

## 3D Culture



1. Bring hydrogel to room temperature.



2. Prepare cells in culture medium.

Recommend cell concentration of  $0.5\text{--}2 \times 10^6$  cells/mL  
 If cells cultured in medium supplemented with FBS (e.g. 10%) or critical growth factors, prepare cell suspension in 30% FBS with 3X critical growth factors.



3. Pipette mix 1 mL VitroGel with 500  $\mu$ L cell suspension.

Keep VitroGel and cell suspension at 2:1 v/v mixing ratio.

4. Transfer hydrogel mixture to a well plate.

Plate Type	6 well	12 well	24 well	48 well	96 well
Volume/well	1200 $\mu$ L	600 $\mu$ L	300 $\mu$ L	150 $\mu$ L	50 $\mu$ L



5. Wait 10-15 min at room temperature.

6. Carefully cover hydrogel with additional medium.

Plate Type	6 well	12 well	24 well	48 well	96 well
Volume/well	1200 $\mu$ L	600 $\mu$ L	300 $\mu$ L	150 $\mu$ L	50 $\mu$ L



7. Incubate and change cover medium every 48 hours.

## 2D Hydrogel Coating Culture



1. Bring hydrogel to room temperature.



2. Pipette mix 1 mL VitroGel with 500  $\mu$ L cell medium.

Keep VitroGel and cell medium at 2:1 v/v mixing ratio.  
 If cells cultured in medium supplemented with FBS (e.g. 10%) or critical growth factors, prepare the cell culture medium with 30% FBS or 3X critical growth factors.

3. Transfer hydrogel mixture to a well plate.



Plate Type	6 well	12 well	24 well	48 well	96 well
Volume/well	1200 $\mu$ L	600 $\mu$ L	300 $\mu$ L	150 $\mu$ L	50 $\mu$ L



4. Wait 10-15 min at room temperature.

5. Carefully add medium with cells on top of the hydrogel.

Recommend cell concentration of  $1\text{--}5 \times 10^5$  cells/mL



Plate Type	6 well	12 well	24 well	48 well	96 well
Volume/well	1200 $\mu$ L	600 $\mu$ L	300 $\mu$ L	150 $\mu$ L	50 $\mu$ L



6. Incubate and change cover medium every 48 hours.

## Hydrogel Preparation for Animal Injection



1. Bring hydrogel to room temperature.



2. Prepare cell suspension in PBS.

Adjust the cell/molecular concentration accordingly to experiment (prepare cell suspension at 2X desired concentration for later mixed with VitroGel for 1X final concentration)



3. Pipette mix VitroGel with cell suspension at 1:1 (v/v) ratio.

Example: 1 mL VitroGel to 1 mL cell suspension in PBS.  
See protocol online for mixing ratio if using different medium for cell suspension.



4. Transfer hydrogel mixture to a syringe.



5. Stabilize mixture for 15 min at room temperature or by putting on ice or at 4 °C for 5-10 min.



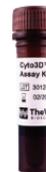
6. Hydrogel mixture is ready for animal injection.

More on *in vivo* injection applications:  
[thewellbio.com/applications/in-vivo](http://thewellbio.com/applications/in-vivo)

## Don't Forget These...

### Cyto3D® Live-Dead Assay Kit

Live-dead cell viability analysis for 3D & 2D culture.



- ▶ Fast one-step staining
- ▶ Sensitive
- ▶ No washing step needed
- ▶ Excellent for high-throughput

Cyto3D® Live-Dead Assay Kit  
Cat No. **BM01**

### VitroGel® Organoid Recovery Solution

Recover organoids/cells from VitroGel hydrogels or an animal-based ECM within 15 minute!



- ▶ Fast ECM Dissociation
- ▶ High yield, high viability
- ▶ Safe harvesting
- ▶ Stable formulation
- ▶ Room temp operation
- ▶ 3D and 2D ECM

VitroGel® Organoid Recovery Solution  
Cat No. **MS04-100**