



Home for Cells and Beyond

PGbioInk™ Using Guides

The **PepGel PGbioInk™** kit consists of a vial of **PGbioInk** patented peptides nanofiber solution and a vial of **PGworks** trigger solution and a growth kit (optional). The PGbioInk nanofibrils are formulated into a basic or a customer desired cell medium in neutral pH. A 3D microenvironment can be formed accordingly for cell performance. With PGbioInk, cells no longer suffer acidic or chill conditions; all operating and growth procedures can be completed at room temperature or 37°C in neutral pH.

PRODUCT:	PepGel PGbioInk™ Research Kit
CONTENT:	PGbioInk solution and PGworks solution
QUANTITY:	20 mL of PGbioInk and 1 mL of PGworks or 10 mL of PGbioInk and 0.5 mL of PGworks 6 mL of PGbioInk and 0.3 mL of PGworks 2 mL of PGmatriax and 0.3 mL of PGworks
STORAGE:	Stored at 4°C
LOT NUMBER:	See product label

FOR IN VITRO RESEARCH USE ONLY. PLEASE READ MATERIAL USING AGREEMENT FOR MORE DETAILS.

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FOR FIRST TIME USER, PLEASE READ THE FOLLOWING THREE MESSAGES

MESSAGE I: Mixing Ratio Notice

The PGbioInk solution contains 1% W/V standard peptides. Most cells from soft tissue grow well in the peptide concentration from 0.1% to 0.5%. If you are first time user, we recommend using 4-6 mixing ratios in the range of 0.1-0.6% W/V final peptide concentration for 3D cell encapsulation to identify the best mixing ratio for your cells. The following **Table 1** presents two mixing ratios at 0.2% and 0.5% concentration as example, respectively. Please use the following table as reference to mix PGbioInk solution and cell suspension.

Remember: add the PGworks to your cell suspension FIRST before you mix PGbioInk solution with cell suspension. If you still have questions, please contact technical support by email to customerservice@pepgel.com

Table 1: Examples of Mixing ratios of PGbioInk solution, cell suspension and PGworks* solution. (See NOTE for PGworks concentration)

Well Plate	0.2% W/V			0.5% W/V		
	Cell suspension (μ L)	PGworks solution (μ L)	PGbioInk solution (μ L)	Cell suspension (μ L)	PGworks solution (μ L)	PGbioInk solution (μ L)
12	780	20	200	480	20	500
24	390	10	100	240	10	250
48	195	5	50	120	5	125
96	78	2	20	48	2	50

* PGworks is always 2% of the total volume of PGbioInk + cell suspension together.

Note:

1. Cells will not perform well without appropriate growth factors, it is users' preference what growth factors are needed for their cells, or contact customerservice@pepgel.com for suggestion.
2. PGgrow kit is also available for stem cells (i.e., hiPSC)

MESSAGE II: Examples of Cell Density and Gel Concentration

***Table 2: Cell density and gel concentration recommendation for 3D cell cultures in PGbioInk Products**

Cells	PGbioInk [£]	Gel concentration (%)	Gelation time (min)	Cell seeding density (Cell/mL)
rESC 101	PGR2TbioInk D	0.5	60	1 x10 ⁵
Jurkat Clone E6-1 (ATCC)	PGbioInk R	0.2	60	1 x10 ⁵ - 2 x10 ⁵
Jurkat Cell (ASC)	PGbioInk R	0.2	60	1 x10 ⁵ - 2 x10 ⁵
CHO-S (ASC)	PGbioInk P/ PGbioInk D	0.2-0.4	60	1 x10 ⁵ - 2 x10 ⁵
MCF-7 (ATCC)	PGbioInk D	0.2-0.3	60	2.8 x10 ⁵
Hela cell	PGbioInk D	0.2	60	8 x10 ⁴
Head Neck cell	PGbioInk D	0.5	30	4 x10 ⁴
hiPSC derived from fibroblast (ASC)	PGbioInk D-hiPSC (with PGgrow-hiPSC**)	0.5	30	1.8 x10 ⁵ - 2 x10 ⁵
Episomal hiPSC (Thermofisher)	PGbioInk D-hiPSC (with PGgrow-hiPSC**)	0.5	30	2 x10 ⁵ - 3 x10 ⁵
AD MSC (ATCC)***	PGbioInk D	0.3	60	1.4 x10 ⁶

[£]PGR2TbioInk D= PGR2TbioInk DMEM; **PGbioInk D**=PGbioInk DMEM; **PGbioInk D-hiPSC**=PGbioInk DMEM-hiPSC; **PGbioInk R**=PGbioInk RPMI; **PGbioInk P**=PGbioInk PURE, **PGbioInk with other basal medium would be also available upon request**

***Note:** Results reported here are from 48-24 well-plate under culturing condition at 37°C and 5% CO₂, and can only be used as reference. It is users' responsibility to use appropriate cell medium and growth supplement or consult with PepGel.

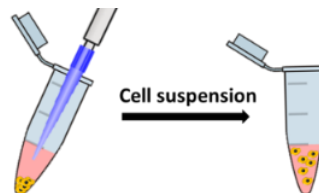
****PGgrow is optional but highly recommended for hiPSC culturing in PGbioInk D-hiPSC.** Without PGgrow, It is users' responsibility to choose appropriate growth supplement.

***** AD MSC grows better at O₂ level of 3% or in the presence of antioxidants at regular cell culturing condition.**

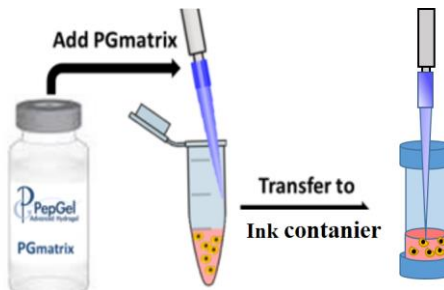
A. Protocols for BioInk preparation for 3D Bioprinting

I CELL ENCAPSULATION

1. Bring the PGbioInk solution and PGworks solution to room temperature (15 - 25 °C) or 37 °C (37 °C water bath)
2. Suspend cells in desired cell culture medium with appropriate growth factors then add PGworks solution to the cell suspension according to the Mixing Ratio in **Table 1** on page 2, pipet well without introducing air bubbles (always immersing pipet tip in cell solution during pipetting).



3. Mix the PGbioInk solution carefully into the cell suspension of step 2 at the Mixing Ratio indicated in **Table 1** on page 2 (pipet well without introducing air bubbles). Transfer the mixture into the center of the ink container. (**Note:** Please select the PGbioInk solution accordingly that matches your cell culture medium or contact PepGel for special medium requirement by email to customerservice@pepgel.com). For cell seeding density and gel concentration, please see examples in **Table 2** on page 3.



4. Incubate the ink container at 37°C (5% CO₂) for 30-60 min to complete the gelation. (**Note:** 30 min gelation time is recommended for 0.5% PGbioInk concentration, and 60 min gelation time for PGbioInk concentration lower than 0.5%). Then it should be ready for bioprinting (Example of PGbioInk 3D bioprinting parameters shown in **Table 3** on page 5).

Table 3: PGbioInk 3D bioprinting parameters

Example 1. Inkredible E⁺ Printer

PGbioInk	Gel concentration	Printer Brand	Printing Pressure	Printing Speed	Conical Nozzles*
PGmatrix DMEM	0.5%	Inkredible E ⁺	1-4 kPa	5 mm/s	27 G (0.23 mm)

*High Precision Conical Bioprinting Nozzles

Example 2. Ultimaker 2 Go Printer

PGbioInk	Gel concentration	Printer Brand	Printing Pressure	Printing Vacuum	Nozzle* (Diameter)	Printing Speed	Jetting Frequency
PGmatrix DMEM	0.5%	Ultimaker 2 Go	37-45 kPa	22-28 kPa	0.25 mm	2.5 mm/s	15 Hz

*062 MINSTAC with Jeweled Orifice Dispensing Nozzles

REFERENCE

1. Huang H., J. Shi, J. Laskin, Z. Liu, D. S. McVey, and X. S. Sun. Design of a shear-thinning recoverable peptide hydrogel from native sequences and application for influenza H1N1 vaccine adjuvant, *Soft Matter*, 2011, 7 (19), 8905 – 8912.
2. Huang, H.; Ding, Y.; Sun, X.; Nguyen, T. A. Peptide hydrogelation and cell encapsulation for 3D culture of MCF-7 breast cancer cells. *PLOS ONE*, 2013, 8 e59482.
3. Li, X., Galliher-Beckley, A.J., Nietfeld, J.C., Huang, H., Sun, X., Faaberg, K.S., and Shi, J. Peptide nanofiber hydrogel adjuvanted live virus vaccine induces cross-protective immunity to porcine reproductive and respiratory syndrome virus, *Vaccine*, 2013, 31, 4508-4515
4. Kumar, D.; Kandl, C.; Hamilton, C. D.; Shnayder, Y.; Tsue, T. T.; Kakarala, K.; Ledgerwood, L.; Sun, X.; Huang, H.; Girod, D.; Thomas, S.M. Mitigation of Tumor-Associated Fibroblast-Facilitated Head and Neck Cancer Progression With Anti-Hepatocyte Growth Factor Antibody Ficlatusumab. *JAMA Otolaryngol Head Neck Surg.* 2015, 141, 1133-1139
5. Kumar, D.; Kandl, C.; Hamilton, C. D.; Shnayder, Y.; Tsue, T. T.; Kakarala, K.; Ledgerwood, L.; Sun, X.; Huang, H.; Girod, D.; Thomas, S.M. 2015, Anti-HGF antibody ficlatusumab mitigates tumor-associated fibroblast-facilitated head and neck cancer progression, *J of American Medical Association (JAMA)-Otolaryngology* 141(12):1133-9
6. Miller PG, Shuler ML. Design and demonstration of a pumpless 14 compartment microphysiological system. *Biotechnology and bioengineering.* 2016 Oct 1;113(10):2213-27.
7. Liang J, Sun XS, Yang Z, Cao S. Anticancer Drug Camptothecin Test in 3D Hydrogel Networks with HeLa cells. *Scientific Reports.* 2017;7.
8. Liang J, Liu G, Wang J, Sun XS. Controlled release of BSA-linked cisplatin through a PepGel self-assembling peptide nanofiber hydrogel scaffold. *Amino Acids.* 2017:1-7.