

VitroGel[®] MSC

Catalog Numbers:
VHM03, VHM03S

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

Product Description

VitroGel[®] MSC is a xeno-free (animal origin-free) hydrogel system developed to support three-dimensional (3D) cultures of mesenchymal stem cells (MSCs). This hydrogel system can be used to make hydrogel cell beads for MSC scale-up. Microcarriers are not required for MSC scale-up.

VitroGel MSC is ready-to-use with an optimized formulation to support the rapid expansion of MSCs. Cells directly thawed from liquid nitrogen or passaged from 2D culture vessels can be immediately mixed with the hydrogel solution for 3D culture or Hydrogel-Cell beads generation. This hydrogel system is compatible with most MSC culture media and tissue culture vessels. By using the VitroGel Cell Recovery Solution, the cell harvesting after 3D culture is simple and effective.



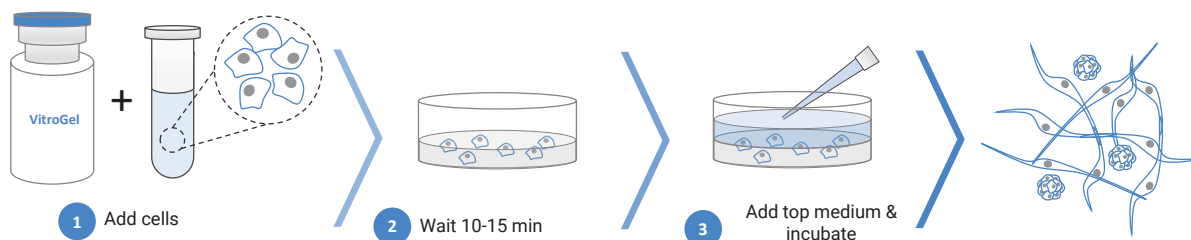
VitroGel MSC is ready-to-use. Just mix with your cells. There is no cross-linking agent or the need to adjust the hydrogel concentration. Simple 20 minute protocol.

SPECIFICATIONS	
Formulation	Xeno-free. Polysaccharide based functional hydrogel
Use	3D cell culture, 2D hydrogel coating, Hydrogel-Cell bead formation
Operation	Ready-to-use at room temperature
Biocompatibility	Biocompatible, safe for animal studies
Injection	Contact support@thewellbio.com for more information.
Cell Harvesting	Use VitroGel Cell Recovery Solution (Cat# MS03-100)
pH	Neutral
Storage	Store at 2-8°C. Ships at ambient temperature.
Stability	24 months from date of manufacture.
Uses	60 uses for each 2 mL bottle at 50 µL/test 300 uses for each 10 mL bottle at 50 µL/test

PROTOCOLS

Visit 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

3D Cell Culture Protocol



1. Bring VitroGel MSC to room temperature or warm at 37°C.
2. Prepare the MSC suspension in the culture medium.
 - Recommended cell concentration > 0.8 x 10⁶ cells/mL.
 - Optional: If culture medium contains critical supplement (e.g. 2% Human Platelet Lysate (HPL), prepare cell suspension with 3X supplement (e.g. 6% HPL).

- Add 1 mL VitroGel MSC hydrogel to 500 μL cell suspension and gently pipette up and down 5-10 times to mix thoroughly. **(Keep VitroGel and cell medium at 2:1 v/v mixing ratio.)**
- 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.

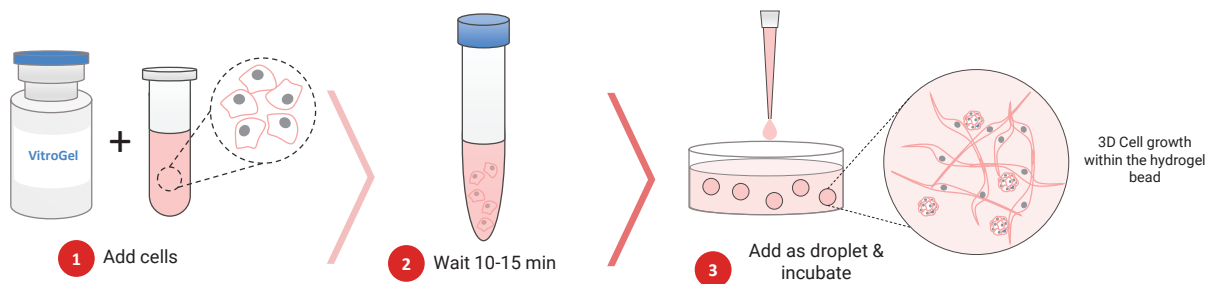
	6 well plate	12 well plate	24 well plate	48 well plate	96 well plate
Volume per well	1200 μL	600 μL	300 μL	150 μL	50 μL

- 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.
- 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

- Place the well plate in an incubator and change the cover medium every 48 hours.
(Note: We recommend to only change 50-80% of the top medium without disturbing the hydrogel).

Hydrogel-Cell Bead Protocol Can be adapted for MSC scale-up



- Bring VitroGel MSC to room temperature or warm at 37°C.
- Prepare the MSC suspension in the culture medium.
 - Recommended cell concentration > 0.8×10^6 cells/mL.
 - Optional: if culture medium contains critical supplement (e.g. 2% Human Platelet Lysate (HPL), prepare cell suspension with 3X supplement (e.g. 6% HPL)
- Add 1 mL VitroGel MSC hydrogel to 500 μL cell suspension and gently pipette up and down 5-10 times to mix thoroughly. **(Keep VitroGel and cell medium at 2:1 v/v mixing ratio.) Wait 10-15 minutes at room temperature for stabilization.**
- Add cell culture medium to the well plate. 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 of cell culture medium per well	3000 μL	1500 μL	750 μL	300 μL	100 μL

- Using a pipettor with a 100 μL tip, carefully add the Hydrogel-Cell mixture from step 3 into the well plate as droplets (roughly 5-10 droplets per 100 μL of Hydrogel-Cell mixture). The ratio between hydrogel-cell mixture and cell culture medium in the well plate is about 1:5 (v/v) (e.g. 600 μL hydrogel-cell mixture for 3 mL cell culture medium in each well of a 6-well plate).

Optional Tips:

- 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
 - Create a droplet on the pipette tip. Lower the droplet and allow to contact the surface of the culture medium to release the droplet.
- Place the well plate in an incubator and change the medium every 48-72 hours.
(Note: We recommend to only change 50-80% of the top medium without disturbing the hydrogel beads).

2D Hydrogel Coating Protocol

1. Bring VitroGel MSC to room temperature or warm at 37°C.
2. Add 1 mL VitroGel MSC 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 culture medium contains critical supplement (e.g. 2% Human Platelet Lysate (HPL), prepare culture medium with 3X supplement (e.g. 6% HPL) to mix with VitroGel MSC to get 1X final concentration of supplement.
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 hydrogel. (Recommend cell concentration of 5×10^5 cells/mL). 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 cover medium every 48 hours.
Note: We recommend to only change 50-80% of the top medium without disturbing the hydrogel.

Cell Recovery from VitroGel MSC Protocol

- For 3D cell culture and 2D hydrogel coating, refer to Protocol-1 of the VitroGel Cell Recovery Solution Protocol.
- For hydrogel-cell bead culture, refer to Protocol-2 of the VitroGel Cell Recovery Solution Protocol
- Contact support@thewellbio.com for further suggestions.

Animal Injection Protocol for *In Vivo* Studies

Please contact support@thewellbio.com for injection options for *in vivo* studies.

Related Products

- VitroGel® Cell Recovery Solution (MS03-100)
- Other versions of VitroGel - www.thewellbio.com/hydrogels

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