

## VitroGel® Dilution Solution

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

### PRODUCT DESCRIPTION

VitroGel® Dilution Solution is a ready-to-use solution use to mix with the VitroGel system to adjust the hydrogel concentration for different hydrogel strength.

The VitroGel Dilution Solution is room temperature stable and neutral in pH which helps maintain a good physiological condition for hydrogel formation. The VitroGel Dilution Solution can accelerate the hydrogel formation in combine with cell medium. The VitroGel Dilution Solution can be used to prepare a VitroGel thin gel coating plate or an injectable hydrogel.

The VitroGel Dilution Solution is offered in two types:

TYPE 1: Contains sucrose to maintain the best osmolarity.

TYPE 2: Sucrose free formulation for cells sensitive to the sugar level in the medium

#### MS01-50 and MS01-100 (VitroGel Dilution Solution TYPE 1)

Specifications	
Use	Dilute/adjust the hydrogel concentration of the VitroGel system
Formulation	Contains sucrose for maintaining best osmolarity
pH	Neutral
Physical State	Liquid
Color	Clear
Storage	Room temperature (+15-30°C)
Stability	30 months from date of manufacture

#### MS02-50 and MS02-100 (VitroGel Dilution Solution TYPE 2)

Specifications	
Use	Dilute/adjust the hydrogel concentration of the VitroGel system
Formulation	Sucrose free formulation for cells sensitive to the sugar level in the medium
pH	Neutral
Physical State	Liquid
Color	Clear
Storage	Room temperature (+15-30°C)
Stability	30 months from date of manufacture

### GUIDELINE FOR USE

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

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. 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  $\mu$ 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.

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 ( $\mu$ 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 Hydrogel Matrix (VHM01)
- All versions of VitroGel High Concentration - [www.thewellbio.com/hc-hydrogels](http://www.thewellbio.com/hc-hydrogels)
- VitroGel Cell Recovery Solution (MS03-100)

## REFERENCES

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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>
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