

VitroGel® 3D

Catalog Numbers:
TWG001 (10 mL)
TWG001S (2 mL)

Usage restrictions: For Research Use Only. Not For Use In Diagnostic Procedures.

PRODUCT DESCRIPTION

VitroGel® 3D is a pure and unmodified hydrogel that allows the 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 hydrogels closely mimic the natural extracellular matrix (ECM) environment. The hydrogel system is ready-to-use at room temperature, has a neutral pH, transparent, permeable and compatible with different imaging systems. The tunability of the hydrogel gives the ability to create an optimized environment for cell growth. The solution transforms into a hydrogel matrix by simply mixing with the cell culture medium. No cross-linking agent is required. The hydrogel strength can be adjusted with VitroGel® Dilution Solution. Cells cultured in this system can be easily harvested out with our VitroGel® Cell Recovery Solution. The hydrogel can also be tuned to be injectable for in vivo studies.

“Mix & Match” Unique to the VitroGel system is the ability to blend the different types of VitroGel to create a customized multi-functional hydrogel.

SPECIFICATIONS	
Use	Good for cell spheroid formation, suspension cells or cells requiring low cell-matrix interactions.
Formulation	Xeno-free. Unmodified polysaccharide based hydrogel.
Hydrogel strength	10 ~ 4,000 Pa of G' depending on dilution ratio. Use VitroGel Dilution Solution.
Physical State	Liquid
pH	Neutral
Color	Transparent
Cell Recovery	Use VitroGel Cell Recovery Solution
Storage	Store at 2-8°C. Ships at room temperature.
Stability	15 months from date of manufacture

USAGE TABLE						
10 mL VitroGel in a 24-well plate at 300 µL sample per well	at 1:1 dilution 3.5 X 24-well plate		at 1:3 dilution 7 X 24-well plate		at 1:5 dilution >10 X 24-well plate	
	µL/well	mL/plate	µL/well	mL/plate	µL/well	mL/plate
VitroGel	120 µL	2.88 mL	60 µL	1.44 mL	40 µL	0.96 mL
Dilution Solution	120 µL	2.88 mL	180 µL	1.44 mL	200 µL	4.8 mL
Cells	60 µL	1.44 mL	60 µL	1.44 mL	60 µL	1.44 mL

GUIDELINE FOR USE

[Download the full handbook for detail usage at www.thewellbio.com/handbook](http://www.thewellbio.com/handbook)

- Bring VitroGel to room temperature and warm cell culture medium to 37°C if needed.
- Adjust the concentration of VitroGel for different cell types by diluting the VitroGel with VitroGel Dilution Solution. After dilution, gently mix the diluted VitroGel with a cell suspension (in the desired media) without introducing bubbles. Please check the Table 1 below for suggested solution/medium volume of different dilutions.

Table 1. Volumes of solution/medium for different hydrogel dilutions for 3D cell culture (each well of a 24-well plate)

Dilution Ratio	VitroGel	Dilution Solution	Cell Medium with Cells
1:0	240 µL	0 µL	60 µL
1:1	120 µL	120 µL	60 µL
1:2	80 µL	160 µL	60 µL
1:3	60 µL	180 µL	60 µL
1:5	40 µL	200 µL	60 µL

If cells are to be cultured in complete cell culture medium with 10% FBS or other critical growth factors/supplement, prepare the cell suspension by following the step below:

- a. Prepare 100% FBS with 10X of critical growth factors.
- b. Prepare cells in regular 1X cell culture medium. (Do not make the medium at a high concentration as the ionic molecules would affect the hydrogel formation.)
- c. Mix the solution from step a) and b) to get cell suspension in 50% FBS with 5X critical growth factors
- d. Mix the diluted VitroGel with cell suspension at 4:1 v/v ratio (eg.400 μ L diluted VitroGel with 100 μ L cell suspension).

Note: If the cells need to culture at a higher FBS concentration (eg. 20%), prepare cells suspension directly in 100% FBS. Prepare the diluted VitroGel by mixing VitroGel with VitroGel Dilution Solution and wait 30-60 min before mixing it with cell suspension. Wait 20-30 min at room temperature (or 37°C) before adding the cover medium on top.

3 Transfer the hydrogel mixture to a well plate. Gently tilt/swirl the well plate to ensure there is an even coating on the bottom of each well.

Note: If the cells need to culture at a higher FBS concentration (eg. 20%), prepare cells suspension directly in 100% FBS. Prepare the diluted VitroGel by mixing VitroGel with VitroGel Dilution Solution and wait 30-60 min before mixing it with cell suspension. Wait 20-30 min at room temperature (or 37°C) before adding the cover medium on top.

Table 2. Recommended hydrogel volume for WELL PLATES

WELL PLATE	Volume of hydrogel (μ L)	Volume of Cover Medium (μ L)
6 well plate	1200	1200
12 well plate	600	600
24 well plate	300	300
48 well plate	150	150
96 well plate	75	75

Table 3. Recommended hydrogel volume for PLATE INSERTS

PLATE INSERTS	Volume of hydrogel (μ L)	Volume of Cover Medium (μ L)
6 well plate	800	800
12 well plate	400	400
24 well plate	200	200
48 well plate	100	100
96 well plate	50	50

4. Wait 10-20 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. After soft gel formation, GENTLY tilt the well plate to check if hydrogel has formed and attached firmly to the bottom of the well plate.
6. Carefully cover hydrogel with additional medium to further stabilize the hydrogel. See Table 2 or Table 3 for recommended volume of cover medium.
7. Place the well plate in an incubator and change the cover medium every 48 hours.
Note: We recommend to only change 60-80% of the top medium without disturbing the hydrogel.

RELATED PRODUCTS

- VitroGel Dilution Solution TYPE 1 (MS01-100)
- VitroGel Dilution Solution TYPE 2 (MS02-100)
- VitroGel Cell Recovery Solution (MS03-100)
- Other versions of VitroGel - www.thewellbio.com/hydrogels

REFERENCES

1. Xiao, M., Qiu, J., Kuang, R., Zhang, B., Wang, W., & Yu, Q. (2019). Synergistic effects of stromal cell-derived factor-1 α and bone morphogenetic protein-2 treatment on odontogenic differentiation of human stem cells from apical papilla cultured in the VitroGel 3D system. *Cell and Tissue Research*, 378(2), 207–220. <https://doi.org/10.1007/s00441-019-03045-3>
2. Wang, F., Nan, L., Zhou, S., Liu, Y., Wang, Z., Wang, J., Feng, X., & Zhang, L. (2019). Injectable Hydrogel Combined with Nucleus Pulposus-Derived Mesenchymal Stem Cells for the Treatment of Degenerative Intervertebral Disc in Rats. *Stem Cells International*, 2019, 1–17. <https://doi.org/10.1155/2019/8496025>
3. Kim, E. J., Yang, C., Lee, J., Youm, H. W., Lee, J. R., Suh, C. S., & Kim, S. H. (2019). The new biocompatible material for mouse ovarian follicle development in three-dimensional in vitro culture systems. *Theriogenology*. <https://doi.org/10.1016/j.theriogenology.2019.12.009>
4. Huang, J. 3D Cell Culture on VitroGel System. *HSOA Journal of Cytology and Tissue Biology*. <https://doi.org/10.24966/CTB-9107/S1001>