TheWell BIOSCIENCE

Growing Cells in New Dimensions

Product Data Sheet

Revision v1.0

VitroGel® HEK293

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

Catalog Numbers:
VHM05, VHM05S

Product Description

VitroGel® HEK293 is a xeno-free (animal origin-free) functional hydrogel system developed to support three-dimensional (3D) cultures of human embryonic kidney 293 (HEK293) cells.

VitroGel HEK293 is ready-to-use with an optimized formulation to fully support the 3D growth of HEK293 cells. Both our standard 3D cell culture protocol and the 3D static suspension culture protocols can be used for rapid cell expansion.

Cells directly thawed from liquid nitrogen or passaged from 2D culture vessels can be immediately mixed with the hydrogel solution for static suspension cultures. The high-quality 3D spheroids enhance the cell performance for protein expression and downstream applications. HEK293 cells cultured in VitroGel HEK293 can be easily scale-up for large-scale bioreactors or spin flasks. By using the VitroGel Cell Recovery Solution, the cell harvesting after 3D culture is simple and effective.

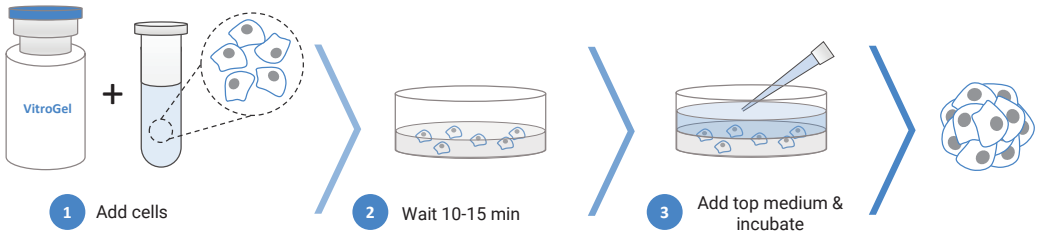
VitroGel MSC hydrogels are ready-to-use. Just mix with your cells. There is no cross-linking agent or the need to adjust the hydrogel concentration. Simple 5 or 20 minute protocol depending on the protocol method use.

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	Injectable hydrogel for *in vivo* studies and laboratory automation
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 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% HPL).

TheWell BIOSCIENCE
US TF: +1 866.3D.CELLS TEL: +1 973.855.4955 | Email: info@thewellbio.com | Web: www.thewellbio.com
TheWell Bioscience, Inc. 675 US Highway 1, North Brunswick, NJ 08902 USA

3. Add 1 mL VitroGel HEK293 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.)
4. 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.

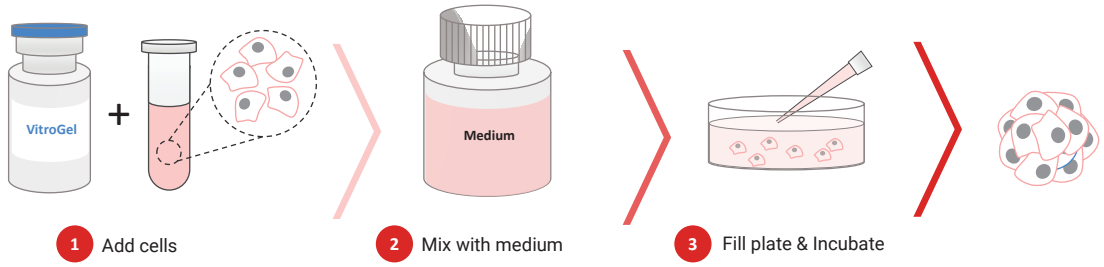
	6 well plate	12 well plate	24 well plate	48 well plate	96 well plate
Volume per well	1200 µL	600 µL	300 µL	150 µL	50 µL

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 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

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 MSC to room temperature or warm at 37°C.
2. Prepare the cell suspension in the culture medium.
   • Recommended cell concentration 0.5 x 10^6 cells/mL. For high seeding density, the concentration can be up to 10^7 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 1 mL VitroGel HEK293 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.)
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 mix the medium and mixture homogeneously.
5. Add the mixture to the well plate and incubate at 37°C with 5% CO_2. The recommended volume of mixture 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	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.
- For long term culture, add additional medium directly to the mixture after day 3.
- If additional culture medium is added more then once or the initial cell seeding density is higher than 2 x 10^6 cells/mL, an orbital shaker with a speed setting of 10-40 rpm may be required to help maintain the cell suspension.

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

Please check Protocol-3 from the **VitroGel Cell Recovery Solution** protocol 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.

TheWell BIOSCIENCE
US TF: +1 866.3D.CELLS TEL: +1 973.855.4955 | Email: info@thewellbio.com | Web: www.thewellbio.com
TheWell Bioscience, Inc. 675 US Highway 1, North Brunswick, NJ 08902 USA

2D Hydrogel Coating Protocol

1. Bring VitroGel HEK293 to room temperature or warm at 37°C.
2. Add 1 mL VitroGel HEK293 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. 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 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 x 10^5cells/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.

Prepare Hydrogel for Animal Injection Protocol

1. Bring VitroGel HEK293 to room temperature or warm at 37°C.
2. Prepare the cell suspension in the culture medium (adjust the cell concentration to 3X of desired concentration of final injection).
3. Add 1 mL VitroGel 293 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.
4. Transfer the hydrogel mixture to a syringe.
5. Let mixture stabilize at room temperature for 10-20 min. The hydrogel is now ready for animal injection.

Cell Recovery from VitroGel HEK293 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 futher suggestions.

Related Products

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

TheWell BIOSCIENCE
US TF: +1 866.3D.CELLS TEL: +1 973.855.4955 | Email: info@thewellbio.com | Web: www.thewellbio.com
TheWell Bioscience, Inc. 675 US Highway 1, North Brunswick, NJ 08902 USA