PepGel

Home for Cells and Beyond

PGmatrix-LiveShip using guide

PGmatrix-LiveShip (PG-LS) allows sending and receiving fresh spheroids/organoids under ambient condition. The fresh spheroids/organoids are ready-to-use for downstream applications such as drug screening, bioprinting, and stem cell differentiation into somatic cells. In addition, shipping spheroids alive saves valuable time by freeze-thaw cycles from traditional cryogenic shipping. The physiological functions (gene expression and Pluripotency, and differentiation capability etc) of shipped alive cells are perfectly maintained. PG-LS kit consists of a vial of PGmatrix nanofiber solution, a vial of PGworks trigger solution and a vial of PGgrow. With PGmatrix, cells no longer suffer acidic or chill conditions; cultured cells/spheroids are easily harvested from the matrix; all operating procedures can be completed at room temperature or 37°C in neutral pH.

PRODUCT:	PepGel™ PGmatrix-LiveShip Research Kit
CONTENT:	PGmatrix-LiveShip solution and PGworks solution
QUANTITY:	20 mL of 4% PG-LS, 4 mL of PGworks, 500 µL PGgrow-hiPSC
	10 mL of 4% PG-LS, 2 mL of PGworks, 250 µL PGgrow-hiPSC
	6 mL of 4% PG-LS, 1 mL of PGworks, 100 µL PGgrow-hiPSC
STORAGE:	Stored at 4°C

FOR IN VITRO RESEARCH USE ONLY. PLEASE READ MATERIAL USING AGREEMENT FOR MORE DETAILS. FOR IN VIVO TEST, PLEASE ASK FOR PG IN VIVO PRODUCTS.

CONTACT:

PepGel LLC

101 BIVAP Bldg.
1980 Kimball Ave
Manhattan, KS 66506
E-mail: info@pepgel.com
Phone: 785-226-6917
Fax: 785-532-7193
Web: www.pepgel.com

To Order: customerservice@pepgel.com, or online www.pepgel.com

A. Cell encapsulation and 3D culturing- hiPSC as an example

I. Encapsulate cells from subculture.

1. Bring the PGmatrix-LiveShip solution and PGworks solution to room temperature (15 – 25 °C) or 37 °C (37 °C water bath).
2. To prepare cell medium stock solution, thaw PGgrow* and add it into mTeSR1 complete medium at ratio 1:1000 v/v (PGgrow : mTeSR1 complete medium).
3. Suspend cells in mTeSR1 complete medium supplemented with PGgrow, then add PGworks solution to the cell suspension according to the Mixing Ratio in **Table 1**, pipet well without introducing air bubbles (always immersing pipet tip in cell solution during pipetting).

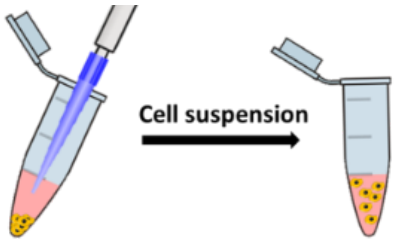
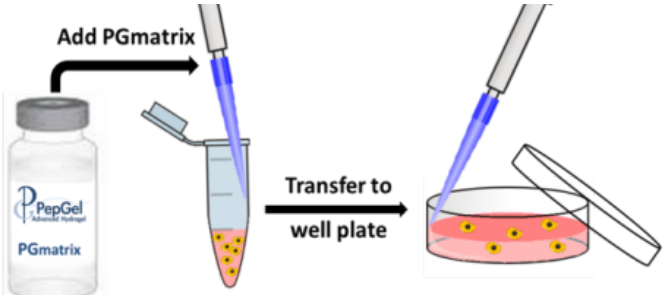


Table 1. Mixing ratios of PGmatrix solution, cell suspension and PGworks* solution and maximum plating volume for 1 well for 24-well plate plates.**

0.5% W/V			Maximum plating volume for 1 well (µL)	Feeding medium on top of hydrogel for 1 well (µL)
Cell suspension (µL)	*PGworks solution (µL)*	*PG-LS solution (µL)*		
240	10	250	500	1500-2000

NOTE: *PGworks is always 2% of the total volume of PGmatrix + cell suspension together.
** Nontreated culture plate is recommended for 3D cell culturing in PGmatrix.

4. Mix the PGmatrix solution carefully into the cell suspension of step 3 at the Mixing Ratio indicated in **Table 1** (pipet well without introducing air bubbles). Transfer the mixture into the center of each well, then swirl the plate to uniformly cover the entire well bottom surface. Recommend hiPSC seeding density can be 2 x10^5 cell/mL; it can generate around



25000 hiPSC spheroids with diameter of 30-50 µm at day 5.

5. Incubate the plate at 37°C (5% CO_2) for 30 min to complete the gelation.
6. After gelation, add 1.5-2 mL of mTeSR1 complete medium supplemented with PGgrow on top of the gel of each well to prevent the matrix from drying. (**Tips**: Gently add cell culture medium along the wall of each well on the top surface without disturbing the hydrogel).
7. To feed the cells, change the medium very gently by replacing part (2/3) of medium above the gel with fresh medium every day start from day 3 of each passage. (Tips, usually 60% of the medium is replaced at each time to avoid disruption gel surface).

*** PGgrow is used as a cell culture supplement, PGgrow should be diluted into mTeSR1 complete medium immediately before use (see step 2), and use within two weeks after dilution. Medium used for cell culture in this protocol are all supplemented with PGgrow.**

II. Thawing hiPSC *

1. Bring the PGmatrix-LiveShip solution and PGworks solution to room temperature (15 – 25 °C) or 37 °C (37 °C water bath).
2. To prepare cell medium stock solution, thaw PGgrow and add it into mTeSR1 complete medium at ratio 1:1000 v/v (PGgrow : mTeSR1 complete medium).
3. Thaw the vial with frozen hiPSC by gently agitating in a 37 °C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water.
4. Remove the vial from the water bath as soon as the contents start to thaw. Pre-warm mTeSR1 complete medium to room temperature and add 1 mL to the vial and pipette until the cell suspension is totally thawed.
5. Transfer the cell suspension to a 15 mL conical tube, and use another 1 mL of mTeSR1 complete medium to rinse the vial for the remaining cells, and combine the solution to the conical tube.
6. Dilute the mixture to 10 mL by using mTeSR1 complete medium. And centrifuge at 200 g for 5 min.
7. Remove the supernatant and resuspend the cell pellet gently in mTeSR1 complete medium supplemented with PGgrow (the medium stock solution from step 2).
8. Follow the steps 3-7 in section I **"Encapsulate cells from subculture"** for the hiPSC encapsulation in 3D PGmatrix-hiPSC.

   *** The recommended hiPSC seeding density from thawing in hiPSC-qualified PGmatrix can be higher (i.e. 3 x10^5 cell/mL) than encapsulation cells from subculture in order to maintain good cell viability and proliferation.**

B. Ready-to-use spheroids shipping- hiPSC as an example

At culturing of Day 5, hiPSC spheroids are ready to ship, follow the protocol below to transfer the PGmatrix-LiveShip hydrogel with hiPSC spheroids into the conical tube.

1. Remove 1.2 mL-1.7 mL of medium above the gel for one well of 24-well plate, then gently pipet the remaining medium (around 0.3 mL) and gel to form a uniform gel and cell slurry.
2. Transfer the gel and cell slurry to a 2 mL conical tube.
3. Rinse the well plate using 0.5 mL fresh medium, and gently add to conical tube on the top of slurry.
4. Add additional fresh medium (about 1 mL) to fulfill conical tube, to avoid the excess air in the tube during transportation.
5. Seal the conical tube with parafilm, and put in a thermal box, surrounded with bubble wrap to maintain the tube in the upright position. Standard overnight shipping can be used.

C. Ready-to-use spheroids harvesting protocol-hiPSC as an example

For example: procedures for one 2 mL conical tube containing 500 uL of PGmatrix hydrogel encapsulated with hiPSC spheroids, and 1500 uL complete medium above the gel.

Spheroids harvest:

1. Upon receiving, remove about 1500 uL medium above the gel, and transfer it to a 15 mL conical tube.
2. Mechanically disrupt the remaining gel and medium thoroughly by pipetting.
3. Transfer the mixture to the 15 mL conical tube, and mix well.
4. Rinse the 5 mL conical tube by adding 1000 uL medium and combine the solution to the 15 mL conical tube, and mix well.
5. Then add additional 4 mL DPBS to the 15 mL conical tube, and mix well.
6. Centrifuge the 15 ml tube at 250 g for 5 min, and discard the supernatant and collect the hiPSC spheroids pellet for further uses.

Spheroids breakup:

1. Add 1.5 mL 1X TrypLE to spheroids in the 15 mL conical tube (per one (1) 1.5 ml conical tube), mix gently to disrupt the pellet, then incubate at 37°C for 10 -15 min, until majority of single cells are observed.

   (See details hipsc spheroid breakup on Page 8 of hiPSC-qualified PGmatrix using guide) http://www.pepgel.com/PDF/hiPSC_qualified_PGmatrix_Using_Guides_11_2021.pdf

2. After incubation, add 1.5 mL mTeSR complete medium to neutralize the trypsinization, and centrifuge at 200-250 g for 5min.
3. Discard supernatant and re-suspend the pellet in mTeSR1 complete medium for further application.