PROTOCOL

VitroGel® HEK293

CAT NO. VHM05, VHM05S

RECOMMENDED MATERIALS AND REAGENTS

- VitroGel® HEK293 (Cat# VHM05)
- 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 HEK293 to room temperature or warm at 37°C.
2. Prepare the cell suspension in the culture medium.
   - Recommended cell concentration 0.5-2 x 10^6 cells/mL.
   - Optional: If culture medium contains critical supplement (e.g. 10% FBS, prepare cell suspension with 3X supplement (e.g. 30% FBS).
3. Add 1mL VitroGel HEK293 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 volume of hydrogel for well plates is 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 volume of cover medium for 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

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).

3D Static Suspension Culture Protocol

1. Bring VitroGel HEK293 to room temperature or warm at 37°C.
2. Prepare the cell suspension in the culture medium.
   - Recommended cell concentration 0.5-2 x 10^6 cells/mL.
   - Optional: If culture medium contains critical supplement (e.g. 10% FBS, prepare cell suspension with 3X supplement (e.g. 30% FBS).
3. Add 1mL VitroGel HEK293 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. Add cell culture medium to the cell-hydrogel mixture at 3:1 v/v ratio (e.g. mix 4.5 mL cell culture medium with 1.5 mL of cell-hydrogel mixture). Carefully pipette up and down to mix the medium and mixture homogeneously.
5. Add the mixture to the well plate and incubate at 37°C with 5% CO2. The recommended volume of mixture for well plates is list below.

	6 well plate	12 well plate	24 well plate	48 well plate	96 well plate
Volume per well	3000 µL	1500 µL	750 µL	300 µL	100 µL

Note:

- For 3-4 day culture with low cell seeding density, no medium change is needed.
- If long term culture, add additional medium directly to the mixture after day 3.
- If additional culture medium is added more then one time or the initial cell seeding density is higher than 2 x 10^6 cells/mL, an orbital shaker may be needed at a speed of 10-40 rpm to maintain the cell suspension.

Subculture of 3D HEK293 spheroids from 3D static suspension culture:

Please check the check the Protocol-3 of VitroGel Cell Recovery Solution to harvest the HEK293 spheroids from the hydrogel.

- The collected cell spheroids can be directly resuspended with cell culture medium and mixed with VitroGel HEK293 for subculture
- Or, the collected cell spheroids can be dissociated into single cells by using trypsin. Remove the trypsin by centrifuging and resuspend the cells with cell culture medium for subculture.

2D Hydrogel Coating Protocol

1. Bring VitroGel HEK293 to room temperature or warm at 37°C.
2. Add 1mL VitroGel HEK293 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. 10% FBS, prepare culture medium with 3X supplement (e.g. 30% FBS) to mix with VitroGel HEK293 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 volume of hydrogel for 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 x 10^5 cells/mL). The recommended volume of cell medium for 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).

Protocol for Cell Recovery from VitroGel HEK293

- For 3D cell culture and 2D hydrogel coating, refer to Protocol-1 of the VitroGel Cell Recovery Solution Protocol.
- For 3D static suspension culture, please refer to Protocol-3 of the VitroGel Cell Recovery Solution Protocol."