PROTOCOL

VitroGel® MSC

CAT NO. VHM03, VHM03S

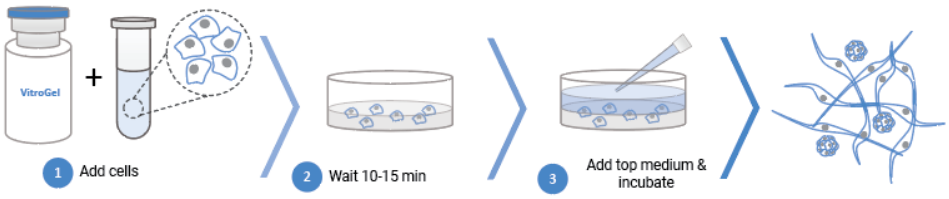
TheWell BIOSCIENCE

Growing Cells in New Dimensions

RECOMMENDED MATERIALS AND REAGENTS

- VitroGel® MSC (Cat# VHM03)
- Cells
- Cell culture medium
- Conical tubes (15 mL or 50 mL)
- Micropipette; low retention pipette tips
- Centrifuge
- Cell culture plate

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 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).
3. Add 1 mL VitroGel MSC to 500 µL cell suspension and gently pipette up and down 5-10 times to mix thoroughly. (Keep VitroGel and cell suspension at 2:1 v/v mixing ratio).
4. Transfer the hydrogel mixture to a well plate. Gently tilt/swirl the well plate to ensure there is an even covering on the bottom of each well. The recommended volumes of hydrogel mixture for specific well plate types are list below.

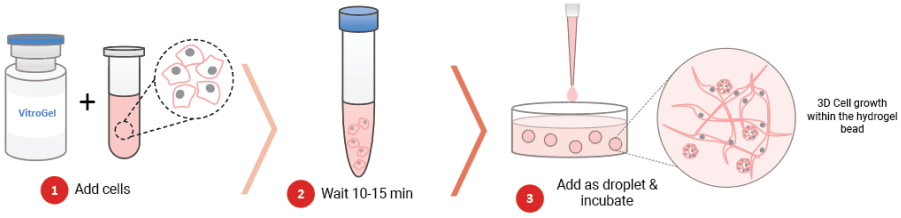
	6 well plate	12 well plate	24 well plate	48 well plate	96 well plate
Volume per well	1200 µL	600 µL	300 µL	150 µL	50 µL

5. 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.
6. Carefully add additional medium to cover the hydrogel. The recommended volumes of cover medium for specific well plate types are list 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

7. 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



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 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).
3. Add 1 mL VitroGel MSC to 500 µL cell suspension and gently pipette up and down 5-10 times to mix thoroughly. (Keep VitroGel and cell suspension at 2:1 v/v mixing ratio). Incubate the hydrogel-cell mixture at room temperature for 10-15 minutes. The hydrogel-cell mixture will be further used in step 5.
4. Add cell culture medium to the well plate. The recommended volumes of cell medium for specific well plate types are 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

5. Using a pipettor with a 100 µL tip, carefully pipette 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: Control the final size of the hydrogel-cell beads by adjusting the volume of the droplets. For small beads, 1-5 µL per droplet and for large beads, 20-50 µL per droplet).
   Tip: Press the pipette plunger to create a droplet on the pipette tip, lower the pipette tip to release the droplet by contacting the surface of culture medium.
6. 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 1mL VitroGel MSC to 500µL cell culture medium and gently pipette up and down 5-10 times to mix thoroughly.
   Note: 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 covering on the bottom of each well. The recommended volumes of hydrogel mixture for specific well plate types are 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 x 10^5 cells/mL). The recommended volumes of cell medium for specific well plate types are 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.

Protocol for Cell Recovery from VitroGel MSC

- 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."

Animal Injection Protocol for *In Vivo* Studies

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