FAQ

You are warmly welcome for more questions if your question is not on the list below, please send your question to info@pepgel.com

1. How long it will take for PepGel product to arrive?
   PepGel products will typically arrive within a week in USA once we received your order. PGmatrix kit will be shipped by using ground shipping under room temperature. However, PGgrow or any other growth factors specially for stem cells cultures from PepGel can be shipped overnight with dry ice.

   For international order, we will use international priority shipping. It usually takes 3-4 days arriving destination country, and plus the time for customer clearance.

2. What storage condition and shelf life of PGmatrix are?
   PGmatrix kit needs to be refrigerated upon arrival for long term storage. PGmatrix kit is stable for 10 months when stored at 4 °C.

3. Can gel strength be altered by changing peptide concentration?
   Yes, you can alter the PGmatrix concentration (0.2%-1%) to either increase or reduce the gel strength in the range of 200 Pa to 1200 Pa. However, to gain stronger gel strength by special order higher concentration PGmatrix or PepGel hybrid PGmatrix hydrogels. For example, PGmatrix-spheroids has gel strength up to 4000 Pa or PGmatrix hybrids up to 20,000 Pa, please contact us at customerservice@pepgel.com for technical support If higher gel strength is needed for cell culturing or bioprinting.

3. How to choose cell seeding density and gel concentration in PepGel?
   Typically, 4-10^4 -2x10^5 cell/mL and gel concentration in rang of 0.2-0.5% can be chosen for various cancer cells, epithelial cells, T cells and stem cells etc. (Please refer to PGmatrix using guides at page 4 and 17) Primary cells, Mesenchymal Stem cells, hepatocyte etc need to have high seeding density 3 x10^6 -6x10^6 cell/mL to form spheroids and maintain high cell viability. Please contact customerservice@pepgel.com for more technical support.

4. How long can cells grow within PepGel?
   Usually, cells can form well shaped spheroids within PGmatrix and maintained up to 2 weeks.
   If you prefer long term 3D culturing inside PGmatrix over 4 weeks, please contact customerservice@pepgel.com about the PGmatrix-spheroid kit for more technical support.

5. Any tips for spheroids harvesting from PepGel?
   1). ***Initial gel disruption*** before gel dilution is crucial to have a better spheroids separation from PGmatrix networks: Without removing the upper layer cell medium, mechanically disrupt the gel ***THOROUGHLY*** by pipetting the gel and medium mixture.
   2). High centrifuge force needs to be used to separate spheroids from PGmatrix networks. We recommend **500g-700 g** for cancer cell lines, and **250-400** g for stem cells (i.e. hiPSC)
   3). If you observe cloudy solution (un-fully disrupted gel) besides cell pellets at bottom of conical tube after centrifuge, we suggest go through another cycle of “gel disruption – gel dilution -- centrifuge” to remove the remaining gel fibers.
   4). If you still have cell isolations issue, you may reduce the gel concentration (0.2%-0.3%) for cell culture (Cells have secretion inside PGmatrix networks, resulting either strengthen or weaken the gel strength depending on cell types that might affect the spheroids harvesting efficiency) or consult with PepGel customerservice@pepgel.com for technical support.

6. How long the spheroid colonies need to be trypsinization to become single cells.
   To break the spheroid colonies, follow your conventional culture protocol, add enzyme solution or other dissociation agents. The trypsinization time for spheroids disruption depends on the cell types, spheroid size and number. See the detailed procedure for spheroid colonies break up in 3D Spheroid Culture in PGmatrix Using Guide. http://www.pepgel.com/PDF/PGmatrix_using_guide_11_2021.pdf

Typically, for cancer cell, add Trypsin-EDTA solution (i.e., 0.25%Trypsin+0.02%EDTA) to the spheroids pellet, the recommended Typsin-EDTA solution amount per well can be equal to the gel plating volume (i.e., 500 µL for 24-well plate). Hela cell, Head and Neck cancer cell needs 10-15 min to break the over 90% spheroids (50-100 um) into single cells, while certain cell lines need longer trypsinization up to **20 min – 30 min** to break the spheroids (50-150 um), like 4t1, PANC 1 and HepG2 etc. (After 5-10 min incubation with trypsin-EDTA solution, pipet the cell solution up and down to mechanically break up the spheroids and observe the *spheroid* size under the microscope to determine if it needs to extend incubation time and for how long). Customers need to choose the appropriate condition for their cell spheroids breakup.

7. Is PGmatrix for high content physiological spheroids?
   Yes, for renewable cells, for example, 5 x 10^{-4} HepG2 cells seeded in one well of 24 well-plate can produce at least 1 x 10^{-4} physiological spheroids with diameter range of 50 to 100 µm in 6-7 days. One published paper demonstrates that Hela cells form spheroids in PepGel system and produces in vivo like extracellular vesicles (EV). EV small RNA profile exhibited a much higher similarity (96%) to in vivo circulating EVs derived from cervical cancer patient plasma. Please review reference [link here] for detailed information
   http://www.pepgel.com/PDF/PepGel_3D_culture_produces_in_vivo_like__exosomes.pdf.

8. Can PGmatrix be printed into small chambers for lab-on-chip settings?
   Yes, all PGmatrix based kits can be manually printed by pipetting or extrusion printer or ink-jet printer, please review Bioink protocol for details for PGmatrix-Spheroids for example.
   http://www.pepgel.com/PDF/PGmatrix_BioInk_using_guide_11_2021.pdf

9. What difference between PGmatrix and PGmatrix-Spheroid or PGmatrix-hiPSC?
   PGmatrix-Spheroid (PG-S) is PGmatrix derivatives. PG-S has stronger gel strength that is more suitable for bioprinting and has better cytocompability compared to PGmatrix. If you prefer to have long term cell cultures over 4 weeks, please consult with PepGel customerservice@pepgel.com for more information.
   PGmatrix-hiPSC is ideal PGmatrix for hiPSC 3D culturing for proliferation and sphepriods.

10. Can PGmatrix be used in microfluidic?
    Yes, please review the following published paper as references.
    http://www.pepgel.com/pdfs/Miller_et_al-2016-Biotechnology_and_Bioengineering.pdf
    http://www.pepgel.com/PDF/PepGel__3D_cell_culture_DNA_transfection_in_Lab_on_a_Chip.pdf

11. Can spheroids or cells in PGmatrix be directly Immuno-staining
    Yes, Same fluorescent stain concentration for 2D samples can be used for staining cells in 3D PGmatrix.
    The gel itself will not be stained, but fluorescence from cells in different planar locations and background fluorescence from soluble proteins might be enhanced. Here are several suggestions that can improve staining results.
    1). Sample preparation. If you can, it is better to make the sample into a thin slice before imaging. For example: put a small droplet of the gel (with cells in it) onto a glass slide, add DAPI, put a cover slide on top, gently squeeze it to disperse the droplet then mount for imaging. This method will not destroy organoids. If you are trying to image some structure in the gel that must be physically intact, we suggest that you try to freeze the sample, use a microtome to obtain thin slices. The main point is that you need to minimize the thickness of the gel that you are trying to image.

    2). Staining. It usually takes longer to properly stain the cells in PGmatrix. Please increase incubation time after applying stain. To wash away extra stain solution, please incubate for 5-10 min after adding DPBS and repeat the washing step several more times compared to the protocol for 2D cultures.

3). Imaging settings. Please try to adjust the brightness and contrast of your light source, which should improve the imaging result. If your microscope and camera have this adjustment, you can adjust the wavelength range of the image to minimize interference from green and red light, which is usually present in DAPI images as background.

For example, Figure A shows the histogram of brightness in software of Zeiss microscope. By limiting the brightness range to only include green light, the background can by largely reduced (Alexa fluor 488, green). Figure B shows that hiPSC cultured in 3D PG-matrix was fixed and incubated with anti-Oct4 antibodies labeled with Alexa Fluor 488 shown fluorescence image.