Corning® Synthegel™ hiPSC Suspension Matrix Kit

Guidelines for Use

CORNING

The Corning Synthegel hiPSC Suspension Matrix 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 spheroids isolation is an easy and straightforward process. For example: in one 6-well plate seeded with hiPSC at a density of 5 x 10^4 cell/mL, when 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). After harvesting, spheroids can be used directly for various downstream tasks (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 Suspension Matrix proprietary nanofiber solution for 3D suspension culture, a vial of Corning Synthegel X-Link solution, and a vial of lyophilized Synthegel hiPSC Grow Mix. The Synthegel hiPSC Suspension Matrix nanofibrils are formulated into a basal cell culture medium, with neutral pH, which forms a 3D microenvironment suitable for spheroid growth. With Corning Synthegel hiPSC Suspension Matrix, cells no longer suffer acidic or chill conditions since all operating procedures can be completed at room temperature or 37°C and in neutral pH.

Additionally, without the need to stir the cells, shearing force is eliminated and cultured cells are easily harvested.

3D Suspension hiPSC Culture Protocol

Bring the Synthegel hiPSC Suspension Matrix kit (Synthegel hiPSC Suspension Matrix and Synthegel X-Link solution) and culture medium to room temperature or 37°C

Step 1. Complete culture medium stock solution preparation

1. Add 300 µL sterilized DPBS (without Mg^{2+} and Ca^{2+}) into one (1 mg) vial of lyophilized Synthegel hiPSC Grow Mix, pipette gently, recap tightly, and invert the vial 4 to 5 times to obtain the homogeneous Synthegel hiPSC Grow Mix solution*.
2. 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.
3. Add an aliquot of the reconstituted Synthegel hiPSC Grow Mix solution into 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 medium stock solution.

NOTE: Synthegel hiPSC Grow Mix is used as a cell culture supplement and added into mTeSR1 complete medium just prior to use, any remaining media should be used within two weeks. Medium used for cell culture in this protocol are all supplemented with Synthegel hiPSC Grow Mix. Addition of ROCK Inhibitor is not necessary for 3D hiPSC culture when used in conjunction with Synthegel hiPSC Suspension Matrix containing Synthegel hiPSC Grow Mix.

Step 2. hiPSC Culture in Synthegel hiPSC Suspension Matrix Using 6-well plate

(Example: For two wells of a 6-well plate with Corning Synthegel hiPSC Suspension Matrix kit).

NOTE: For flask culture using Synthegel hiPSC Suspension Matrix kit please see Appendix A as reference.

1. Prepare 14.3 mL cell suspension Mixture A (Figure 1) using complete culture medium stock solution at a cell density of 5 x 10^4 cell/mL.
2. Cell Encapsulation: Add 2.6 mL Synthegel hiPSC Suspension Matrix solution to Mixture A, from Step 1, (at a 1:5.5 Synthegel hiPSC Grow Mix: Mixture A ratio) to make Mixture B. Mix gently with a pipet to avoid introduction of bubbles.
3. Add 78 µL Synthegel X-Link solution to Mixture B, from Step 2, (at a 1:33.3) Synthegel X-Link solution: Mixture B ratio) to make Mixture C with a final cell density of 4-5 x 10^4 cell/mL. Mix gently with a pipet to avoid introduction of bubbles.
4. Aliquot 8.49 mL of Mixture C into each of two wells of a 6-well-plate.
5. Incubate the plate at 37°C (5% CO_2) for 4 to 6 days, depending on cell growth.
6. Two to three (2 to 3) mL hiPSC medium stock solution can be added to each well to feed cells on Days 1, 3, and 4. GENTLY pipette to distribute the fresh medium uniformly into the 3D suspension culture, then harvest cells by Day 5. (Maximum volume of suspension culture medium per well for 6-well plate can be 10 to 11 mL).

   NOTE: To avoid introducing air bubbles, keep the pipet tip within the solution or mixture and pipette at a controlled rate.

NOTE: Higher cell seeding density is recommended when directly seeding thawed hiPSC into Synthegel hiPSC Suspension Matrix.

- To use cells thawed from 3D (Synthegel 3D hiPSC Matrix or Synthegel hiPSC Suspension Matrix system) subculture, higher cell seeding density is recommended (i.e., 1-2 x 10^5 cell/mL).
- To use cells thawed from 2D subculture, higher cell seeding density (i.e., 2-4 x 10^5 cell/mL) and smaller well formats (24-well) are recommended. In addition, if culturing a fragile hiPSC cell line or others (i.e., embryonic stem cells) directly from thawed 2D subcultures, it is recommended to begin culture for 1-2 passages in the Synthegel hiPSC Matrix embedded system.

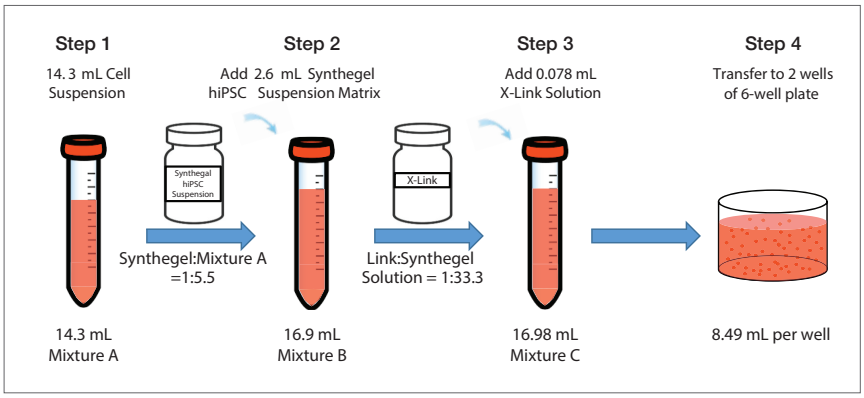

Figure 1. Corning Synthegel hiPSC Suspension Matrix cell culture (example for 6-well plate). Pipette without introducing air bubbles.

Step 3. Cell Recovery

1. Mechanically disrupt the 3D suspension culture thoroughly by pipetting the solution (5 to 6 times) in the well, and transfer to a 15 mL conical centrifuge tube. **NOTE:** 15 mL tube size is only for 1 well of a 6-well plate, for multiple wells use larger tubes.
2. Use 2 mL Dulbecco's Phosphate-Buffered Saline (DPBS, without Mg^{2+} and Ca^{2+}) to rinse the well and transfer to the centrifuge tube.
3. Centrifuge at 700 g for 5 min. by using a centrifuge equipped with a swing bucket rotor. Discard the supernatant and collect the pellet containing cells spheroids.

NOTES:

For passage or cryopreservation of collected cells, 1X TrypLE™ solution is recommended to dissociate the hiPSC spheroids.

Recommended trypsinization procedure for hiPSC spheroids: Add 6 to 7 mL 1X TrypLE solution to the cell pellet, collected after centrifugation, from each well of a 6-well plate. Incubate at 37°C for 15 min., and pipette gently to help breakup the spheroids. Observe the cell cluster size under a microscope to determine if additional incubation time is needed. Once greater than 90% of the cells are observed as single cells, passaging or cryopreservation can begin. Optionally, the spheroids can be cryopreserved or passaged as is.

A more detailed protocol on spheroid breakup can be found in the Corning® Synthegel™ 3D hiPSC Matrix Kit Guidelines for Use (CLS-AN-737DOC).

Example of Culturing hiPSC cells in Corning Synthegel hiPSC Suspension Matrix kit

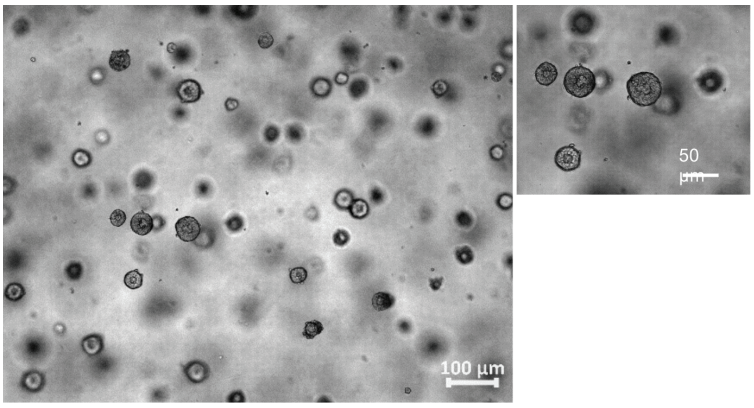

Figure 2. Morphologies of hiPSC spheroids. HiPSC spheriods in Corning Synthegel hiPSC Suspension matrix, mTeSR medium, Day 5. Initial seeding density is 1 x 10^5 cell/mL.

Table 1. Cell growth performance in Corning Synthegel hiPSC Suspension Matrix kit.

Cell Line	Seeding Density (cell/mL)	Seeding Amount (cell/well)	Culture Basal Medium	Culturing Duration (days)	Viability	Proliferation	Harvested Cell per Well
hiPSC derived from Fibroblast (Applied Stem Cell)	4.5 x 10^4	4 x 10^5	mTeSR Plus or MTeSR 1	5	93% - 97%	15 - 20	6 - 8 x 10^6

NOTE: hiPSCs noted in Table 1, were cultured in a 6-well plate at 37°C and 5% CO_2. The optimum culturing duration and conditions will need to be determined empirically for each cell line.

Step 4. Conditioned Medium Recovery

1. Mechanically disrupt the 3D suspension culture medium thoroughly by pipetting the solution. Transfer the mixture to a 15 mL conical centrifuge tube.

NOTE: 15 mL tube size is recommended for use with a single well of 6-well plate, a larger size tube should be used for multiple wells.

2. Centrifuge at 700 g for 5 min. by using a centrifuge equipped with a swing bucket rotor. Collect the supernatant as the conditioned medium and cell pellet separately.

3. **Example: Culturing hiPSC cells in Corning Synthegel hiPSC Suspension Matrix kit**

   Table 1. Cell growth performance in Corning Synthegel hiPSC Suspension Matrix kit.

Step 5. hIPSC Cryopreservation

1. After enumeration, centrifuge solution at 100-200g for 5 min. (cell type dependent).
2. Carefully remove supernatant and discard. Resuspend hiPSC pellet (single or small cell clusters) in ESC-Sure Human ESC Freezing Medium or complete growth medium with 5% to 10% DMSO to a final concentration of 1 x 10^6 cells/mL.

NOTE: Do not add DMSO directly to cells in growth media.

3. Aliquot into cryopreservation vials and allow it to sit at room temperature for 15 min. to allow diffusion of cryoprotectant into the cells.
4. Slowly freeze cells at 1°C/min. in a Corning CoolCell® freezing container (Cat. 432000) placed in a -80°C freezer for 24 hours. Do not store cells at -80°C for extended periods as it negatively impacts cell viability.
5. Transfer vials to liquid nitrogen for long-term storage.

Appendix A

hiPSC Culture in Synthegel hiPSC Suspension Matrix Using a T-Flask

Example of physiological hiPSC spheroids cultured in Synthegel hiPSC Suspension Matrix using a T-150 flask. Culture conditions, procedures and hiPSC growth performance are detailed below.

1. **Culture flask:** T-150 flask with final culture volume of 150 mL
2. **Culture medium:** Complete mTeSR 1 medium supplemented with Synthegel hiPSC Suspension Grow Mix following Step 1.
3. **hiPSC seeding density:** 4.5 x 10^4 cell/mL
4. **Culture condition:** Follow Steps 1 and 2 for matrix formation and cell encapsulation using the ratio below:

   107.3 mL Cell suspension (5.5 to 6 x 10^6 cells) + 19.5 mL Synthegel hiPSC Suspension Matrix + 0.585 mL Synthegel X-Link solution.

   Ratio of Matrix: Cell suspension = 1:5.5

   Ratio of Grow Mix: Matrix = 1:33.3

   ***NOTE:** These ratios above can be used for smaller or larger flasks.*

5. **Cell feeding:** Add 30 mL complete culture medium at Day 4.
6. **Cell recovery:** Follow Section 1.0 for spheroids harvesting at Day 5.

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