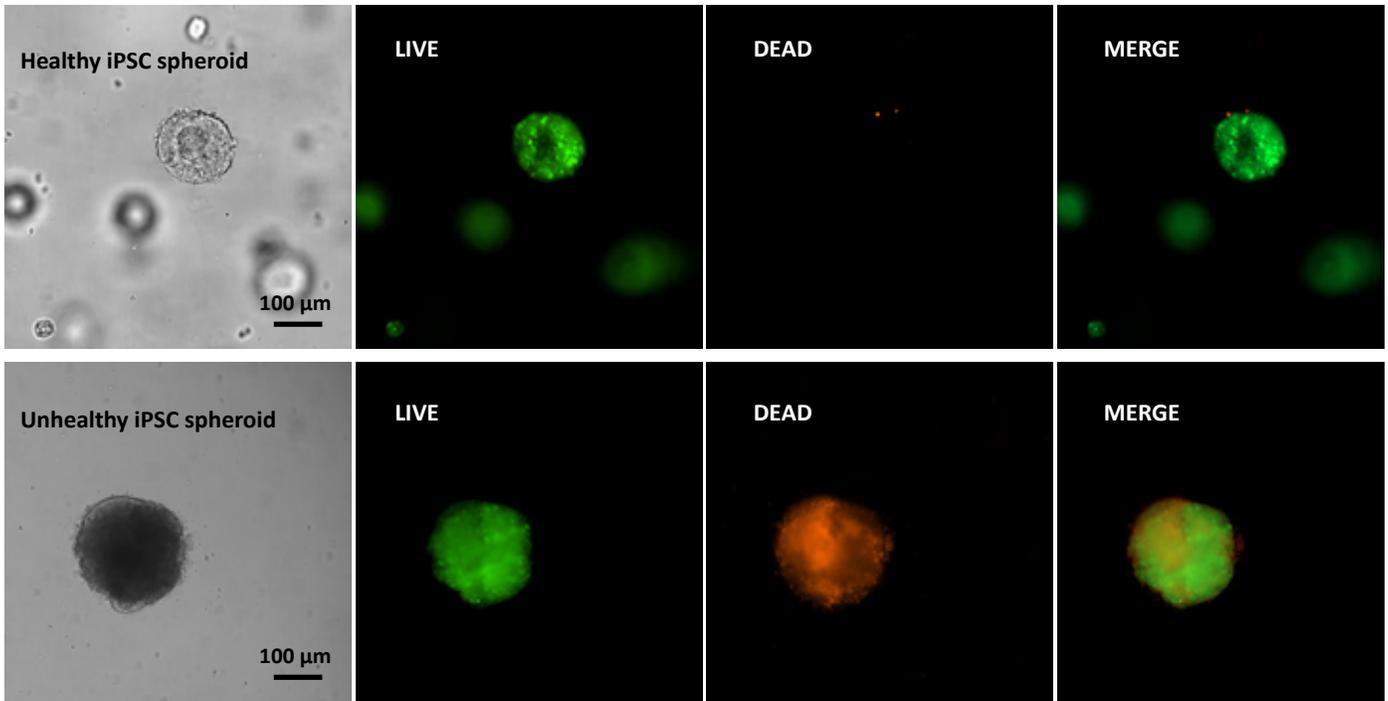
## Easy setup and use





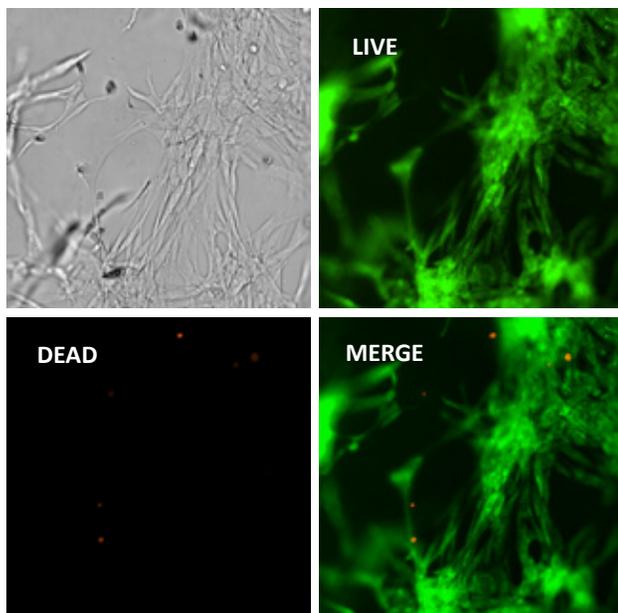
### Live-dead cell viability analysis by using Cyto3D Live-Dead Assay Kit

Glioblastoma cells (SF 298, about 60% cell viability) were 3D cultured in VitroGel system for 2 days. 2  $\mu\text{L}$  of Cyto3D reagent was added to each well containing 50  $\mu\text{L}$  hydrogel and 50  $\mu\text{L}$  cover medium. The mixture was incubated at 37  $^{\circ}\text{C}$  for 5-10 min. The cells were then observed under a fluorescent microscope. The images show the Live (green) and Dead (orange) cells within the 3D hydrogel matrix. The z-stack images of cells within hydrogel were then 3D reconstructed and showed in the 4D view images.



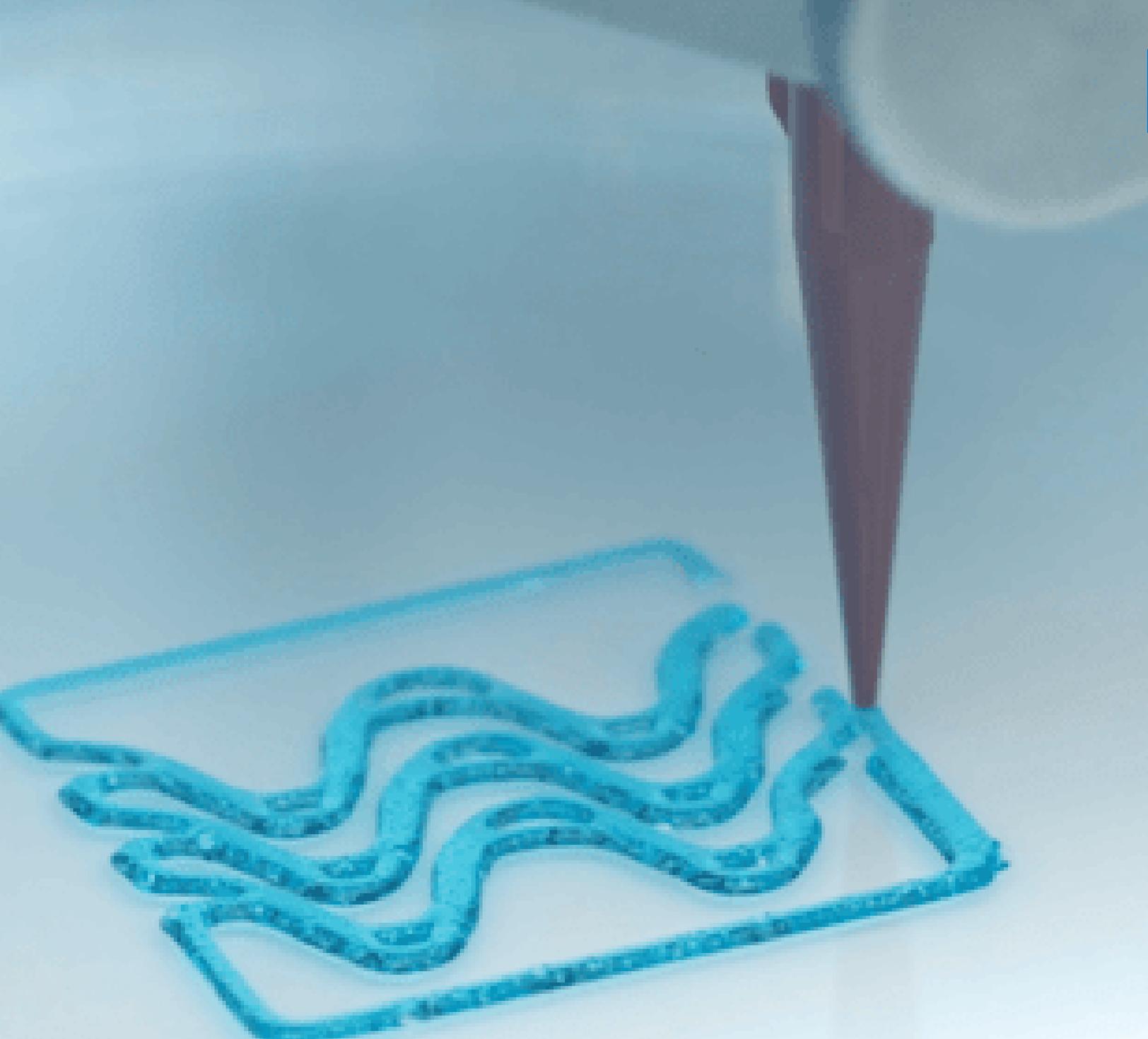
### Live-dead cell viability images of stem cell spheroids

Stem cells were static suspension-cultured in VitroGel STEM (CAT# VHM02) for 5 days. 2 μL of Cyto3D reagent was added to each well containing 100 μL cell suspension. The mixture was incubated at 37 °C for 5-10 min. The cells were then observed under a fluorescent microscope. The images show the Live (green) and Dead (orange) stem cell spheroids cultured in a 3D hydrogel matrix. The live-dead dyes of Cyto3D Live-Dead Assay Kit can successfully penetrate large cell spheroids for cell viability analysis.



### Live-dead cell viability images of normal Human Dermal Fibroblast (NHDF) culture on top of 2D hydrogel coating surface

NHDF cells were culture on the surface of VitroGel RGD at 1:3 dilution for 24 hours. 2 μL of Cyto3D reagent was added to each well containing 50 μl hydrogel and 50 μl cell medium. The mixture was incubated at 37 °C for 5-10 min and then observed under a fluorescent microscope.

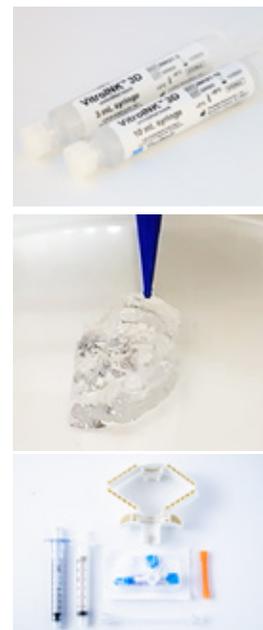


**VITROINK®**

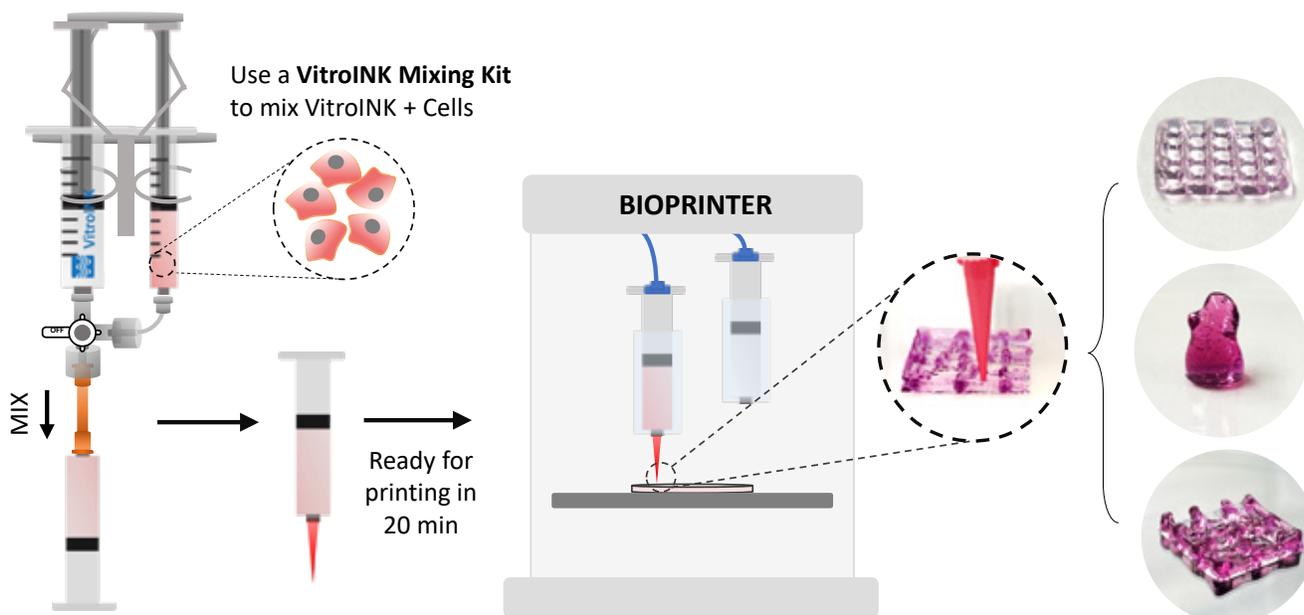
**EASY-TO-USE BIOINKS  
NO UV / NO HEAT CURING  
NO CHEMICAL CROSSLINKING**

# VITROINK® | XENO-FREE FUNCTIONAL BIOINK FOR 3D BIOPRINTING

VitroINK is a family of the ready-to-use, xeno-free tunable bioink system that requires no UV, no temperature/pH curing, or chemical cross-linking. The system is ready-to-use at room temperature, neutral in pH, transparent, and has excellent visibility after printing and cell culture. Due to the unique shear-thinning and rapid recovery mechanical property, VitroINK can maintain the printed structure without UV or other special curing methods. Adding cell culture medium after printing can further stabilize the printed structure and support cell growth. Cells can be pre-mixed with VitroINK using our VitroINK Mixing Kit for mixing ratios at 3:1 or 10:1. Different versions of VitroINK may incorporate multiple biological functional ligands to promote cell attachment, cell-matrix interactions, cell proliferation, motility/migration and differentiation for many various applications.



- Ready-to-use
- No UV, temperature/pH curing, or chemical cross-linking
- Multiple functional versions
- Wide printing temperature range
- Directly add cell medium for long term culture

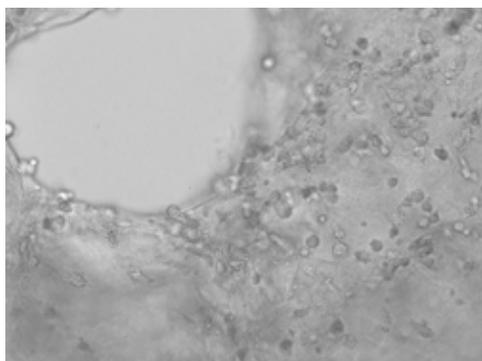


# VITROINK® 3D

VitroINK 3D is a ready-to-use tunable bioink for 3D bioprinting. The pure hydrogel matrix structure is good for cell spheroid formation and supporting the growth of cells requiring low cell-matrix interactions.



VitroINK 3D Bioink Starter Kit contains both bioink and the complete mixing kit to prepare the cell/bioink mixture for 3D bioprinting. The kit includes a 3 mL or 10 mL VitroINK 3D and a complete mixing kit that includes a dispenser, 1 mL syringe, 3 mL syringe, connectors, and mixing head to get you started.



Glioblastoma cell line (SNB 75) cultured in VitroINK 3D (3:1 bioink: cells mixing) at day 4.

## Why VitroINK 3D?

**READY -TO-USE:** Ready to print at room temperature, directly mix with cells without additional preparation

**XENO-FREE TUNABLE BIOINK**

**NO UV, TEMPERATURE/pH CURING OR CHEMICAL CROSS-LINKING REQUIRED**

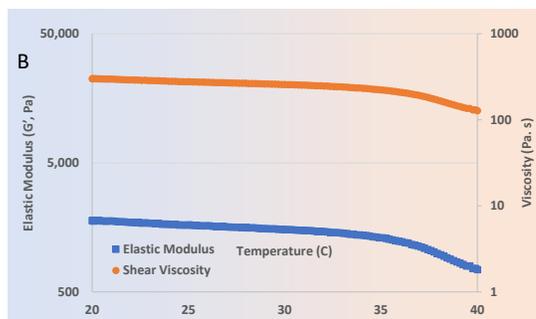
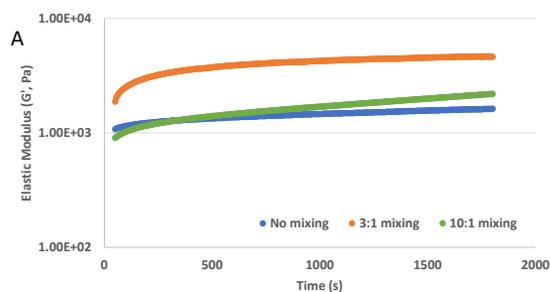
**EXCELLENT VISIBILITY:** After printing and cell culture

**EXCELLENT FOR LONG TERM CULTURE**

## SPECIFICATIONS

- Xeno-free tunable bioink for 3D bioprinting
- Ready-to-use at room temperature
- No UV, temperature/pH curing or chemical cross-linking
- Neutral pH
- Transparent. Excellent visibility after printing and cell culture
- Pre-mix with cells using our VitroINK Mixing Kit

| Product                          | Size  | SKU #    |
|----------------------------------|-------|----------|
| VitroINK 3D                      | 3 mL  | INK01-3  |
|                                  | 10 mL | INK01-10 |
| VitroINK 3D - Bioink Starter Kit | 3 mL  | ISK01-3  |
|                                  | 10 mL | ISK01-10 |



## Rheological properties of VitroINK 3D under the time sweep and temperature sweep tests.

A) The time sweep test of VitroINK 3D without or with 3:1 and 10:1 (v/v) mixing with DMEM medium. The elastic modulus was tested at room temperature and showed a feasible range for extrusion printing before incubation with cell culture medium. B) the elastic modulus and viscosity properties of VitroINK 3D under the temperature sweep testing (20 to 40°C). The elastic modulus and viscosity are relatively stable at 20 to 36°C. When experiencing temperature higher than 36, the elastic modulus and viscosity decrease. The data indicates VitroINK has a wide range of working temperatures and can be easily used at room temperature or physiological temperature.

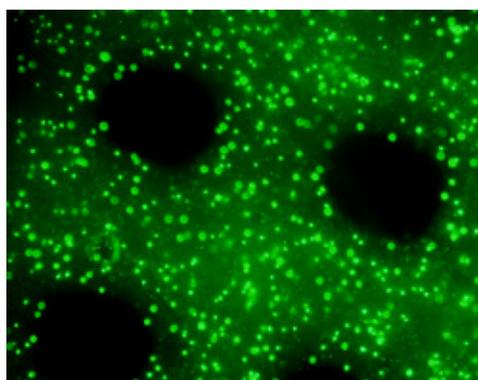
## VITROINK® RGD

VitroINK® RGD ready-to-use, xeno-free (animal origin-free) bioink system modified with RGD cell adhesive peptide, promoting the cell attachment and cell-matrix interactions.



This RGD modified bioink can enhance cell adhesion, proliferation, motility/migration and differentiation in different applications.

VitroINK RGD Bioink Starter Kit contains both bioink and the complete mixing kit to prepare the cell/bioink mixture for 3D bioprinting. The kit includes a 3 mL or 10 mL VitroINK RGD and a complete mixing kit that includes a dispenser, 1 mL syringe, 3 mL syringe, connectors and mixing head to get you started.



Patient-derived Non-Small Cell Lung Cancer (NSCLC) cells in VitroINK RGD. Over 90% cell viability after printing.

### Why VitroINK RGD?

**READY -TO-USE:** Ready to print at room temperature, directly mix with cells without additional preparation

**POPULAR FOR MANY APPLICATIONS:** Widely used RGD modified hydrogel that is versatile for many different uses

**NO UV, TEMPERATURE/pH CURING OR CHEMICAL CROSS-LINKING REQUIRED**

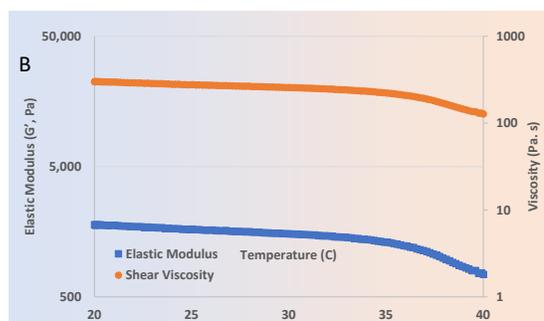
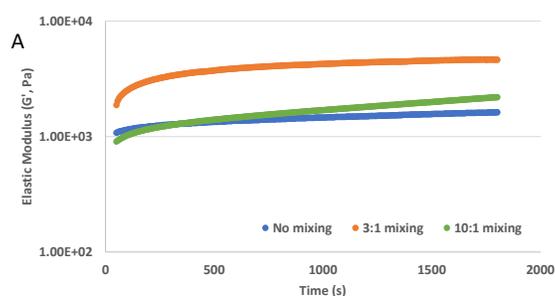
**EXCELLENT VISIBILITY:** After printing and cell culture

**EXCELLENT FOR LONG TERM CULTURE**

### SPECIFICATIONS

- Xeno-free tunable bioink for 3D bioprinting
- Promote cell adhesion, proliferation, motility/migration and differentiation
- Ready-to-use at room temperature
- No UV, temperature/pH curing or chemical cross-linking
- Neutral pH
- Transparent. Excellent visibility after printing and cell culture
- Pre-mix with cells using our VitroINK Mixing Kit

| Product                           | Size  | SKU #    |
|-----------------------------------|-------|----------|
| VitroINK RGD                      | 3 mL  | INK02-3  |
|                                   | 10 mL | INK02-10 |
| VitroINK RGD - Bioink Starter Kit | 3 mL  | ISK02-3  |
|                                   | 10 mL | ISK02-10 |



### Rheological properties of VitroINK RGD under the time sweep and temperature sweep tests.

A) The time sweep test of VitroINK RGD without or with 3:1 and 10:1 (v/v) mixing with DMEM medium. The elastic modulus was tested at room temperature and showed a feasible range for extrusion printing before incubation with cell culture medium. B) the elastic modulus and viscosity properties of VitroINK RGD under the temperature sweep testing (20 to 40°C). The elastic modulus and viscosity are relatively stable at 20 to 35°C. When experiencing temperatures higher than 36, the elastic modulus and viscosity decrease. The data indicates VitroINK has a wide range of working temperatures and can be easily used at room temperature or physiological temperature.

# VITROINK® MIXING KIT

VitroINK Mixing Kit was designed to provide a robust mixing of the bioink and cells.

Mixing cells with the bioink is a critical step for 3D bioprinting. Because of the differences in viscosity of the cell suspension and bioink, the traditional manual mixing methods create a lot of air bubbles and non-uniform mixture which leads to the unstable printed structure, difficulty for cell observation, and affects cell viability.

With VitroINK Mixing Kit, cells can be prepared with VitroINK bioink for a 3:1 mixing ratio using the 3 mL syringe or a 10:1 mixing ratio using the 1 mL syringe. To prepare the bioprinter cartridge, the VitroINK and cell suspension are placed in the mixer and are dispensed through the the mixing head. Wait 10-20 minutes for the mixture to stabilize and the cartridge is ready for printing. There is no UV, no temperature/pH curing, or chemical cross-linking for the VitroINK system. Adding cell culture medium to cover the printed structure can further stabilize and support cell growth. The bioprinted cells are ready for incubation.

## Why VitroINK Mixing Kit?

**EASY-TO-USE:** Simply connect the syringes of the bioink and cells for sample preparation

**REDUCE AIR BUBBLES:** Dramatically reduce air bubbles for smooth 3D bioprinting

**ROBUST MIXING RESULTS:** Achieve homogeneous mixture consistently

## SPECIFICATIONS

- Disposable mixing kit components for VitroINK bioink:
- (1) Dispenser
- (1) 1 mL syringe
- (1) 3 mL syringe
- (1) connector and tubing
- (1) mixing head
- Sterile
- Use for 3:1 or 10:1 mixing ratio

| Product             | Size                       | SKU #   |
|---------------------|----------------------------|---------|
| VitroINK Mixing Kit | Complete Pack              | IMK00-1 |
|                     | 3:1 Mixing Component Pack  | IMK03-1 |
|                     | 10:1 Mixing Component Pack | IMK01-1 |



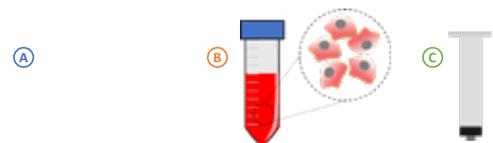
VitroINK Mixing Kit – Complete Pack comes with the following:

- 1 3 mL syringe & 1 mL syringe
- 2 Dispenser
- 3 Connector and tubing
- 4 Mixing head



You need:

- A VitroINK
- B Cell suspension in a culture medium
- C Your bioprinter cartridge







**TheWell**  
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*Growing Cells in New Dimensions*

Pioneering personalized medicine. Improving the quality of life for every patient.

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