PROTOCOL

VITROGEL® ORGANOID

CAT NO. VHM04-1, VHM04-2, VHM04-3, VHM04-4, VHM04-K (includes VHM04-1S, VHM04-2S, VHM04-3S, VHM04-4S)

VitroGel® ORGANOID is a xeno-free (animal origin-free) hydrogels that support the growth of patient-derived organoids or organoids developed from pluripotent stem cells (PSCs).

The VitroGel ORGANOID Discovery Kit (Cat# VHM04-K) includes all four types of 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.

BY TESTING ALL FOUR TYPES, YOU CAN CONCLUDE WHICH HYDROGEL PERFORMS BEST FOR YOUR ORGANOID CULTURE.

CHOOSE THE CULTURE METHOD THAT FITS YOUR PROJECT

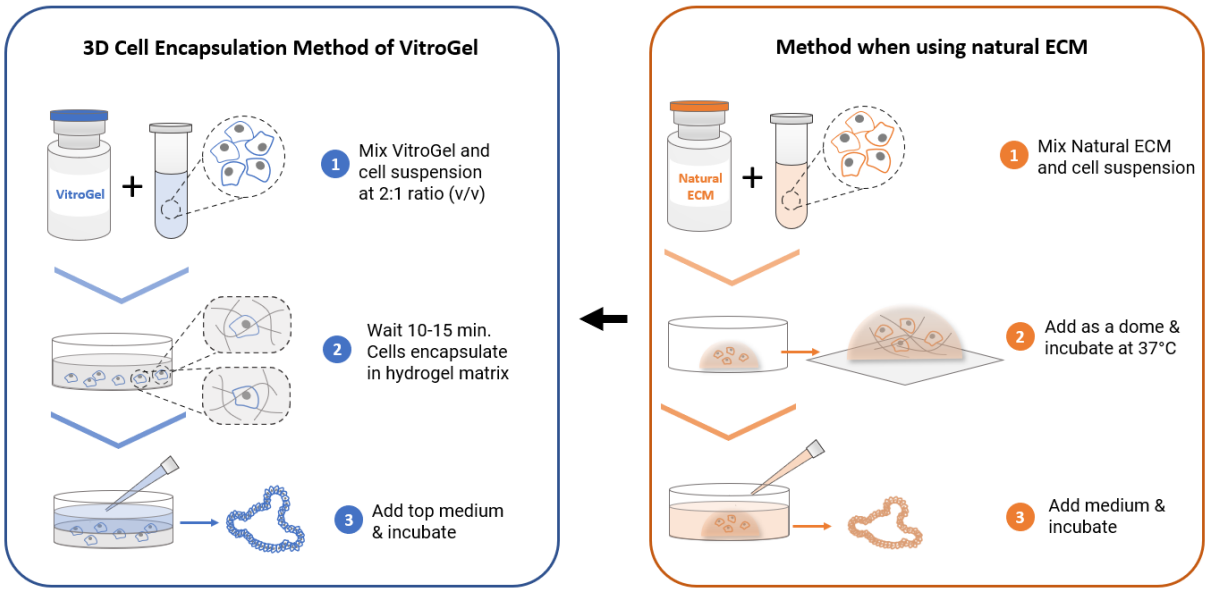
VitroGel ORGANOID system can culture organoids in variety of methods:

- 3D cell culture encapsulation (page 1)
- 2D hydrogel coating (page 3)
- Hydrogel-Cell droplet (A unique method only to VitroGel ORGANOID, Page 4)

Review all the following VitroGel ORGANOID protocol methods compared to the natural ECM methods and choose the one that best fits your project.

PROTOCOLS:

3D Cell Culture 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
- 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 to room temperature or warm at 37°C.

2. Prepare the cell/organoid suspension in the culture medium.
   Optional: If cells cultured in medium 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 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. 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

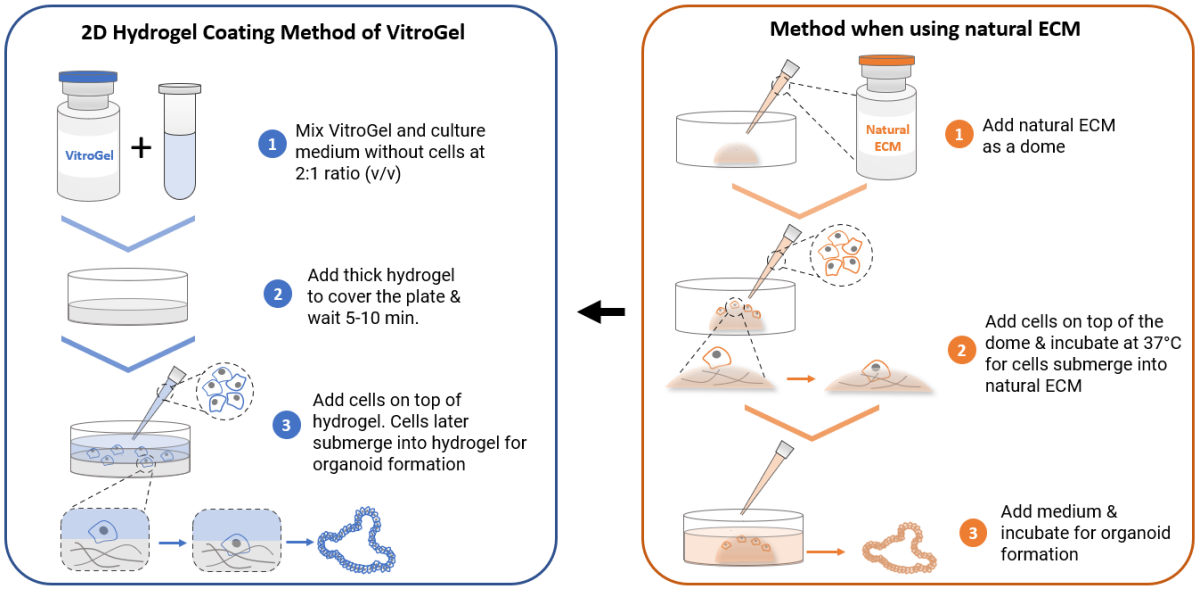
5. Wait 10-15 min at room temperature for a soft gel formation.
   Note: During the hydrogel forming process, do not disrupt the hydrogel by tilting or shaking the well plate.

6. Carefully cover the hydrogel with additional medium.
   The recommended volume of cover 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: Recommend changing 50-80% of the same type of top medium without disturbing the hydrogel. However, change 100% of the top 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
- Growth factors (depending on cultur 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 or warm at 37°C.

2. Add 1 mL VitroGel ORGANOID-1 hydrogel to 500 µL cell culture 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

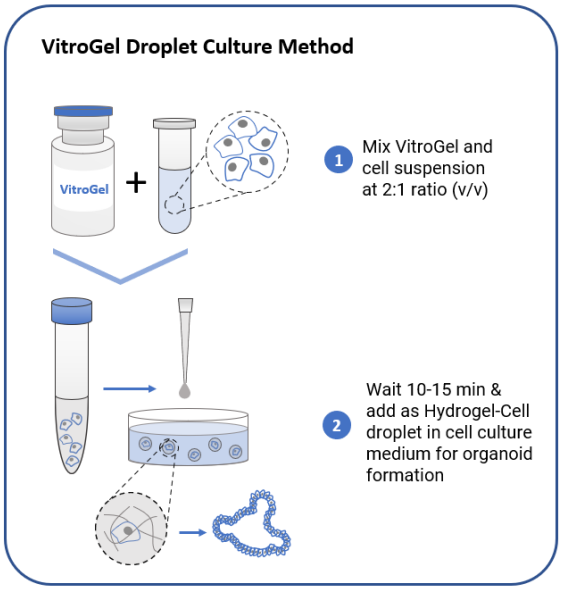
4. Wait 10-15 min at room temperature for a soft gel formation.
   Note: During the hydrogel forming process, do not disrupt the hydrogel by tilting or shaking the well plate.

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: Recommend changing 50-80% of the same type of top medium without disturbing the hydrogel. However, change 100% of the top medium if switching to a different medium during organoid culture.

Hydrogel-Cell Droplet Protocol



A UNIQUE METHOD ONLY TO VITROGEL

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 cultur 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 or warm to 37°C.

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: Recommend changing 50-80% of the same type of medium without disturbing the hydrogel. However, change 100% of the top medium if switching to a different medium during organoid culture.

ORDERING INFORMATION FOR FULL PRODUCT

SKU	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)