READY-TO-USE HYDROGEL

PROTOCOL

VitroGel® ORGANOID

CAT NO. VHM04-1, VHM04-2, VHM04-3, VHM04-4, VHM04-K

TheWell BIOSCIENCE
Growing Cells in New Dimensions

VitroGel® ORGANOID (1-4) are xeno-free (animal origin-free) hydrogels that supports the growth of patient-derived organoids or organoids developed from pluripotent stem cells (PSCs), co-culture, and PDX model.

VitroGel ORGANOID versions:

- VitroGel ORGANOID-1
- VitroGel ORGANOID-2
- VitroGel ORGANOID-3
- VitroGel ORGANOID-4

Each type of VitroGel ORGANOID (1-4) is formulated with various bio-functional ligands, mechanical strengths, and degradability to fulfill the needs of different organoid culture conditions.

The **VitroGel ORGANOID Discovery Kit** (Cat# VHM04-K) includes all four types of organoid hydrogels. **Use the Discovery Kit to screen which organoid hydrogel version works best for your organoid culture.**

All 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 multiple culture methods including 2D hydrogel coating, 3D cell encapsulation and the unique hydrogel-cell droplet method.

Organoids cultured in this system can be easily harvested out with our enzyme-free VitroGel Cell Recovery Solution (Cat No. MS03-100).

MULTIPLE CULTURE PROTOCOLS THAT FIT YOUR PROJECT

VitroGel ORGANOID system can culture organoids in variety of methods:

- **3D Dome Method (Page 2)**
- **2D Dome Method (Page 3)**
- **2D Hydrogel Coating (Page 4)**
- **3D Cell Culture Encapsulation (Page 5)**
- **Hydrogel-Cell Droplet (A method unique only to VitroGel ORGANOID, Page 6)**

Full control of growth factors with the HYDROGEL SYSTEM

Because the xeno-free VitroGel ORGANOID system is growth factor-free, researchers have full control of the growth factors for enriching the hydrogel system for organoid formation. If the complete organoid culture medium contains critical growth factors/supplements (e.g., R-spondin, Nogin, EGF, B-27 supplement, KOSR, etc.), we recommend preparing a mixing medium/cell suspension with 3X critical growth factors/supplement to mix with VitroGel ORGANOID hydrogel. The mixing ratio of VitroGel ORGANOID hydrogel and mixing medium (or cell suspension) is 2:1 ratio (gel: medium at 2:1 v/v), which makes the concentration of growth factors/supplement in the final hydrogel as 1X.

Note: The 3X growth factors/supplement medium is only used for mixing with the hydrogel solution.
For medium or cells to be added on top of the hydrogel, use regular 1X concentration. Please review the protocols in each method for more information.

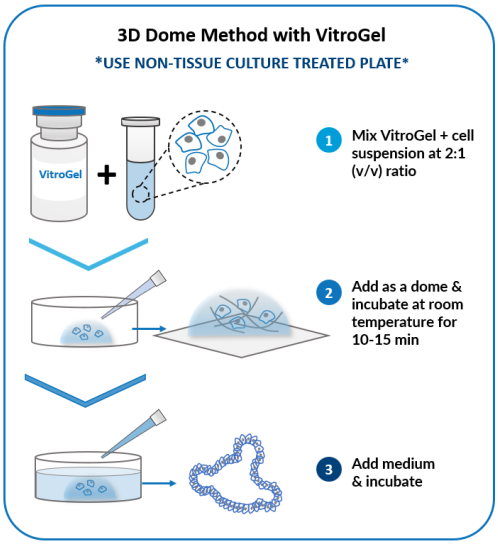
Besides the growth factors, for some organoids (such as mouse intestinal crypts), the hydrogel concentration may need to be optimized. Simply dilute the VitroGel ORGANOID hydrogel solution with DI water (e.g. 1:1 v/v ratio) before mixing the hydrogel solution with the cell medium/cell suspension to help establish a soft hydrogel condition to increase the success rate of the organoid formation.

READY-TO-USE HYDROGEL

3D DOME PROTOCOL

RECOMMENDED MATERIALS AND REAGENTS

- VitroGel ORGANOID
- Organoid culture medium
- Conical tubes (15 mL or 50 mL)
- Serological pipettes
- Supplement/growth factors (depending on culture experiment)
- **NON-TISSUE CULTURE TREATED cell culture vessels (24-well plate)**
  (Using tissue culture treated plates may cause the dome to float)



VitroGel ORGANOID-1 is used as an example below. Replace VitroGel ORGANOID-1 with other versions of choice.

1. Bring VitroGel ORGANOID-1 hydrogel to room temperature.

2. Prepare the cell/organoid suspension in the culture medium.
   Optional: If cells are cultured in a medium with critical supplement/growth factors, prepare cell suspension with 3X critical supplement/growth factors for later mixed with VitroGel for 1X final concentration

3. Add 200 µL VitroGel ORGANOID-1 hydrogel to 100 µL cell suspension and gently pipette up and down 5-10 times to mix thoroughly. (Keep VitroGel and cell suspension at 2:1 v/v mixing ratio)

4. Add 25 µL of the hydrogel-cell mixture to the center of each well of a 24-well plate (non-tissue culture treated).

5. Let the hydrogel stabilize at room temperature for 10-15 min for a soft gel formation.

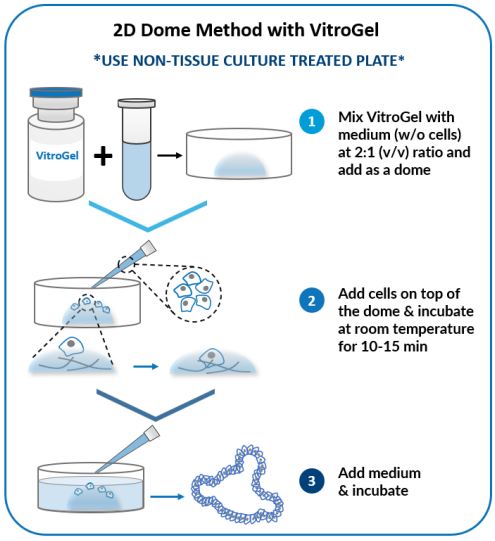
6. Carefully add 1 mL of culture medium to each well of the 24-well plate without disturbing the hydrogel.

7. Place the well plate in an incubator and change the medium according to the experiment's design.
   Note: Recommend changing 50-80% of the same type of medium without disturbing the hydrogel. However, change 100% of the medium if switching to a different medium during organoid culture.

2D DOME PROTOCOL

RECOMMENDED MATERIALS AND REAGENTS

- VitroGel ORGANOID
- Organoid culture medium
- Conical tubes (15 mL or 50 mL)
- Serological pipettes
- Supplement/growth factors (depending on culture experiment)
- **NON-TISSUE CULTURE TREATED cell culture vessels (24-well plate)**
  (Using tissue culture treated plates may cause the dome to float)



VitroGel ORGANOID-1 is used as an example below. Replace VitroGel ORGANOID-1 with other versions of choice.

1. Bring VitroGel ORGANOID-1 hydrogel to room temperature.

2. Add 200 µL VitroGel ORGANOID-1 hydrogel to 100 µL cell medium and gently pipette up and down 5-10 times to mix thoroughly.
   (Keep VitroGel and cell medium at 2:1 v/v mixing ratio)
   Optional: If cells need to be cultured in a medium with critical supplement/growth factors, prepare the medium with 3X critical supplement/growth factors to mix with VitroGel for 1X final concentration.

3. Add 25 µL of the hydrogel-cell mixture to the center of each well of a 24-well plate (non tissue culture treated).

4. Carefully add cells on top of the hydrogel dome.

5. Let the hydrogel stabilize at room temperature for 10-15 min for a soft gel formation.

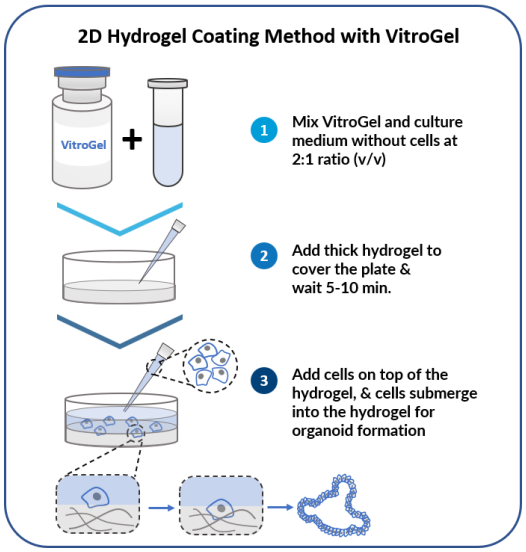
6. Carefully add 1 mL of culture medium to each well of the 24-well plate without disturbing the hydrogel.

7. Place the well plate in an incubator and change the medium according to the experiment's design.
   Note: Recommend changing 50-80% of the same type of medium without disturbing the hydrogel. However, change 100% of the medium if switching to a different medium during organoid culture.

2D HYDROGEL COATING PROTOCOL

RECOMMENDED MATERIALS AND REAGENTS

- VitroGel ORGANOID
- Organoid culture medium
- Conical tubes (15 mL or 50 mL)
- Serological pipettes
- Tissue culture-treated cell culture vessels
- Supplement/growth factors (depending on culture experiment)



Video protocol can be found online:
https://www.thewellbio.com/video-protocols

VitroGel ORGANOID-1 is used as an example below. Replace VitroGel ORGANOID-1 with other versions of choice.

1. Bring VitroGel ORGANOID-1 hydrogel to room temperature.

2. Add 1 mL VitroGel ORGANOID-1 hydrogel to 500 µL cell culture medium (mixing medium) and gently pipette up and down 5-10 times to mix thoroughly. **(Keep VitroGel and cell medium at 2:1 v/v mixing ratio)**
   Optional: If critical growth factors are needed in the hydrogel to support the organoid culture, prepare the cell culture medium with 3X critical growth factors.
3. Transfer the hydrogel mixture to a well plate. Gently tilt/swirl the well plate to ensure there is an even coverage on the bottom of each well.
   The recommended volume of hydrogel for specific well plates is listed below.

	6 well plate	12 well plate	24 well plate	48 well plate	96 well plate
Volume per well	1200 µL	600 µL	300 µL	150 µL	50 µL

4. Wait 5-10 min at room temperature for a soft gel formation.
   Note: During the hydrogel forming process, do not disrupt the hydrogel by tilting or shaking the well plate.
5. Carefully add medium with cells on top of the hydrogel.
   The recommended volume of cell medium for specific well plates is listed below.

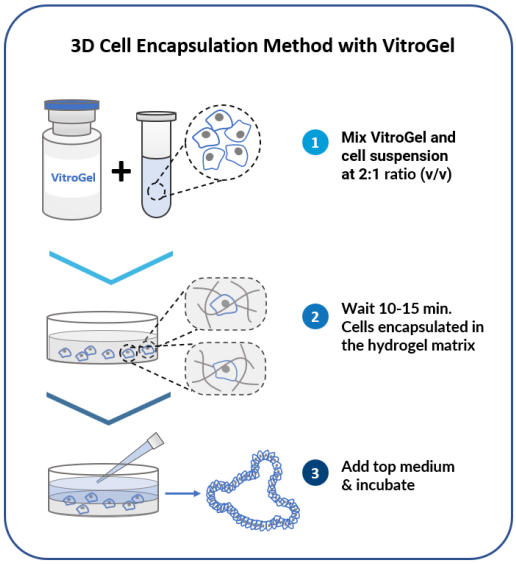
	6 well plate	12 well plate	24 well plate	48 well plate	96 well plate
Volume per well	1200 µL	600 µL	300 µL	150 µL	50 µL

6. Place the well plate in an incubator and change the top medium according to the experiment design.
   Note: Change 50-80% of the top medium with the same type of culture medium used. Change 100% of the top medium if switching to a different medium during organoid culture. Avoid disturbing the hydrogel during medium changes.

3D CELL ENCAPSULATION PROTOCOL

RECOMMENDED MATERIALS AND REAGENTS

- VitroGel ORGANOID
- Organoid culture medium
- Conical tubes (15 mL or 50 mL)
- Serological pipettes
- Tissue culture-treated cell culture vessels
- Supplement/growth factors (depending on culture experiment)



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VitroGel ORGANOID-1 is used as an example below. Replace VitroGel ORGANOID-1 with other versions of choice.

1. Bring VitroGel ORGANOID-1 hydrogel to room temperature.
2. Prepare the cell/organoid suspension in the culture medium.
   Optional: If cells cultured in medium are supplemented with critical growth factors, prepare cell suspension with 3X critical growth factors.
3. Add 1 mL VitroGel ORGANOID-1 hydrogel to 500 µL cell culture medium (mixing medium) and gently pipette up and down 5-10 times to mix thoroughly. **(Keep VitroGel and cell medium at 2:1 v/v mixing ratio)**
   Optional: If critical growth factors are needed in the hydrogel to support the organoid culture, prepare the cell culture medium with 3X critical growth factors.
4. Transfer the hydrogel mixture to a well plate. Gently tilt/swirl the well plate to ensure there is an even coverage on the bottom of each well.
   The recommended volume of hydrogel for specific well plates is listed below.

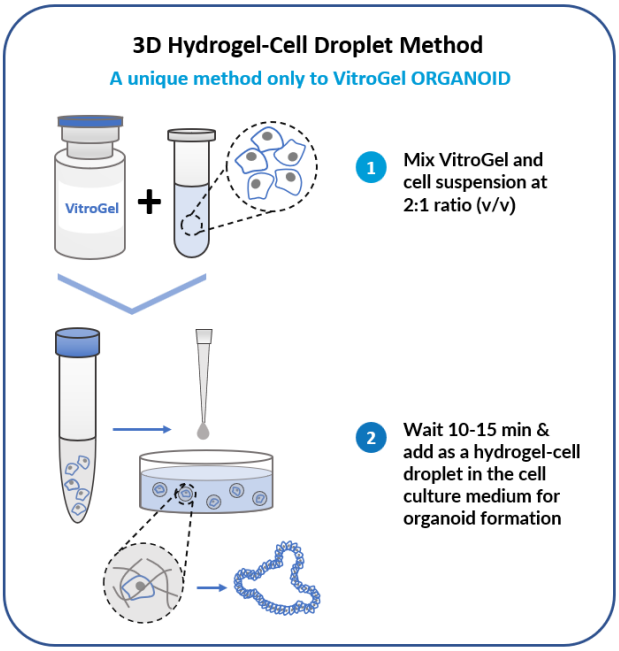
	6 well plate	12 well plate	24 well plate	48 well plate	96 well plate
Volume per well	1200 µL	600 µL	300 µL	150 µL	50 µL

5. Wait 10-15 min at room temperature for a soft gel formation.
   Note: During the hydrogel forming process, do not disrupt the hydrogel by tilting or shaking the well plate.
6. Carefully cover the hydrogel with additional medium.
   The recommended volume of cell medium for specific well plates is listed below.

	6 well plate	12 well plate	24 well plate	48 well plate	96 well plate
Volume per well	1200 µL	600 µL	300 µL	150 µL	50 µL

7. Place the well plate in an incubator and change the top medium according to the experiment's design.
   Note: Change 50-80% of the top medium with the same type of culture medium used. Change 100% of the top medium if switching to a different medium during organoid culture. Avoid disturbing the hydrogel during medium changes.

3D HYDROGEL-CELL DROPLET PROTOCOL



A METHOD UNIQUE ONLY TO VITROGEL

Video protocol can be found online:
https://www.thewellbio.com/video-protocols/

RECOMMENDED MATERIALS AND REAGENTS

- VitroGel ORGANOID
- Organoid culture medium
- Conical tubes (15 mL or 50 mL)
- Serological pipettes
- Tissue culture-treated cell culture vessels
- Growth factors (depending on culture experiment)

VitroGel ORGANOID-1 is used as an example below. Replace VitroGel ORGANOID-1 with other versions of choice.

1. Bring VitroGel ORGANOID-1 hydrogel to room temperature.
2. Add 1 mL VitroGel ORGANOID-1 hydrogel to 500 µL cell suspension and gently pipette up, down 5-10 times to mix thoroughly, and let mixture stabilize at room temperature for 10-15 min.
   (Keep VitroGel and cell suspension at 2:1 v/v mixing ratio)
   Optional: If critical growth factors are needed in the hydrogel to support the organoid culture, prepare the cell suspension with 3X critical growth factors.
3. Add cell culture medium to the well plate.
   The recommended volume of hydrogel for specific well plates is list below.

	6 well plate	12 well plate	24 well plate	48 well plate	96 well plate
Volume per well	3000 µL	1500 µL	750 µL	300 µL	100 µL

4. Carefully add the Hydrogel-Cell mixture into the well plate as droplets. (roughly 5-10 droplets per 100 µL of Hydrogel-Cell mixture.
   Optional Tips:
   a. Control the size of Hydrogel-Cell beads by adjusting the volume of the droplets.
      -For small beads, 1-5 µL per droplet
      -For large beads, 20-50 µL per droplet
   b. Create a droplet on the pipette tip. Lower the droplet and allow to contact the surface of the culture medium to release the droplet.
5. Place the well plate in an incubator and change the medium according to the experiment design without disrupting the hydrogel beads.
   Note: Change 50-80% of the top medium with the same type of culture medium used. Change 100% of the top medium if switching to a different medium during organoid culture. Avoid disturbing the hydrogel during medium changes.

CELL HARVESTING FROM ORGANOID CULTURE

Use our enzyme-free **VitroGel Cell Recovery Solution** (Cat. No. MS03-100) to harvest organoid cells easily and efficiently from the hydrogel.

Read the VitroGel Cell Recovery Solution protocol for more details.
https://www.thewellbio.com/product/cell-harvesting-solution/

A link to a video protocol for oganoid cell harvesting can be found here:
https://www.thewellbio.com/3d-organoid-cell-harvesting/

ORDERING INFORMATION

Cat. No.	Product Name
VHM04-1	VitroGel ORGANOID-1 (10 mL)
VHM04-2	VitroGel ORGANOID-2 (10 mL)
VHM04-3	VitroGel ORGANOID-3 (10 mL)
VHM04-4	VitroGel ORGANOID-4 (10 mL)
VHM04-K	VitroGel ORGANOID Discovery Kit
MS03-100	VitroGel Cell Recovery Solution