Corning® Synthegel™ 3D hiPSC Suspension Matrix Kit

Guidelines for Use

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The Corning Synthegel hiPSC Suspension Matrix Kit is a powerful bio-tool for large-scale manufacturing of physiological 3D human induced Pluripotent Stem Cells (hiPSCs) spheroids in a lab setting. Cell encapsulation and spheroid isolation is an easy and straightforward process. For example: one 6-well plate seeded with hiPSC at a density of 5 x 10^4 cell/mL cultured for 5 days using the Corning Synthegel hiPSC Suspension Matrix Kit, can yield approx. 50 million total cells (approx. 600,000 to 700,000 spheroids with diameter ranging from 30 µm to 50 µm). Additionally, daily media exchanges are not required to maintain hiPSC as in more traditional planar culture. After retrieving, spheroids can be used directly for various downstream applications (e.g., drug screening, bioprinting for tissue engineering, and somatic cells differentiation). The Corning Synthegel hiPSC Suspension Matrix Kit consists of a vial of Corning Synthegel hiPSC proprietary peptide solution for 3D suspension culture and a vial of Corning Synthegel X-Link solution. The Synthegel hiPSC Suspension Matrix nanofibrils are formulated into a basal cell culture medium containing Corning Synthegel 3D hiPSC Grow Mix. This allows for a neutral pH that forms a 3D microenvironment suitable for spheroid growth. With Corning Synthegel hiPSC Suspension Matrix Kit, cells no longer suffer acidic or chill conditions since all operating procedures can be completed at room temperature or 37°C and at neutral pH. Additionally, there is no need to stir the cells as typically required in suspension culture, so cells are not exposed to shear force. There is also flexibility on the volume of Synthegel hiPSC Suspension Matrix Kit used per vessel which allows users to scale-up as needed utilizing any standard cell culture vessel.

NOTE: In addition to the Corning Synthegel hiPSC Suspension Matrix Kit, Corning Synthegel 3D hiPSC Grow Mix (Cat. No. 354792) needs to be purchased separately.

3D Suspension hiPSC Culture Protocol

Bring the Synthegel hiPSC Suspension Matrix Kit (Synthegel hiPSC Matrix Peptide solution and Synthegel X-Link solution) and culture medium to room temperature.

Step 1. Complete culture medium stock solution preparation

1. Add 300 µL sterile DPBS (without Mg2+ and Ca2+) into one (1 mg) vial of lyophilized Synthegel hiPSC Grow Mix. Pipette gently to obtain a homogeneous solution.
   - The reconstituted Synthegel hiPSC Grow Mix solution is stable at -20°C for up to 6 months, therefore, aliquots should be prepared, with working volumes, to avoid repeated freeze-thaw cycles.
2. Add an aliquot of the reconstituted Synthegel hiPSC Grow Mix solution into pre-warmed cell culture medium (i.e., mTeSR™1 complete medium) at a ratio of 1:1000 v/v (Synthegel hiPSC Grow Mix solution: mTeSR1 complete medium) to prepare hiPSC complete medium.
   - All medium used for cell culture in this protocol needs Synthegel hiPSC Grow Mix and should be used within 2 weeks of addition.

Synthegel hiPSC Grow Mix contains ROCK Inhibitor and does not require additional ROCK supplementation.

Step 2. Prepare Synthegel hiPSC Suspension Matrix Kit

NOTE: Corning Synthegel hiPSC Suspension Matrix Kit can be used at volumes between 0.3 and 0.85 mL/cm^2. This allows the volume to be adjusted depending on how many cells are needed. See table below for an example of a culture using 0.3 or 0.85 mL/cm^2.

Corning Synthegel Thickness (mL/cm^2)	Volume of Corning Synthegel hiPSC Suspension Matrix Kit per T-75 Flask	Cells Required to Seed (assuming 50,000 cells/mL)	Cells at Harvest (assuming 3 population doublings)
0.3	22.5 mL	1.125 x 10^6 cells	9 x 10^6 cells
0.85	63.75 mL	3.15 x 10^6 cells	2.52 x 10^7 cells

1. The table below can be used to calculate number of cells, media, X-Link solution and Synthegel matrix (peptide) solution needed based on the listed % of total volume determined above.
   - We recommend starting with a cell density of between 40-100,000 cells/mL.

Media/Cells	Corning X-Link Solution	Corning Synthegel hiPSC Suspension Matrix (Peptide)
84%	0.5%	15.5%

Example: A single well of a 6-well plate at 0.85 mL/cm^2

Total volume of Synthegel hiPSC Suspension Matrix Kit at 0.85 mL/cm^2

- 0.85 mL/cm^2 x 9.5 cm^2 (surface area) = 8.075 mL

Volume of each component of Synthegel hiPSC Suspension Matrix

- Media/cells: 84% of 8.075 mL = 6.80 mL
- Cells (i.e., desired cell density of 50,000 cells/mL): 8.075 mL x 50,000 cells/mL = 4 x 10^5 cells in 6.80 mL media
- X-Link Solution: 0.5% of 8.075 mL= 40 µL
- Synthegel Peptide: 15.5% of 8.075 mL = 1.25 mL

2. Prepare 6.80 mL cell suspension Mixture A (Figure 1) containing 4 x 10^5 cells using hiPSC complete medium.
3. Cell Encapsulation: Add 1.25 mL Synthegel peptide solution to Mixture A. Mix gently with a pipet to avoid making bubbles.
4. Add 40 µL Synthegel X-Link solution to Mixture B resulting in the formation of Mixture C with a final cell density 5 x 10^4 cell/mL. Mix gently with a pipet to avoid making bubbles.
5. Transfer 8.09 mL of Mixture C to a single well of a 6-well-plate.

Step 3: Feeding strategy

NOTE: Unlike iPSCs grown on two-dimensional formats, iPSC grown in the hiPSC Suspension Matrix Kit may not require daily media exchanges.

1. Incubate the plate at 37°C (5% CO_2) for 4 to 6 days. Additional complete media containing Grow Mix can be added on Day 4 or 5 of culture by gently pipetting or swirling.
   - Do not add more than 30% of the initial Synthegel hiPSC Suspension Matrix Kit volume to culture as too much media can dilute the suspension matrix and result in undesired cell sedimentation and or attachment.

   Example: 24% of 8.075 mL = 1.9 mL total of media. Feed media can be added on a single day (Day 3, 4 or 5) or divided across Days 3 to 5.

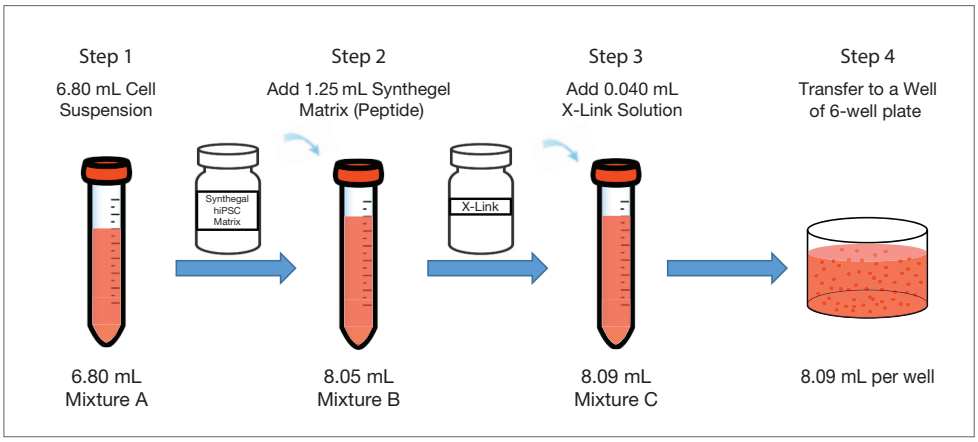

Figure 1. Corning Synthegel hiPSC Suspension Matrix Kit cell culture (example for 6-well plate).

Step 4. Harvest of hIPSC spheroids

Example: A single well of a 6-well plate

1. Mechanically disrupt the 3D suspension culture by pipetting 5 to 6 times in the well. Transfer to a 15 mL centrifuge tube.
2. Use 2 mL Dulbecco's Phosphate-Buffered Saline (DPBS, without Mg2+ and Ca2+) to rinse the well and transfer to the centrifuge tube.
3. Centrifuge at 500 g for 5 minutes by using a centrifuge equipped with a swing bucket rotor. Discard the supernatant, and collect the pellet containing cell spheroids.

Step 5. Cell dissociation

NOTE: For dissociation of spheroids, Accutase® cell detachment solution (Corning) or 10X TrypLE™ is recommended.

1. Add 6 to 7 mL Accutase cell detachment solution to the cell pellet, and pipet several times to resuspend pellet.
2. Incubate at room temperature for 15 to 20 min. Visualization under the microscope can help ensure spheroid dissociation.
3. Pipette gently to help breakup the spheroids to desired size.
4. Process cells as needed.

To start a culture from freeze-thaw, a higher starting cell seeding density is recommended.

- Thaw of 3D cultured cells: 1-2 x 10^5 cell/mL.
- Thaw of 2D cultured cells: 2-4 x 10^5 cell/mL

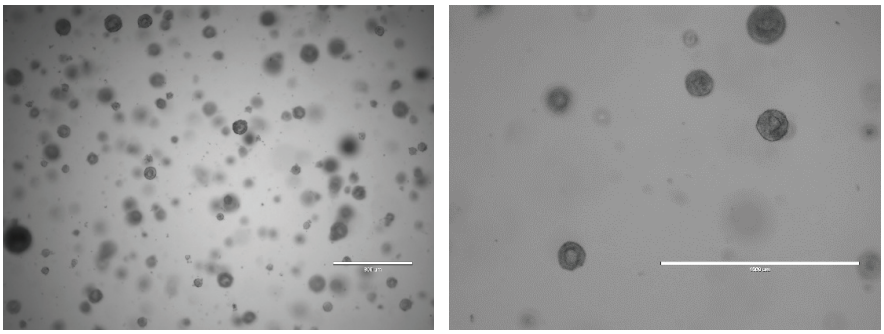

Figure 2. Morphologies of hiPSC spheroids after 5 days of culture in Corning Synthegel hiPSC Suspension Matrix Kit with mTeSR Plus medium. Initial seeding density was 4.5 x 10^4 cell/mL.

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