VitroGel®

Technical Tips and Frequently Asked Questions (FAQ)

GENERAL

What is VitroGel ?

VitroGel® is a xeno-free functional hydrogel system that closely mimics the natural extracellular matrix (ECM) environment. Modified with multiple cellular functional ligands, VitroGel allows for a robust 3D cell culture platform and can be used as an injectable delivery system for drug discovery, tissue engineering, cell therapy, and personalized medicine.

VitroGel comes in two different variations, ready-to-use and high concentration.

The ready-to-use VitroGel is a series of user-friendly functional hydrogels offering an excellent balance of simplicity and versatility. The hydrogels have optimized formulations of multi-functional ligands and concentration. The hydrogel solution is stable at room temperature and can mix with cells/culture medium directly to use.

VitroGel High Concentration hydrogels give scientists the full control to adjust both mechanical strength and functional ligands of the cell culture environment. The high concentration formulation allows the maximum flexibility to manipulate the mechanical strength of hydrogel by adjust the dilution ratios of the hydrogel solution. VitroGel High Concentration Hydrogels also can use as a set of building blocks to create a functional micro-environment by blending ("mix and match") different versions.

Which VitroGel hydrogel product should I choose?

Ready-To-Use Hydrogel

For many users, this is the preferred hydrogel system for many applications. It is a ready-to-use hydrogel where you need only to add your cells, cover medium, and incubate. The hydrogels come with different versions of optimized formulations for different cell types and applications: It is a ready-to-use hydrogel where you need only to add your cells, cover medium, and incubate.

- VitroGel Hydrogel Matrix (SKU: VHM01): a general-purpose hydrogel that is good for many cell lines and primary cells. This hydrogel can support the formation of tumor spheroid, the cellular network structure of stromal or fibroblast cells, co-culture, invasion or 3D cell migration.
- VitroGel ORGANOID (SKU: VHM04): supports a wide range of organoids from patient-derived samples, stem cells, tissues, co-culture and PDX resources.
- VitroGel STEM (SKU: VHM02): generate high-quality 3D stem cells directly from liquid nitrogen. Excellent for stem cell expansion, 3D scale-up or downstream applications.

- VitroGel MSC (SKU: VHM03): support 2D hydrogel coating, 3D cultures of mesenchymal stem cells (MSCs) and make hydrogel cell beads to replace microcarrier for MSC scale-up.
- VitroGel HEK293 (SKU: VHM05): support 3D culture and scale up of human embryonic kidney 293 (HEK293) cells for cell-based bioproduction.
- VitroGel Angiogenesis Assay Kit (VHM06-K1): a revolutionary tool for researchers to study the effect of both hydrogel properties and culture medium on angiogenesis process

High Concentration Hydrogels

For more advanced users, the VitroGel High Concentration hydrogels give scientists full control to manipulate the biophysical and biological properties of the cell culture environment. We have many types of hydrogel with different binding ligands, making our system a "Mix and Match" where you can control what is in the hydrogel for your 3D cell culture. All the high concentration hydrogels come with VitroGel Dilution Solutions to adjust the final hydrogel strength from 10 to 4000 Pa. Customized hydrogels can reach more than 20,000 Pa.

- VitroGel® 3D High Concentration Kit (SKU: TWG001) is a pure and unmodified hydrogel that allows the maximum flexibility to manipulate the 3D cell culture environment for different needs. The unmodified hydrogel matrix structure is good for cell spheroid formation, suspension cells, or cells requiring low cell-matrix interactions.
- VitroGel® RGD High Concentration Kit (SKU: TWG003) is modified with a high concentration of RGD cell adhesive peptides, promoting the cell attachment and cell-matrix interactions during the 3D cell culture. It can achieve high levels of integrin-binding activities to promote intercellular networks even after hydrogel dilution.
- VitroGel® COL High Concentration Kit (SKU: TWG009) is modified with collagen-mimetic peptide, which specifically binds the integrin α2β1, promoting many bioactivities such as osteoblastic differentiation *in vitro* and enhancing osteoblastic activity *in vivo*.
- VitroGel® IKVAV High Concentration Kit (SKU: TWG007) is modified with laminin-derived functional peptide, which is actively involved in different biological activities such as neuronal progenitor cell differentiation, promoting cell adhesion, neurite outgrowth, angiogenesis, and tumor growth.
- VitroGel® YIGSR High Concentration Kit (SKU: TWG008) is modified with laminin-derived functional peptide, which involves endothelial cell adhesion, cell proliferation, and motility/migration.
- VitroGel® MMP High Concentration Kit (SKU: TWG010) is modified with Matrix Metalloproteinases (MMP) for a biodegradability matrix.

We can customize our hydrogels with different functional ligands. Please contact at support@thewellbio.com if you need a customized product.

If you need help finding the right hydrogel product for your research project, please fill this product help form.

What is the chemical composition of the VitroGel?
The hydrogel is a xeno-free synthetic polysaccharide-based hydrogel system.

What is the difference between VitroGel Dilution Solution Type 1 and Type 2?
VitroGel Dilution Solution Type 1 contains sucrose to maintain the best osmolarity. Dilution Type 2 is sucrose-free. For most cell lines, VitroGel Dilution Solution Type 1 is recommended. VitroGel Dilution Solution Type 2 is for scientists working with cells that are sensitive to sugar.

Does VitroGel have viscoelastic properties?
Yes, all VitroGel hydrogels are tested with a dynamic rheometer for viscoelastic properties. For VitroGel High Concentration hydrogels, the elastic modulus (G') can be adjusted from 10 to 4,000 Pa by changing the hydrogel concentration with the VitroGel Dilution Solution.

Does VitroGel contain any natural ECM proteins like fibronectin, collagens, or laminins?
VitroGel does not contain any natural ECM proteins. Scientists can add growth factors and proteins to the hydrogel system for a well-defined system.

What is the mechanical property of VitroGel? Can I adjust the stiffness?
We test the elastic modulus (G') of VitroGel with a dynamic rheometer. The G' is dependent on the hydrogel dilution and the cell culture medium used. The G' of non-diluted VitroGel High Concentration is around 4,000 Pa. If a higher hydrogel strength is required, please contact us at support@thewellbio.com for a customized hydrogel that can reach over 20k Pa. You can also check our BioInk product (VitroINK), which can reach the G' over 45k Pa.

What is the difference between hydrogel elastic modulus and hydrogel stiffness?
We use the elastic modulus (G') to represent the strength of VitroGel, which is tested by a dynamic rheometer. A simple conversion between the elastic modulus and stiffness is to calculate at 1:3 ratio. Fox example, if the hydrogel is 100 Pa in G', which is equal to 300 Pa in stiffness. Therefore, the 4000 Pa G' of VitroGel High Concentration is around 12,000 Pa in stiffness.

Will adjusting the temperature induce the hydrogel formation of VitroGel?
Adjusting the temperature will not induce the hydrogel formation. VitroGel can maintain the liquid form at room temperature. The hydrogel formation can be induced by mixing with an ionic solution such as a cell culture medium. Increasing the temperature would reduce the viscosity of the hydrogel solution.

Can molecules penetrate or diffuse through VitroGel?
Yes, VitroGel supports the diffusion of molecules of different sizes from small molecules to big proteins (e.g., IgG). Drug compounds, antibodies, staining dyes can be added on the cover medium to penetrate the hydrogel. VitroGel can be mixed with molecules for control release experiments.

Does VitroGel have autofluorescence?

VitroGel is a xeno-free system. We do not detect any autofluorescence in our various imaging tests. We recommend that scientists perform a control experiment to determine background fluorescence.

Can I harvest cells after culturing with VitroGel?

Yes, scientists can easily harvest cells with our enzyme-free, ready-to-use VitroGel Cell Recovery Solution in 20 minutes. VitroGel Cell Recovery Solution is room temperature stable with a neutral pH and has a operating temperature of 37°C. The cell harvesting solution can maintain high cell viability during the recovery process. Harvested cells can be further sub-cultured for both 2D and 3D.

Additional information can be found here.

Can VitroGel be used for both 3D culture and 2D hydrogel coating?

Yes, VitroGel can support 3D cell culture, 2D thick gel coating and 2D thin gel coating culture. Embedding cells in the hydrogel matrix (3D cell culture) can maximize the cell-matrix interaction and potential cell-cell communication in response to different biophysical and biological properties of the hydrogel. Some cells are hard to culture in 3D and as a result, 2D coating is an alternative method for cell culture.

2D thick gel coating uses a layer of hydrogel to change the properties of the substance. It is a method between the traditional 2D culture and the true 3D cell culture. 2D thick gel coating is an alternative approach if scientists do not want to jump into 3D cell culture but want to see how cells behave differently based on different substances. It is also a good method for layer-by-layer co-culture as well.
2D thin gel coating is used as a thin layer of hydrogel to change the surface properties of the substance, which may change the capability for cell attachment but not the stiffness of the substance.

Which culture method should I choose??

VitroGel hydrogel system is versatile for many applications. The hydrogel is flexible for multiple culture methods such as 3D cell culture, 2D hydrogel coating, static suspension culture, hydrogel-cell bead, and used as an injectable carrier.

- 3D cell culture: make a full cellular encapsulation in the hydrogel matrix and enhance the cell-hydrogel matrix interactions
- 2D hydrogel coating: allow cells to interact with/submerge into the functional hydrogel substance and maintain an excellence exposing surface to the top medium. This method is great to generate the bridge between the traditional 2D culture and 3D cellular encapsulation culture.
- Static suspension culture: this is a unique culture method of VitroGel hydrogel system. By simply mixing the VitroGel solution and cells for a soft hydrogel formation, the researchers can further directly mix the hydrogel-cell mixture with additional culture medium to make a hydrogel-cell suspension. This method is great for suspension culture and scale up.
- Hydrogel-cell bead: The hydrogel solution can mix with cells for a soft hydrogel, which then can add to the cell culture medium as droplets for hydrogel-cell bead formation. This culture method not only encapsulates cells within the hydrogel matrix to enhance cell-matrix interactions, but also allow the whole hydrogel-cell bead to suspense in cell culture medium for

optimal medium penetration. Researchers can adjust the size of the hydrogel beads by changing the volume of droplets added to the culture medium.

- Injectable carrier: Simply mix hydrogel solution with cells/compounds at room temperature, the hydrogel is ready for injection in 20 minutes. Under the mechanical shearing force such as injection through a syringe, the hydrogel performs a gel-sol transition and becomes free-flowing status. However, once the shearing force ceased, the mechanical strength of the hydrogel can rapidly recover with a sol-gel transition and become a hydrogel status again. With this injectable property, VitroGel can be used for in vivo cells/drug delivery for cell therapy or controlled release.

Is VitroGel biocompatible for *in vivo* study?

Yes, VitroGel is biocompatible and safe for animal study.

How long can cells be grown in the VitroGel system?

We have tested the growth of cells in the hydrogel system for more than eight weeks. Depending on the cell type and application, the 3D cell culture can last even longer. Scientists may need to change the cover media more frequently once the number and size of cells increases.

Is there a specific type of cell culture plate that should be used?

No specific plate is required. Any regular tissue culture treated plate can be used.

What is the pore size of VitroGel?

The pore size of VitroGel is about 200-500 nm. The hydrogel is flexible, soft, so the cells can push the hydrogel matrix out and molecules can easily penetrate through the hydrogel matrix.

What is the elastic modulus of ready-to-use gels?

The elastic modulus of the ready-to-use VitroGel is about 100-300 Pa. Please keep in mind, the elastic modulus (G') was tested by dynamic rheometer. The G' is different than stiffness (Young's modulus). A simple conversion between G' and stiffness is 1:3 (100 Pa of G' is about 300 Pa of stiffness). Therefore, the stiffness of ready-to-use VitroGel is about 300-900 Pa.

What is the elastic modulus of the high concentration/different dilution VitroGels?

For VitroGel High Concentration hydrogels, the elastic modulus (G') can be adjusted from 10 to 4,000 Pa by changing the hydrogel concentration with the VitroGel Dilution Solution. The G' is about 4000 Pa at 1:0 dilution, 1200-2000 Pa at 1:1 dilution, 600-1000 Pa at 1:2 dilution, 200–500 Pa at 1:3 dilution, and < 500 Pa at dilution higher than 1:3.

What is the difference between VitroGel ORGANOID versions?

The VitroGel ORGANOID has four different version which were formulated with various bio-functional ligands, mechanical strengths, and degradability to fulfill the needs of different organoid culture conditions.

What are the different mechanical strengths between VitroGel ORGANOID versions?

The mechanical strengths of different VitroGel ORGANOID hydrogel are in this order:

VitroGel ORGANOID-3 > VitroGel ORGANOID-4 ≥ VitroGel ORGANOID-2 > VitroGel ORGANOID-1.

Which VitroGel ORGANOID hydrogel should I choose for my organoid type?

The VitroGel ORGANOID Discovery Kit includes four different formulations of VitroGel ORGANOID hydrogels, type 1-4, which have various bio-functional ligands, mechanical strengths, and degradability to fulfill the needs of different organoid culture conditions. Because there are a wide range of cell resources for organoids from stem cells, patient-derived tissue, co-culture, and PDX, it is hard to tell which organoid hydrogel would be best for the researcher's experiment. Therefore, the discovery kit helps perform a quick screening of 4 different formulations and determine the best version for moving on. From our findings, versions 1, 2, or 3 are suitable for gastric organoids. Versions 1 or 3 are suitable for lung organoids. Versions 2 or 3 are suitable for brain organoids, and versions 3 or 4 are suitable for cancer organoids. By saying that, we would still suggest you using the Discovery kit to make a quick screening and find the hydrogel that can fulfill your research goal best.

Hydrogel Formation and Preparation Protocols

How does gelation work in VitroGel?

The hydrogel formation starts when VitroGel is mixed with the cell culture medium. The hydrogel molecules will interact with the ionic molecules, such as Ca2+ or Na+, from the cell culture medium to induce a matrix structure (hydrogel). For 3D culture operation, the hydrogel formation is in two stages: a) soft hydrogel formation, b) hydrogel stabilization.

1. Soft hydrogel formation: The hydrogel formation process is slow when a small amount of cell culture medium is used. At this stage, the hydrogel is soft and possess a shear-thinning and rapid recovering mechanical property, which makes the hydrogel injectable for *in vivo.* The slow hydrogel-forming process and the injectable property of the soft hydrogel create a time frame for easy hydrogel transfer from the mixing tube to the cell culture plate.
2. Hydrogel stabilization: After soft hydrogel formation, adding 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.

More information can be found here.

How do I adjust the hydrogel formation time?

Adjusting the mixing ratio between the VitroGel solution and cell culture medium would change the hydrogel formation rate.

If the hydrogel solidifies too fast after mixing with the culture medium (producing a small solid gel chunk), adjust the mixing ratio by using less cell culture medium. For example, if mixing 2 mL hydrogel solution with 0.5 mL cell culture medium leads to solid gel chunk (particles), then mixing 2 mL diluted hydrogel solution with 0.2-0.4 mL cell culture medium would help to solve the issue.

On the other hand, if the hydrogel formation is too slow, adjust the mixing ratio by using more cell culture medium. For example, if mixing 2 mL diluted hydrogel solution with 0.5 mL cell

culture medium leads to a slow hydrogel formation, then mixing 2 mL diluted hydrogel solution with 1-2 mL cell culture medium would help to solve the issue.

What can I do to prevent bubbles from forming when mixing the VitroGel solution with the cell culture medium?

The bubble issue is related to the increased solution viscosity after mixing the gel solution with cell medium. Here are some suggestions that can help to reduce the formation of bubbles:

- Warm up the VitroGel solution to 37° C to reduce the viscosity of the gel.
- Gently mix the VitroGel solution with the cell medium. Then pipette slowly without introducing bubbles.
- Quickly spin the mixing tube to get rid of bubbles.

Can I add extracellular matrix proteins or other molecular compounds into VitroGel?

Yes. Extracellular matrix proteins or other molecular compounds can be added into the VitroGel system. Before hydrogel formation, add the proteins or the molecular compounds into the cell culture medium and then mix directly with the VitroGel 3D hydrogel solution. Please note that the hydrogel formation time and the final gel stiffness might change due to the salts contained in the proteins or chemical compounds. Please contact us at support@thewellbio.com if you have any questions or concerns about adding additional compounds to the VitroGel system.

Why does the hydrogel sticks loosely to the tissue culture plate?

This issue might be because of the following reasons:

- Using a non-treated tissue culture plate, which has a more hydrophobic surface. This reduces the attachment of hydrogel/cells on the surface of the well-plate. For better performance, we suggest using a treated tissue culture plate with VitroGel.
- Adding the hydrogel as a dome instead of covering the whole bottom of the well plate might also cause this issue. We suggest gently tilting and swirling the well plate after adding the hydrogel to ensure the whole bottom of the well plate is covered by the gel.
- Not waiting long enough before adding the additional medium on the top of the hydrogel. After transferring the hydrogel to the well plate, please wait 10-30 minutes for hydrogel stabilization before adding the top medium. Adding the medium before the hydrogel stabilizes would disrupt the structure of hydrogel. The lower the concentration of the hydrogel, the longer the waiting time is needed.
- After the initial soft hydrogel formation, it is crucial to make sure the hydrogel is stable and attached to the bottom of the well plate before adding the cover medium. If the hydrogel is not stable, it might detach from the bottom of the well after adding the cover medium. During the hydrogel formation, the gel is soft; do not shake the plate or position the plate vertically. Position the plate horizontally.

Can I form a dome shape culture?

Adding the hydrogel as a dome shape is not recommended. The dome shape may not stick to the bottom of the culture plate for long term culture. The hydrogel should cover the whole bottom of the well plate. We suggest gently tilting/swirling the well plate after adding the hydrogel to ensure the gel coats the entire bottom of the well plate. Using a 96 well plate can

reduce the usage volume of the hydrogel to 30-75 µL/well. There is no issue with molecular penetration when adding the cover medium to feed the cells.

Can I use a serum-free medium with VitroGel?
Yes, serum-free medium works with VitroGel.

Is it possible to dilute VitroGel hydrogels with cell culture medium instead of the dilution solution?
For the High Concentration hydrogels, it is not recommended. The concentration of the ionic molecule in medium would be much higher than the dilution solution, so diluting VitroGel with cell culture medium may cause the gel to form too quickly and produce a chunky consistency. We recommend using VitroGel Dilution Solution to adjust the highly concentrated VitroGel.

Alternatively, the Ready-to-use VitroGel hydrogels are a better options for scientists who do not want to worry about dilution and only want to mix the hydrogel with cell suspension directly.

How fast should I transfer the sample from the mixing tube to the culture plate?
After mixing with the cell culture medium, we recommend transferring the mixture to the tissue culture plate immediately. The hydrogel formation starts after mixing the VitroGel solution with the cell culture medium.

If you have multiple samples with different hydrogel conditions or cell types to prepare, we recommend transferring the mixture of sample 1 to the tissue culture plate before mixing the hydrogel with cell culture medium for sample 2.

Can I place the gel mixture in an incubator instead of leaving it at room temperature for gel stabilization?
Yes, it is fine to place the gel mixture in an incubator instead of room temperature, but please note, the gel forms more slowly in an incubator than at room temperature. As a result, for lower concentrated gels, you will need to keep it in an incubator for longer. However, for gels at a higher concentration or VitroGel Hydrogel Matrix, it is not a problem.

Can I pre-coat the culture plate to increase hydrogel attachment?
Although, for most cases, pre-coating the culture plate is not necessary, coating the plate with 1X PBS, Poly-D-Lysine solutionor 10-100 mM $CaCl_2$ can improve the hydrogel attachment. Please check the following protocols for coating the culture plate:

- Add the PBS, Poly-D-Lysine solutionor $CaCl_2$ solution to the culture plate for 30 min.
- Remove the PBS, Poly-D-Lysine solutionor $CaCl_2$ solution, open the lid under the biosafety hood for 10-20 min before adding the hydrogel. The recommend volumes of PBS or $CaCl_2$ for different sizes of well plates are list in Table 1 below.
  Table 1: Recommend volume of PBS, Poly-D-Lysine solution or $CaCl_2$ solution for pre-

coating well plate

	Volume of PBS, Poly-D-Lysine solution, or $CaCl_2$ solution for each well
6-well plate	2000 µL
12-well plate	1000 µL
24-well plate	500 µL
48-well plate	250 µL
96-well plate	100 µL

Cell Preparation and Culture on VitroGel ----------

How do I prepare the cell suspension to mix the hydrogel? Should I add serum?

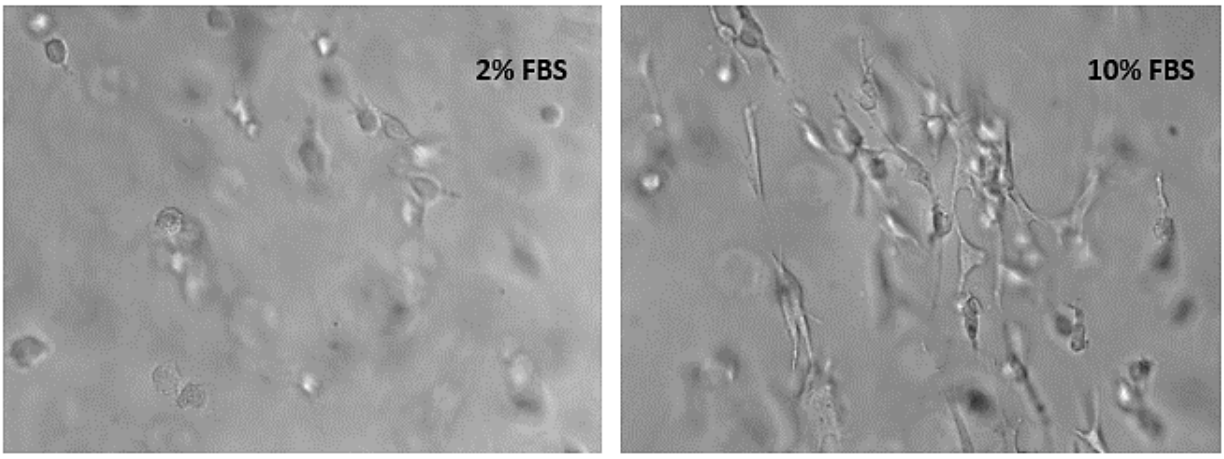
Cells can be prepared in regular complete cell culture medium and be directly mixed with VitroGel. If you want to adjust the concentration of the serum or other critical supplement(s) in the final hydrogel, follow the steps below:

If cells cultured in complete cell culture medium, which is supplement with 10% FBS or other critical supplements, please prepare the cell suspension using the following methods before mixing it with hydrogel solution.

- Prepare the cell suspension with 2X concentration (e.g. 100K) and mix with 100% FBS at 1:1 (v/v) ratio to get 1X cell suspension (e.g. 50K) with 50% FBS.
- Mix VitroGel hydrogel solution with the cell suspension from above at 4:1 (v/v) ratio to get the final cells in the hydrogel at 10K with 10% FBS supplement.

Note: Do not make 2X (or higher) concentration medium. The ionic molecules in the medium would affect hydrogel formation. The high concentration medium might make gel chunky or give the gel a heterogeneous consistency

The serum or critical supplement(s) in the hydrogel would affect cell growth, especially during the first 48 hrs. Please see the example figures below.



Bone marrow cells (OP9) 3D cultured in VitroGel LDP3 with 2% and 10% FBS. The cells were encapsulated in hydrogel matrix with 2% and 10% final FBS concentrations respectively. The images were taken 18 hours after cell seeding.

What cell seeding density should I use?

The final cell concentration can be optimized based on different cell types. We recommend preparing cell suspension at the following concentration for:

- 3D cell culture: 5-2 x 10^6 cells/mL (the cell suspension needs to mix with VitroGel solution at the 4:1 ratio (VitroGel solution: Cell suspension at 4:1 v/v), which makes the final cell concentration in the hydrogel 1-4 x 10^5 cells/mL)
- 2D hydrogel coating: 1-5 x 10^5 cells/mL

How often does the cover media need to be changed for 3D culture?

Typically, we recommend changing the cover medium every other day, similar to regular 2D cell culture. However, it depends on the experiment's needs. Some experiments might require changing every 24 hours and some might not require changing for an entire week. Please contact us at support@thewellbio.com if you are unsure of this.

Shall I change the full amount of the cover medium or just partially?

Changing 100% of the cover medium might cause the disruption of the hydrogel. We recommend adding additional fresh medium without removing the top medium for the first medium change. Afterward, change 50-80% of the cover medium. (Please check the recommended volume of additional cover medium in Table below.)

	Volume of the cover medium at Day 0	Addtional medium volume to add without removing the cover medium at Day 2	Volume of partial medium to change afterwards (Day3)
6 well plate	1200 µL	600 µL	1200 µL
12 well plate	600 µL	300 µL	600 µL
24 well plate	300 µL	150 µL	300 µL
48 well plate	150 µL	50 µL	150 µL
96 well plate	75 µL	25 µL	75 µL

Is it possible to cover cell spheroids with VitroGel and apply differentiation media on top?
Yes, the medium on top can penetrate through the hydrogel.

Can cells move within the hydrogel matrix (migration/invasion)?
Yes, the mobility of cells on VitroGel can be observed in both 3D culture and 2D hydrogel coating culture. Spheroid invasion assay, wound healing assay, or 3D cell migration can be performed with VitroGel.

Can cells grown in the VitroGel be sub-cultured?
Yes. Cells can be harvested from the VitroGel system by using the VitroGel Cell Recovery Solution and be sub-cultured for an additional period by using fresh VitroGel.

How can I use VitroGel for co-culture or sandwich culture (layer by layer)?
The co-culture of two or more different cell types can be performed with the VitroGel system. Besides adding different cell types together with the hydrogel solution for co-culture, each cell type can also be mixed with the hydrogel solution and be added layer by layer. After the first layer of hydrogel becomes stable, carefully overlay the second layer of cells/hydrogel mixture on top of the first layer of cells/hydrogel.

How long before spheroid formation takes place in hydrogel?
Normally, spheroid formation requires about 3-10 days after culture in the hydrogel system. The formation time may vary depending on different cell types. The small cell aggregation (colony formation) might happen overnight.

Imaging and Analysis ----------

Is VitroGel compatible with staining and immunofluorescence protocols for confocal microscopy or other imaging technologies?
Yes. Cells can be stained within the hydrogel or harvested from the hydrogel and then stained. Most fluorescent dyes and immunological reagents can be used at standard protocols. VitroGel hydrogel is transparent and compatible with different imaging systems for cell observation.

Please check the protocols for more details: https://www.thewellbio.com/protocols/

Is it possible to fix cells inside the hydrogel with PFA and performed immunolabelling?
Yes. The cells can be fixed inside the hydrogel with PFA. Immunolabeling of cells works well with VitroGel.

Can I do live/dead assay and cell proliferation assay on VitroGel?
Yes. You can use Cyto3D™ Live-Dead Assay Kit 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.

Please check the product page of Cyto3D™ Live-Dead Assay Kit for more information: https://www.thewellbio.com/product/live-dead-cell-assay-kit-cyto3d/

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Can I do molecular analyses of protein or nucleic acids (DNA/RNA) of cells cultured in VitroGel?
Yes. Cells can be harvested from the hydrogel and be used for molecular analyses according to standard procedures. The hydrogel is transparent, and you can use the molecular assay directly with the hydrogel. Additionally, the hydrogel can be easily dissolved by using a homogenizer/ultrasonic processor, so scientists can lysis cells together with hydrogel to extract DNA/RNA.

Please check the protocols for more details: https://www.thewellbio.com/protocols/

Applications ----------

Is the VitroGel compatible with drug/compound treatments?
Yes, the VitroGel hydrogels are a great system to build 3D cell models for drug/compound screening. VitroGel is compatible for high-throughput screening with an auto liquid handling machine. Molecules can be mixed with the hydrogel or be added directly from the top of the hydrogel. This will allow an easy diffusion through the gel.

Can VitroGel perform transfection studies?
Yes, VitroGel can be used to perform 3D transfection studies.

Can I use VitroGel to cover cells or tissue slides?
Yes, VitroGel can be added directly on top of the cells or tissue slide.

Can I use VitroGel for *in vivo* studies?
Yes, VitroGel can be injected before or after soft hydrogel formation for *in vivo* study.
Before the hydrogel formation: The VitroGel solution can be injected directly into the animal. It will become a hydrogel when it encounters the ionic compounds of the physiological environment.

After soft hydrogel formation: The VitroGel hydrogel has an advanced injectable property after soft hydrogel formation. Mixing the hydrogel solution and cell culture media/PBS at a proper ratio, the final hydrogel becomes injectable for *in vivo* studies. Using this method, cells or other chemical compounds can be mixed in the hydrogel before injection.

Can VitroGel be used for cell therapy?
Yes, VitroGel is an excellent delivery system for cell therapy. Using VitroGel as an injectable delivery system, scientists can achieve better cell retention and higher cell viability for cell therapy.

What type of needles can you use for animal injection?
We normally use 18-30 G needle size.

Can DMSO be used with the VitroGel? If so, what is the maximum amount of DMSO that can be mixed with the hydrogel?
Yes, DMSO can be used with VitroGel. For the maximum amount of DMSO, we suggest no more than 50% of the volume.

Can VitroGel be used for cryopreservation?
Yes, VitroGel can be used for cryopreservation. It can help to promote cell viability.