TheWell BIOSCIENCE
Growing Cells in New Dimensions

Product Data Sheet

Revision v1.1

VitroGel® STEM

Catalog Numbers:
VHM02
VHM02S

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

Product Description

VitroGel® STEM is a xeno-free hydrogel system developed to improve the performance of three-dimensional (3D) static suspension cultures and scale-up of human pluripotent stem cells (hPSCs) to create a high-throughput system to model various tissue and disease states.

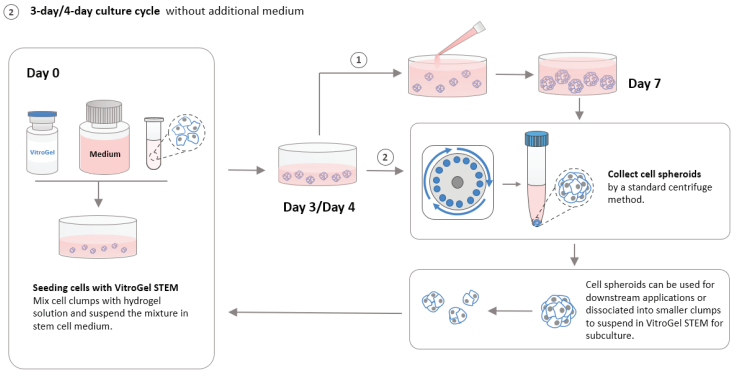
This hydrogel system is ready-to-use with an optimized formulation that fully supports the rapid expansion of high-quality 3D stem cell spheroids with pluripotent properties. hPSCs directly thawed from liquid nitrogen or passaged from 2D matrix coated culture vessels can be immediately mixed with the hydrogel solution for static suspension cultures. Moreover, the optimization protocol is ideal for time-sensitive experiments, as it does not require excessive medium exchanges, which can ultimately save on time and materials.

This hydrogel system is compatible with most hPSC culture media and tissue culture vessels. Due to the unique static suspension culture procedure, the requirement for microcarriers for large-scale bioreactors is eliminated, making the cell harvesting simple and effective. The 3D stem cell spheroids that are developed using this system can be used for further sub-culturing, patterned differentiating, organoid developing, or re-establishing 2D culture morphologies.

SPECIFICATIONS	
Formulation	Xeno-free. Polysaccharide based functional hydrogel
Use	3D static suspension culture for hPSCs
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.
Sizes	10 mL, 2 mL
Uses	10 mL hydrogel good for 90-180 mL suspension culture 2 mL hydrogel good for 15-30 mL suspension culture

Culture cycle of hPSCs with the VitroGel® STEM system

① **7-day culture cycle** with additional medium on day 3/4
② **3-day/4-day culture cycle** without additional medium



"Just add cells" No matrix coating required

VitroGel STEM is ready-to-use. Just mix with your hPSCs. There is no laborous matrix coating required to maintain and expand your stem cells.

Benefits of VitroGel STEM

Flexible

Undifferentiated stem cells can easily be mixed with VitroGel STEM to form cell-hydrogel mixtures, which can simply and efficiently transferred to multiple different types of cell culture vessels, including 96-well plates, T-flasks, shaking flasks, and bioreactors. Vitrogel STEM is compatible with multiple stem cell culture media. Moreover, after expansion, using the Vitrogel STEM system, stem cell spheroids can easily be sub-cultured in 3D for expansion or differentiation, as well as re-established 2D culture on matrix coating plate.

High performance

Stem cell populations can be scaled with VitroGel STEM in combination with bioreactors. At ultra-low agitation speeds, stem cell suspension cultures can be expanded with high cell viability and excellent cell growth rates. Using VitroGel STEM, expanded stem cell pools maintain full pluripotent properties.

Easy to use

VitroGel STEM offers the ability to directly culture stem cells from liquid nitrogen in 3D suspension cultures for the expansion of stem cell pools. Multi-passaged stem cells cultured on 2D culture vessels, such as tissue culture plates or flasks, can also be easily transitioned to 3D using the VitroGel STEM platform. Upon expansion, cells can be efficiently harvested or sub-cultured, without the requirement of additional reagents, for further differentiation.

Cost-effective

VitroGel STEM is not similar to common stem cell culture systems that require expensive matrix coating procedures, which can be laborious and time-consuming, or microcarriers. With VitroGel STEM, there is also no need for typical extraneous laboratory equipment, such as shakers or stirrers, to successfully scale up stem cell populations.

Guide for Use

View the full protocol for futher use of VitroGel STEM at www.thewellbio.com/protocols

Initial static suspension culture of hPSC using VitroGel STEM

1. Harvest hPSC from 2D matrix coating surface; or use cells directly from liquid Nitrogen (centrifuge to get cell pellet and remove the cell dissociation reagent or cell freezing solution).
2. Prepare cell clump suspension in stem cell medium with 10 µm/mL Y-27632. Recommend cell density at 0.5-2 X 10^6 cells/mL for the final cell seeding density in the hydrogel suspension culture around 0.3-1 X 10^5 cells/mL.
   - If needed, break up the clumps for 30-70 µm in size by carefully pipetting the clump suspension up and down. Single cell suspension is not recommended.
   - The typical working range of cell density is around 0.2-5 X 10^6 cells/mL, which make the final cell seeding density in the hydrogel suspension culture around 0.1-3 X 10^5 cells/mL. Depending on the desired culture conditions and the final sizes of stem cell spheroid, the cell seeding density should be optimized for individual cell types.
3. Gently mix VitroGel STEM with cell clump suspension at 2:1 v/v ratio (e.g. mix 2 mL VitroGel STEM with 1 mL of cell clump suspension. Check Table 1 or Table 2 of the recommended volume of different culture vessels for different culture circles.
4. Add stem cell medium (with 10 µm/mL Y-27632) to the cell-hydrogel mixture at 5:1 v/v ratio (e.g. mix 15 mL stem cell medium with 3 mL of cell-hydrogel mixture). Carefully pipette up and down to mix the medium and mixture homogeneously.
5. Add the desire volume of the mixture to the culture vessel and incubate at 37°C with 5% CO_2.
6. Place the well plate in an incubator and change the cover medium every 48 hours.
   Note: Recommend changing 50-80% of the top medium without disturbing the hydrogel.

Add additional medium for 7-day culture circle: On day 3 or day 4, add the desired volume of stem cell medium (without Y-27632) directly to the culture vessel (check Table 1 for the recommended volume of additional medium for different culture vessels).

Notes:

- It is recommended to use the same type of stem cell culture medium for 2D matrix coating culture to culture cells in 3D suspension culture. If a different type of medium is used for 3D culture, the cells may take 1-3 days to adapt the new medium (check the instruction of the new medium providers for medium switch procedure).
- The selection between 3-day or 4-day culture circle or 7-day culture circle is depended on the cell seeding density and the desired conditions of stem cell spheroids.
- Adding additional medium with a culture circle is required whenever the culture medium is turning yellow color (add additional cell culture medium for 3-day or 4-day culture circle may need when the initial cell seeding density in hydrogel suspension is higher than 1 X 10^5 cells/mL).
- If additional culture added more then one time within a culture circle, an orbital shaker may need to apply at low speed 10-40 rpm to maintain the cell suspension.

Table 1. Recommend volume of VitroGel STEM, cell clump suspension and stem cell medium for 7-day culture circle

	WELL PLATE (Volume per well)			T-FLASK			ERLENMEYER FLASK			
	96 well plate	24 well plate	6 well plate	T-25	T-75	T-175	125 mL	250 mL	500 mL	1000 mL
VitroGel STEM	12 µL	60 µL	200 µL	400 µL	1.2 mL	4 mL	6 mL	12 mL	24 mL	50 mL
Cell clump suspension	6 µL	30 µL	100 µL	200 µL	600 µL	2 mL	3 mL	6 mL	12 mL	25 mL
Stem cell medium	90 µL	450 µL	1.5 mL	3 mL	9 mL	30 mL	45 mL	90 mL	180 mL	375 mL
Initial culture volume	108 µL	540 µL	1.8 mL	3.6 mL	10.8 mL	36 mL	54 mL	108 mL	216 mL	450 mL
Additional medium	108 µL	540 µL	1.8 mL	3.6 mL	10.8 mL	36 mL	54 mL	108 mL	216 mL	450 mL

Table 2. Recommend volume of VitroGel STEM, cell clump suspension and stem cell medium for 3-day or 4-day culture circle

	WELL PLATE (Volume per well)			T-FLASK			ERLENMEYER FLASK			
	96 well plate	24 well plate	6 well plate	T-25	T-75	T-175	125 mL	250 mL	500 mL	1000 mL
VitroGel STEM	20 µL	100 µL	400 µL	600 µL	1.8 mL	6 mL	12 mL	24 mL	48 mL	100 mL
Cell clump suspension	10 µL	50 µL	200 µL	300 µL	900 µL	3 mL	6 mL	12 mL	24 mL	50 mL
Stem cell medium	150 µL	750 µL	3 mL	4.5 mL	13.5 mL	45 mL	90 mL	180 mL	360 mL	750 mL
Initial culture volume	108 µL	900 µL	3.6 mL	5.4 mL	16.2 mL	54 mL	108 mL	216 mL	432 mL	900 mL

Related Products

VitroGel® ORGANOID (Cat# VHM04)
Other versions of VitroGel hydrogels - www.thewellbio.com/3d-hydrogels

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