PepGel

Home for Cells and Beyond

PGmatrix3D-Suspension Cell Culture of Physiological hiPSC Spheroids (Organoids)

The **PepGel PGmatrix™3D-Suspension-hiPSC (PG-3DSUSP-hPS)** is a powerful bio-tool for large-scale manufacturing of physiological 3D hiPSC spheroids/organoids at lab setting. It is incredibly easy for cell encapsulation and spheroids/organoids isolation. For example: using PG-3DSUSP-hPS kit 3D culture for 5 days, with seeding density of $1x10^{-5}$ cell/mL, one 6-well plate can produce ~50 million of hiPSC (~700,000-750,000 spheroids with diameter ranging from 30 um to 50 um). After harvesting, spheroids/organoids can be used directly for various downstream tasks such as drug screening, bio-printing for tissue engineering, and somatic cells differentiation. PG-3DSUSP-hPS kit consists of a vial of nanofiber solution for 3D suspension culture (PG-3DSUSP-hPS), a vial of PGworks trigger solution and a vial of PGgrow-hiPSC. The PGmatrix nanofibrils are formulated into a basic or a customer desired cell culture medium in neutral pH. A 3D microenvironment can be formed accordingly for spheroid/organoid growth. With PG-3DSUSP-hPS, cells no longer suffer acidic or chill conditions or sever shearing force from stirring; cultured cells are easily harvested; all operating procedures can be completed at room temperature or 37°C in neutral pH.

PRODUCT: PepGel PGmatrix™3D-Suspension-hiPSC Research Kit

CONTENT: PGmatrix3D-Suspension-hiPSC (PG-3DSUSP-hPS), 8 mL
PGworks solution, 300 uL
PG-grow-hiPSC, 100 uL

STORAGE: Stored at 4°C

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A. **3D Suspension hiPSC Culture Protocol -** example of two wells of a 6-well plate with PGmatrix 3D-Suspension-hiPSC Kit.

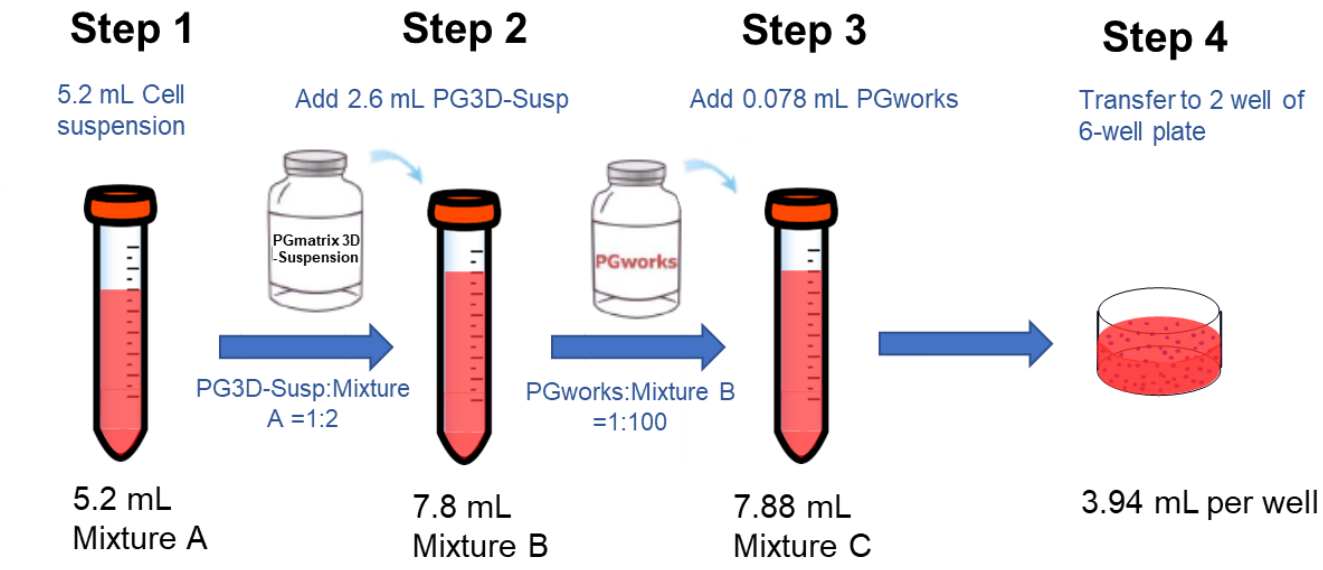
Bring the PGmatrix3D-Suspension-hiPSC kit (PG-3DSUSP-hPS and PGworks) and culture medium to room temperature or 37 °C; add growth factors at customer preference to the culture medium to make complete culture medium.

- **Step 1** Prepare 5.2 mL cell suspension Mixture A (Illustration 1) using the complete culture medium at a cell density of (1.5-2) x10^5 cell/mL.
- **Step 2 Cell Encapsulation:** Add 2.6 mL PG-3DSUSP-hPS solution to the Mixture A from Step 1 at 1:2 (PG-3DSUSP-hPS: Mixture A) to make Mixture B.
- **Step 3** Add 78 µL PGworks to the Mixture B from Step 2 at 1:100 (PGworks : Mixture B) to make Mixture C with a final cell density of 1 x 10^5 -1.5 x 10^5 cell/mL.
- **Step 4** Transfer the Mixture C to two wells of a 6 well-plate, 3.94 mL for each well.
- **Step 5** Incubate the 6-well plate at 37 °C (5% CO_2) for cell culture. Culturing duration can be 4-6 days, depending on cell growth.
- **Step 6** Two to Three (2-3) mL complete culture medium can be added to one well of 6-well plate to feed cells at day 1, day 3 and day 4, respectively, pipet GENTLY to distribute the fresh medium uniformly into the 3D suspension culture medium, then harvest cells by day 5. (Maximum volume of suspension culture medium per well for 6-well plate can be 10-11 mL)

NOTE: * To avoid introducing air bubbles for each step, keep pipetting within the solution or mixture and pipet gently.

Illustration 1. PGmatrix3D-Suspension-hiPSC Cell Culture–example for 6 well plate

(Pipet without introducing air bubble)



B. Cell Recovery

- **Step 1 Disruption:** Mechanically disrupt the 3D suspension culture medium **THOROUGHLY** by pipetting. Then transfer the mixture to a 15 mL conical centrifuge tube (NOTE: 15 mL tube size is only for 1 well of 6-well plate, large size tube should be used for multiple wells).
- **Step 2 Rinse:** Use 2 mL Dulbecco's Phosphate-Buffered Saline (DPBS, without Mg^{2+} / Ca^{2+}) to rinse the well and combine the solution to the centrifuge tube.
- **Step 3 Centrifuge:** Centrifuge at 600 g-700 g for 5 min by using swing bucket centrifuge. Discard the supernatant and collect the cell organoid-like spheroids pellet.

  Notes: For passage or cyropreservation, 1X TrypLE solution is recommended to dissociate the hiPSC spheroids, or use the spheroids as they are. Recommend trypsinization procedure for hiPSC spheroids: 6-7 mL 1X TrypLE solution per well for 6 well plate and incubate at 37 °C for 15 min.

  Details on Spheroid breakup can be found on Page 8 at PGmatrix-hiPSC protocol.
  http://www.pepgel.com/PDF/hiPSC_qualified_PGmatrix_Using_Guides_04_2022.pdf

C. Conditioned Medium Recovery

- **Step 1 Disruption:** Mechanically disrupt the 3D suspension culture medium **THOROUGHLY** by pipetting. Then transfer the mixture to a 15 mL conical centrifuge tube (NOTE: 15 mL tube size is only for 1 well of 6-well plate, large size tube should be used for multiple wells).
- **Step 2 Centrifuge:** Centrifuge at 600 g-700 g for 5 min by using swing bucket centrifuge. Collect the supernatant as the conditioned medium and cell pellet separately.

D. Example of physiological spheroids culturing in PG-3DSUSP-hPS system

Figure 1. hiPSC spheroids presents physiological spherical morphologies

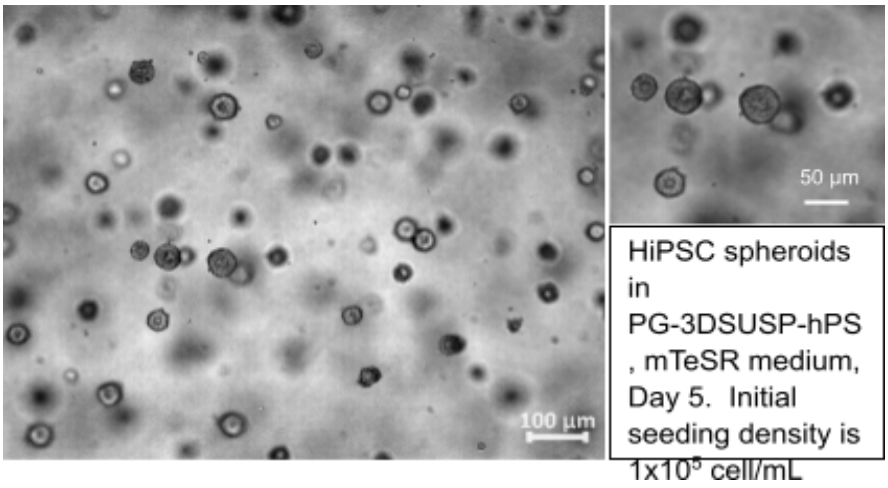


Table 1. Cell growth performance in PG-3DSUSP-hPS system

Cell line	Seeding density (cell/mL)	Culture basal medium	Culturing Duration (days)	Viability	Proliferation	Harvested cell per well
hiPSC derived from Fibroblast (Applied Stem Cell)	1 x10^5	mTeSR Plus	5	93%-97%	16-18	7.0-7.5.0 x10^6

Note: Results reported here are for hiPSCs from Applied StemCell using 6 well plate under culturing condition at 37°C and 5% CO_2 and can be used as reference. The optimum culturing duration depends on cell lines and cell growth.

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