

# VitroGel® 3D High Concentration

Catalog Numbers:  
TWG001

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

## Product Description

VitroGel® 3D High Concentration is a xeno-free hydrogel system that allows the maximum flexibility to manipulate the 3D cell culture environment for different needs. VitroGel 3D High Concentration comes with VitroGel Dilution Solution to adjust the final hydrogel strength from 10 to 4000 Pa. The tunability of the hydrogel gives the ability for researchers to create an optimized environment for cell growth. The VitroGel 3D High Concentration hydrogel matrix structure is good for cell spheroid formation, suspension cells or cells requiring low cell-matrix interactions.

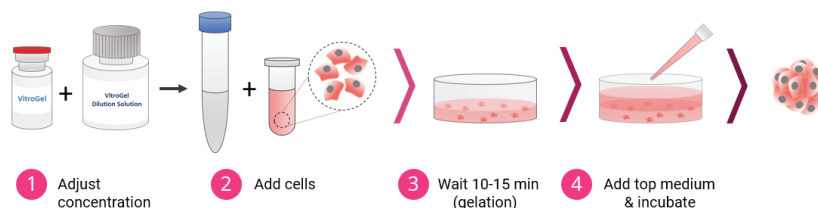
VitroGel® High Concentration hydrogels are our xeno-free, tunable hydrogels for researchers wanting full control to manipulate the biophysical and biological properties of the cell culture environment. The tunability of the hydrogel gives the ability to create an optimized environment for cell growth. The hydrogel system has 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. No cross-linking agent is required. Cells cultured in this system can be easily harvested for further studies. The hydrogel is also injectable for *in vivo* studies. From 3D cell culture, 2D coating to animal injection, VitroGel makes it possible to bridge the *in vitro* and *in vivo* studies with the same platform system.

“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	
Contents	VitroGel® 3D High Concentration, 3 mL VitroGel® Dilution Solution, 50 mL
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
Cell Recovery	Use VitroGel® Cell Recovery Solution (Cat# MS03-100)
Storage	Store hydrogel at 2-8°C. Ships at ambient temperature.
Stability	24 months from date of manufacture
Uses	200 uses at 1:3 dilution for 96 well plate

## VitroGel High Concentration Workflow

VitroGel High Concentration hydrogels are easy-to-use. There is no cross-linking agent required. Work confidently at room temperature.



**Protocol** Visit [www.thewellbio.com/faq-hydrogel](http://www.thewellbio.com/faq-hydrogel) for frequently asked questions on cell culture preparation and operation  
More protocols can be found at [www.thewellbio.com/protocols](http://www.thewellbio.com/protocols)

1. Bring VitroGel to room temperature and warm cell culture medium to 37°C if needed.
2. 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.  
(Recommend cell concentration of  $0.5-2 \times 10^6$  cells/mL)  
See 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  $\mu$ L diluted VitroGel with 100  $\mu$ 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.

Table 2. Recommended hydrogel volume for WELL PLATES

WELL PLATE	Volume of hydrogel ( $\mu$ L)	Volume of Cover Medium ( $\mu$ 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 ( $\mu$ L)	Volume of Cover Medium ( $\mu$ 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 Cell Recovery Solution (MS03-100)
- Other versions of VitroGel High Concentration - [www.thewellbio.com/hc-hydrogels](http://www.thewellbio.com/hc-hydrogels)

## References

1. Xiao, M., Qiu, J., Kuang, R., Zhang, B., Wang, W., & Yu, Q. (2019). Synergistic effects of stromal cell-derived factor-1 $\alpha$  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>

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