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PGmatrix-BioInk-hiPSC Using Guide

PGmatrix-BioInk for hiPSC (PGbioInk) takes the advantage of its unique features including shear-thinning, rapid gel-recovery (self-healing), and tunable gel strength capability, offering the best cyto-compatible hydrogel to meet advanced tissue engineering and organ biofabricaton via 3D bioprinting techniques without using light or chemical crosslinking agent. PGbioInk Kit consists of a vial of PGmatrix-BioInk nanofiber solution, a vial of PGworks trigger solution, and a vial of PGgrow-hiPSC solution. PGbioInk kit creates a microenvironment mimicking in vivo ECM accordingly for cells growing and migrating in 3D manner. All operating procedures can be completed at room temperature or 37°C in neutral pH condition.

NOTE 1: PGmatrix-Spheroid (PG-S) kit with similar mechanical properties and cyto-compatibility as the PGbioink kit can also be used for 3D bioprinting following the PGbioInk-hiPSC protocol.

PRODUCT:	PepGel™ PGmatrix-BioInk for hiPSC Research Kit
CONTENT:	PGmatrix-BioInk solution, PGworks solution, and PGgrow-hiPSC
QUANTITY:	20 mL of 4% PGmatrix-BioInk, 4 mL of PGworks, and 500 µL PGgrow-hiPSC
	10 mL of 4% PGmatrix-BioInk, 2 mL of PGworks, and 250 µL PGgrow-hiPSC
	6 mL of 4% PGmatrix-BioInk, 1 mL of PGworks, and 150 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

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PGmatrix-BioInk for hiPSCs Using Protocol

1. **Cell suspension preparation:** To prepare cell medium stock solution, thaw PGgrow and add into E8 complete medium at ratio 1:1000 v/v (PGgrow : E8 complete medium) and use within two weeks after dilution. Suspend cells/spheroids in stock medium to prepare cell suspension. Recommend final cell seeding density in bioink hydrogel for printing is 2 x10^5-4 x10^5 cell/mL (6000-7000 spheroids/mL). For example: to prepare 2% PGbioInk hydrogel with 4 x10^5 cell/mL, cell density of cell suspension needs to be 9.5 x10^5 cell/mL.

2. **Mixing ratios:** The PGbioInk solution contains 4% W/V standard peptides, specially designed for hiPSCs. **Tables 1** presents mixing ratios of PGbioink solution and PGworks to obtain final gel concentration of 0.5%, 1%, 2%, and 3% as reference, respectively. Then follow procedure #3 below for bioink preparation.

Table 1: Examples of Mixing ratios of 4% PGbioInk solution, PGworks* and cell suspension**
(PGworks is always 16% of the total volume of PGbioInk solution.)

Final concentration***	PGbioInk solution (µL)	PGworks (µL)	Cell suspension	Total volume (µL)
3%	750	120	130	1000
2%	500	80	420	1000
1%	250	40	710	1000
0.5%	125	20	855	1000

NOTES: * add PGworks to your cell suspension **FIRST** before you mix PGbioInk solution with cell suspension.

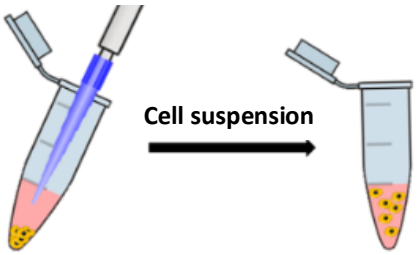
** Complete E8 medium with PGgrow is recommended for 3D culturing of hiPSC in bioprinted constructs.

***Bioink from 0.5 – 1% peptide concentration is suitable for ink-jet printing or manually pipetting for small construct desirable for Lab-on-Chip research, and bioink from 1.5% above is for extrusion printer or other printing devices.

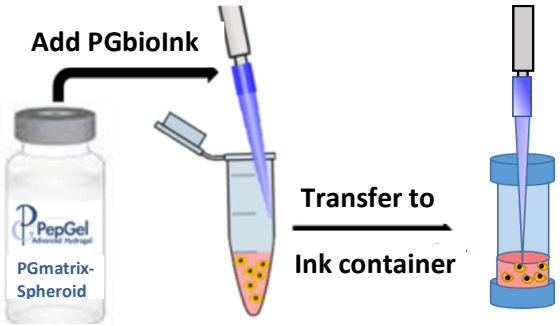
3. Protocol for PGbioInk for hiPSC Preparation

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 completed E8 medium 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).



3. Mix the PGbioInk solution carefully into the cell suspension from step 2 at the Mixing Ratio following **Table** 1 (pipet well without introducing air bubbles). Transfer the mixture into the center of the bioink container (i.e., syringe or pipet).
4. Incubate the bioink container at 37°C (5% CO_2) for 30 min to complete the gelation, then it is ready for bioprinting. (**NOTE:** for hydrogel infusion purpose, the mixture from step 3 can be used, and then perform step 4 for gelation after infusion).
5. Culture the bioprinted construct at 37°C (5% CO_2) using complete E8 medium supplemented with PGgrow. Replace medium to feed cells depends on cell growth without disturbing the construct.



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