Corning® Synthegel™ Spheroid Matrix Kit

Guidelines for Use

CORNING

The Corning Synthegel Spheroid Matrix kit is a powerful tool for high content, 3D biologically formed spheroid culture which provides more accurate *in vivo* predictions for life science research and development. The kit consists of a vial of Corning Synthegel Spheroid Matrix proprietary peptides nanofiber solution and a vial of Corning Synthegel X-Link solution. The Synthegel Spheroid Matrix nanofibrils are formulated into a basal cell culture medium in neutral pH forming a 3D microenvironment for spheroid growth. With Synthegel Spheroid Matrix, cells no longer suffer acidic or chill conditions since all operating procedures can be completed at room temperature or 37°C and neutral pH. Additionally cultured cells are easily harvested from the matrix.

FOR FIRST TIME USERS, PLEASE READ THE FOLLOWING 2 MESSAGES.

MESSAGE 1: Mixing ratio notice.

The Corning Synthegel Spheroid Matrix solution contains 2% W/V standard peptides. Most cells, from soft tissue, grow well in the peptide concentration from 0.2% to 1%. For first time users, experimenting with several mixing ratios, in the range of 0.3% to 2.0% W/V final peptide concentration, is recommended for 3D cell encapsulation to identify the optimal mixing ratio for each application.

Table 1 shows examples of four mixing ratios of 0.3%, 0.5%, 1%, and 1.5% concentrations, and can be used as reference when mixing Synthegel Spheroid Matrix solutions and cell suspension.

NOTES:

- Strict aseptic techniques must be used when handling solutions and cells. All materials (e.g., pipet tips, centrifuge tubes, well plates, etc.) must be sterile prior to use, and all procedures should be performed in a biosafety cabinet (BSC), when appropriate, to minimize the risk of contamination.
- It is recommended that all centrifuge steps be performed in a centrifuge equipped with a swinging bucket rotor to allow pelleting at the bottom of conical tubes. If using microcentrifuge tubes, a fixed angle rotor may be used, but caution should be taken during handling as to prevent loss of sample.
- Corning Synthegel X-Link solution should be added to the cell suspension before mixing the Synthegel Spheroid Matrix solution.

Table 1. Examples of mixing ratios of Corning Synthegel Spheroid Matrix solution, Corning Synthegel X-Link solution* and cell suspension, as well as maximum plating volume per well for different plate formats**.

	0.3% W/V			**0.5% W/V**			
	Corning Synthegel Spheroid Matrix: (cell suspension + Corning Synthegel X-Link solution) = 1:5.7			**Corning Synthegel Spheroid Matrix: (cell suspension + Synthegel X-Link solution) = 1:3**			
Plate Size	Synthegel Spheroid Matrix (µL)	Synthegel X-Link Solution (µL)	Cell Suspension (µL)	Synthegel Spheroid Matrix (µL)	Synthegel X-Link Solution (µL)	Cell Suspension (µL)	**Maximum Plating Volume/per Well (µL)**
6-well	300	24	1,676	500	40	1,460	2,000
12-well	150	12	838	250	20	730	1,000
24-well	75	6	419	125	10	365	500
48-well	37.5	3	209.5	62.5	5	182.5	250
96-well	15	1.2	83.8	25	2	73	60

	1% W/V			**1.5% W/V**			
	Corning Synthegel Spheroid Matrix: (cell suspension + Corning Synthegel X-Link solution) = 1:1			**Corning Synthegel Spheroid Matrix: (cell suspension + Synthegel X-Link solution) = 1:0.33**			
Plate Size	Synthegel Spheroid Matrix (µL)	Synthegel X-Link Solution (µL)	Cell Suspension (µL)	Synthegel Spheroid Matrix (µL)	Synthegel X-Link Solution (µL)	Cell Suspension (µL)	**Maximum plating volume for 1 well (µL)**
6-well	1,000	80	920	1,500	120	380	2,000
12-well	500	40	460	750	60	190	1,000
24-well	250	20	230	375	30	95	500
48-well	125	10	115	187.5	15	47.5	250
96-well	50	4	46	75	6	19	60

*Synthegel X-Link solution is always 8% of the total volume of Synthegel Spheroid Matrix solution.
**Nontreated culture plate is recommended for culturing 3D cells in Synthegel Spheroid Matrix.

MESSAGE 2: Add medium on the top of hydrogel to prevent drying and to feed the cells for long-term culture.

After hydrogel formation (30 min. at 37°C after mixing), cell medium needs to be added on the top of the gel to provide fresh nutrients and prevent drying during long-term culture. Recommended initial volumes of medium overlayed on top of the hydrogel is dependent on different sized well plates (Table 2).

Table 2. The recommended initial volume of medium, per well, to be added on the top of the hydrogel.

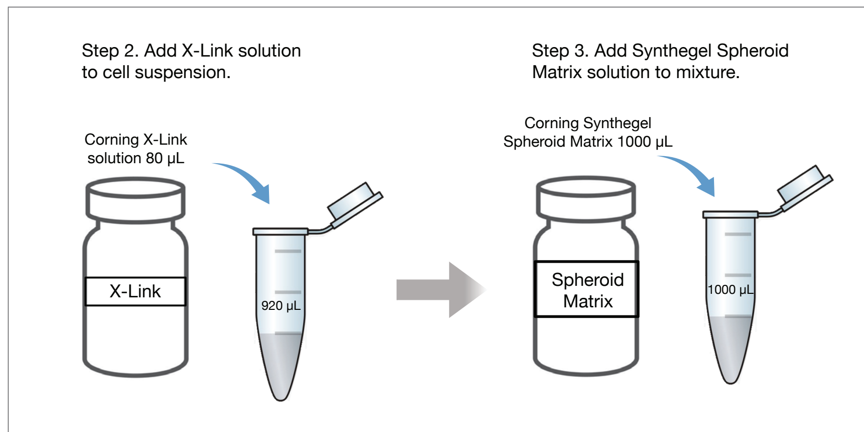
	6-well	12-well	24-well	48-well	96-well
Volume to add (µL) per well	5,000	3,000	1,500	800	300

Synthegel Spheroid Matrix Kit for 3D Cell Culture

Example: 3D spheroid Culture Procedure for a 24-well plate at a ratio of 1:1 (Corning Synthegel Spheroid Matrix: Synthegel X-Link solution + cell suspension) with a 1% (w/v) final Corning Synthegel Spheroid Matrix concentration.

Preparation: Corning Synthegel Spheroid Matrix kit and cell solution should be at room temperature or 37°C prior to starting.

1. Prepare cell suspension in a total of 920 µL of complete medium, (serum or other growth factors can be added as needed), pipetting well without introducing air bubbles.
2. Add Synthegel X-Link solution (80 µL) to the cell suspension from Step 1, pipetting well without introducing air bubbles.
3. Mix Synthegel Spheroid Matrix solution (1000 µL) with the cell suspension from Step 2 at 1:1 ratio, pipetting well without introducing air bubbles.



1% gel concentration for a 2000 µl volume: 920 µL Cell suspension +80 µL Synthegel X-Link solution + 1000 µL 2% Synthegel Spheroid Matrix solution.

4. Transfer 500 mL of the mixture from Step 3 to four wells of a 24-well plate.
5. Place the 24-well plate in a 37°C incubator 30 min. to complete gelation.
6. Gently add 1000 µL of the cell medium on top of the gel to prevent the matrix from drying and to provide nutrients to the cells. **HINT:** Add the medium along the well wall to avoid disturbing the gel. Exchange medium every other day or as needed to provide cells with fresh nutrients being careful not to disturb the gel during exchanges.
7. Place the 24-well plate back into the 37°C incubator for cell growth.

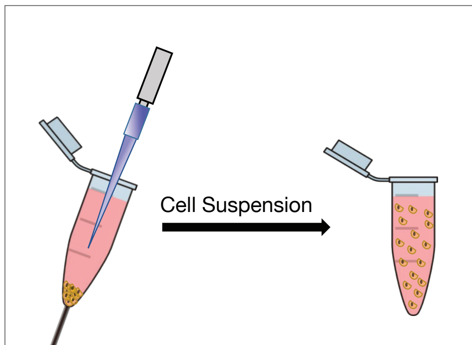
NOTES:

- Spheroids, in the range of 50 to 200 µm, will be generated for most cells in gel concentrations of 0.5% to 1.5%.
- Synthegel X-Link solution is always 8% of the total volume of 2% Synthegel Spheroid Matrix.
- To avoid introducing air bubbles, keep the pipet tip within the solution or mixture during all pipetting steps.

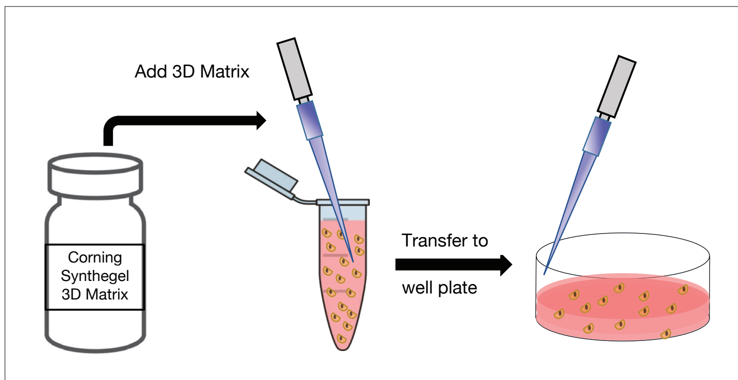
3D Spheroid Culture Protocols

A. Encapsulate Cells from Subculture (2D or 3D) or Cryopreserved Cells

1. Bring Synthegel Spheroid Matrix solution and Synthegel X-Link solution to room temperature (15°C to 25°C) or 37°C (37°C water bath) prior to starting.
2. Add Synthegel X-Link solution to the cell suspension, in desired medium (Table 1), pipetting well without introducing air bubbles.



3. Mix the Synthegel Spheroid Matrix solution carefully into the cell suspension of Step 2 (Table 1) and pipet mix (avoid the introduction of air bubbles). Transfer the mixture into the center of each well, then swirl the plate to uniformly cover the entire bottom surface of the well (for larger area plates, i.e., 6-well plates).
4. Gently shake the plate front to back and side to side to allow the solution to uniformly cover the entire surface. Example of cell seeding densities and gel concentrations are shown (Table 5).



5. Incubate the plate at 37°C (5% CO_2) for 30 to 60 min. to complete the gelation.
6. After gelation, overlay the hydrogel in each well with medium to prevent drying and provide nutrients for the cells (Table 2).

   TIP: Slowly add cell culture medium along the wall of each well without disturbing the hydrogel.
7. Exchange 1/3 to 2/3 the volume of medium, above the hydrogel, with fresh medium every day or every other day. Frequency of medium exchanges will depend on cell feeding strategy for each cell type and will need to be determined empirically. Color change of the medium can also be used as an indicator for timing of medium exchanges.
8. Synthegel Spheroid Matrix supports the formation of spheroids from a single cell in a 3D environment, with the size and compactness dictated by the cell type, concentration of the Synthegel Spheroid Matrix gel, initial cell seeding density and duration of cell growth phase. Examples are shown in Figures 1-4 of various cell type, seeded densities, Synthegel Spheroid Matrix gel concentrations, and duration (Table 3). See Table 2 for recommended medium feeding volume per well for various plate formats.

B. Examples of Spheroids Culturing in 3D Synthegel Spheroid Matrix

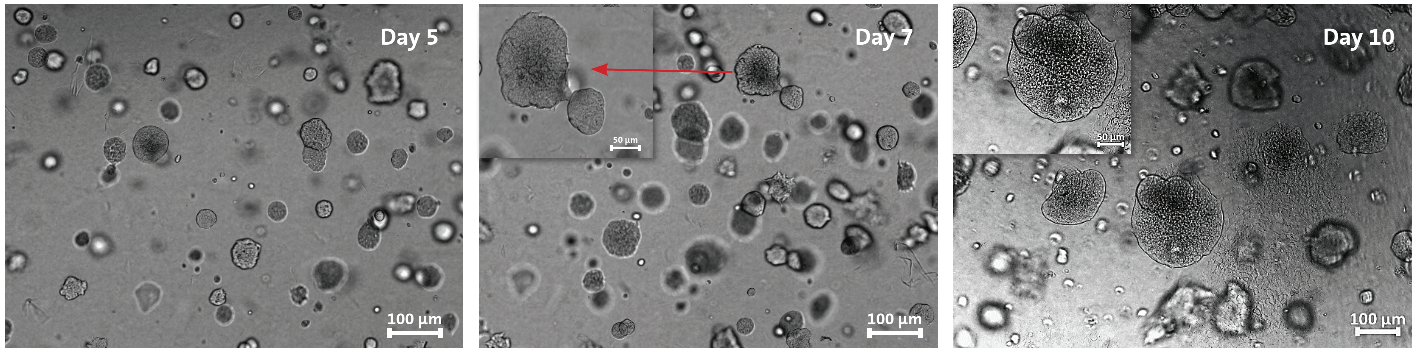


Figure 1. Physiological like morphologies of Head and Neck Cancer cells. 1 x 10^4 cell/mL in 24-well plate, 0.5% Corning Synthegel Spheroid Matrix hydrogel.

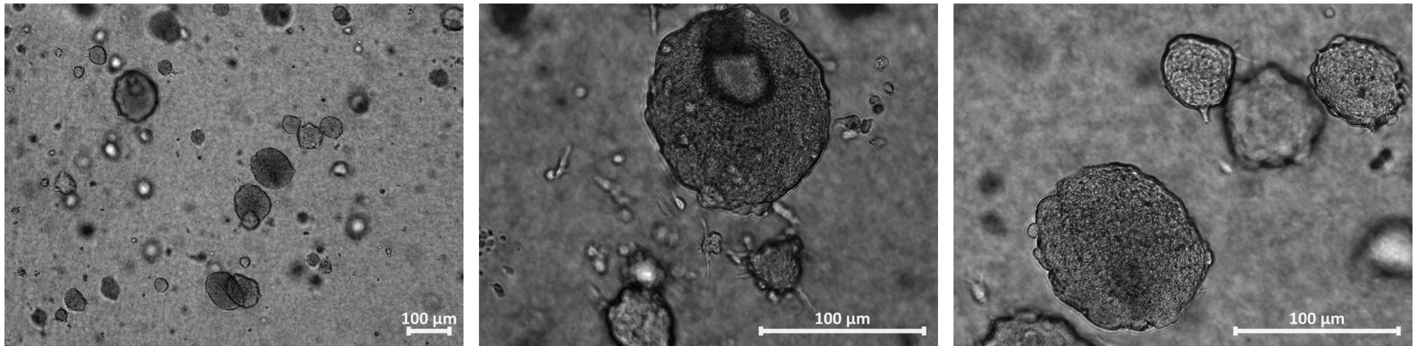


Figure 2. Physiological like morphologies of HepG2 (ATCC HB-8065). 5 x 10^4 cell/mL in 24-well plate, 1% Corning Synthegel Spheroid Matrix hydrogel at Day 11.

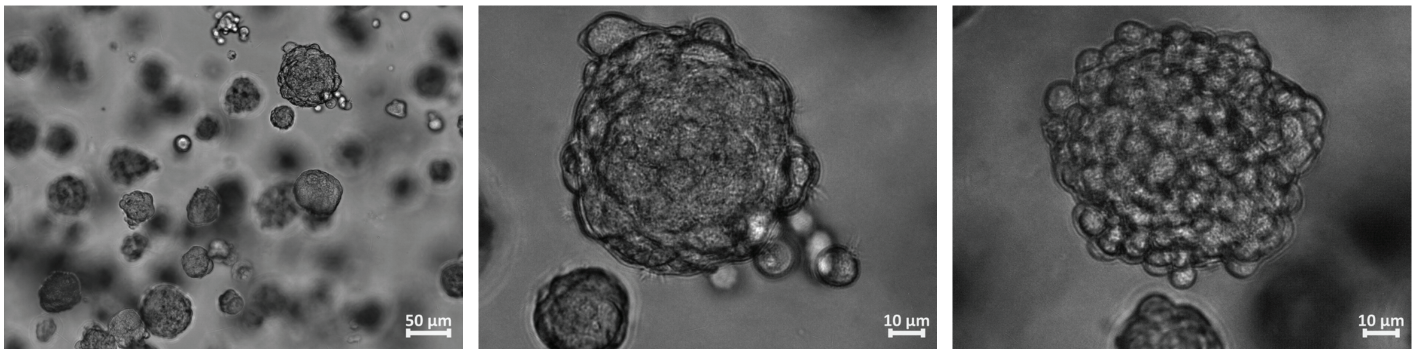


Figure 3. Physiological like morphologies of PANC-1 (ATCC CRL-1469). 3 x 10^4 cell/mL in 24-well plate, 0.5% Corning Synthegel Spheroid Matrix hydrogel at Day 10.

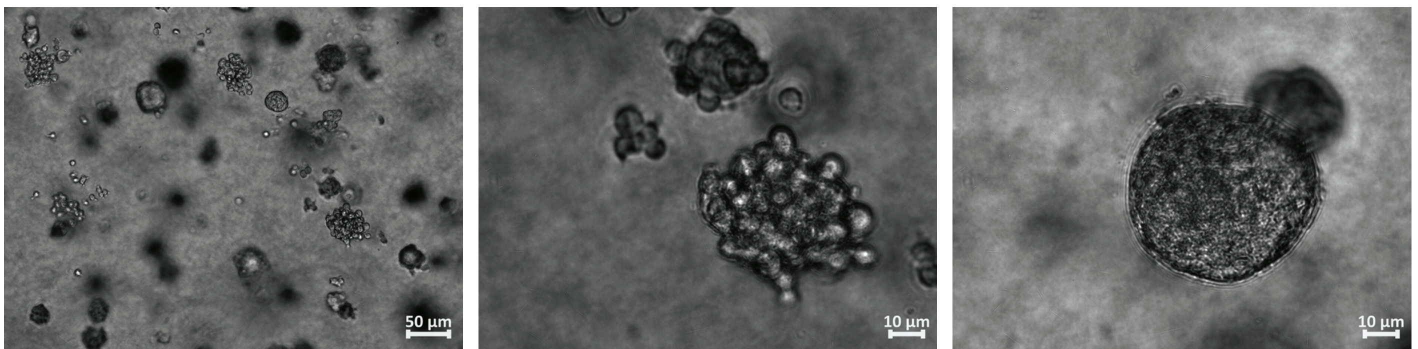


Figure 4. Physiological like morphologies of 4T1 (ATCC CRL-2539). 3 x 10^4 cell/mL in 24-well plate, 0.5% Corning Synthegel Spheroid Matrix hydrogel at Day 5.

Table 3. Impact of cell seeding densities and Synthegel Spheroid Matrix concentrations on spheroid sizes of head and neck cancer cells in Corning 3D Synthegel Spheroid Matrix (24-well plate).

Corning Synthegel Spheroid Matrix Gel Concentration (w/v)	Seeding Density (cell/mL)	Spheroid Size (µm)*		
		Day 4	Day 8	Day 10
0.5% Synthegel Spheroid Matrix	4×10^4	20 - 30	40 - 80	40 - 80
	1×10^4 to 2×10^4	20 - 30	40 - 100	50 - 150
	4×10^3	20 - 30	30 - 80	50 - 150
1% Synthegel Spheroid Matrix	1×10^5	20 - 30	40 - 80	40 - 100
	4×10^4	20 - 30	50 - 100	50 - 200

*5×10^4 cells seeded in one well of 24-well plate can produce at least 1×10^4 physiological spheroids with size range of 50 to 80 µm in 5 to 6 days.

C. Thawing Cells Directly in 3D Synthegel Spheroid Matrix

1. Prior to use, bring the Synthegel Spheroid Matrix and Synthegel X-Link solution to room temperature (15°C to 25°C) or 37°C (37°C water bath).
2. Thaw the vial of cryopreserved cells by gently agitating in a 37°C water bath. To reduce the risk of contamination, avoid exposure of the O-ring and cap to water in the water bath.
3. Remove the vial from the water bath as soon as the contents start to thaw. Spray the outside of vial with 70% ethanol to decontaminate prior to placing into a biosafety cabinet for further manipulations. Add 1 mL of pre-warmed complete culture medium to the vial and pipette gently until the cell suspension is completely thawed.
4. Transfer the cell suspension to the 15 mL conical tube, add 1 mL of complete culture medium to the vial to remove any remaining cells, and combine into the 15 mL conical tube.
5. Add complete culture medium to the 15 mL conical tube to bring the final volume up to 10 mL and centrifuge at 100 to 200 g for 5 to 10 min. Duration and centrifugal force will depend on the cell type and may need to be determined empirically.
6. Remove the supernatant and resuspend the cell pellet by gently pipetting in complete culture medium prior to enumeration.
7. Follow Steps 2-6 in Section A [Encapsulate Cells from Subculture (2D or 3D) or Cryopreserved Cells] for cell encapsulation in 3D Synthegel Spheroid Matrix.

NOTE: One- to two-fold increase in cell seeding density is recommended for direct thaw of cells into 3D Synthegel Spheroid Matrix vs. encapsulation of cells from subculture. This increase in cell density is necessary to offset the loss of cell viability and proliferation that often occurs upon thaw.

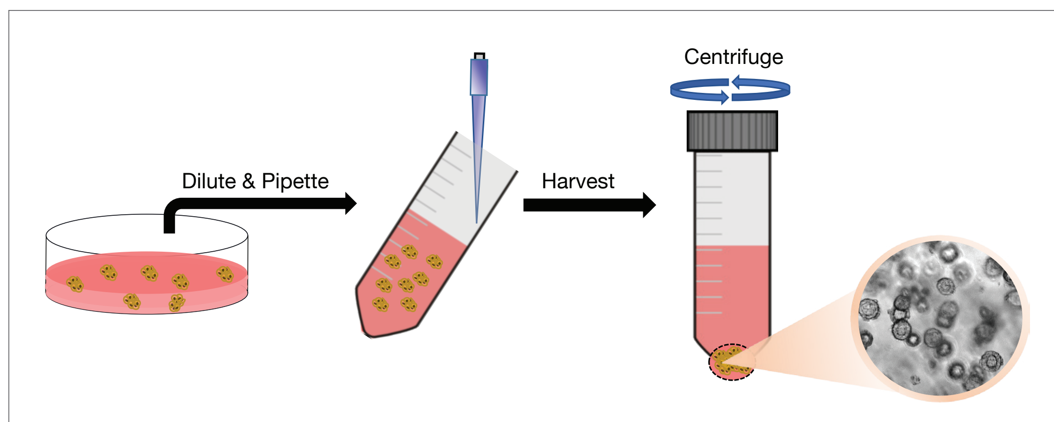
D. Cell Recovery for Passaging, Enumerating, and Downstream Applications

1. **Gel disruption:** Pipette gel and cell medium overlay to mechanically disrupt the gel **THOROUGHLY** (thorough disruption of the gel is very important for spheroid isolation from the gel). Transfer the disrupted mixture to an appropriately sized conical centrifuge tube, as determined by well size (Table 4).
2. **Rinse:** Use PBS or DPBS (without Mg^{2+} and Ca^{2+}) to rinse well and combine into the centrifuge tube. Recommend rinse volume is double the maximum plating volume for each well (Table 1) (i.e., 200 µL/well for 96-well microplate or 1000 µL/well for 24-well plate).
3. **Gel dilution:** Pipette the mixture **THOROUGHLY**. Add additional PBS or DPBS to further dilute the mixture by 20 to 25 folds of the original plating volume (Table 4) (i.e., 2 to 3 mL/well for 96-well microplate or 10 to 15 mL/well for 24-well plate), and mix well.

NOTE: If harvesting wells cultured in the same conditions, the diluted gels can be processed separately or combined into 1 tube to reduce handling. (i.e., 2 wells from a 24-well plate can be combined a single 50 mL conical tube).

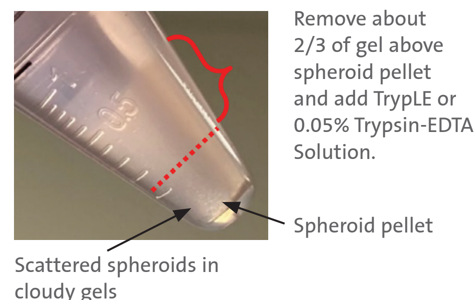
Spheroids recovery

4. **Centrifuge:** Centrifuge at 700 to 800 g for 5 to 6 min. (Higher centrifugal force is typically required for spheroid harvested from 3D Synthegel Spheroid Matrix vs. conventional 2D cell harvests).
    - If final concentration of Synthegel Spheroid Matrix gel used is between 0.3% to 1%, discard the supernatant after centrifugation, and collect the spheroid pellet.



NOTE: Most spheroids collect at the bottom of the centrifuge tube, but occasionally a thin visible layer of cloudy gel is observed right above the spheroids. If this occurs, do not discard the cloudy gel layer when removing the supernatant. Instead repeat Steps 3 and 4 above to dilute the gel and wash away the gel completely by centrifuging to obtain gel-free spheroids.

- If final concentration of Synthegel Spheroid Matrix gel used is between 1.5% to 2% cell culture, a thick layer of cloudy gel right above the spheroid pellet may be observed after centrifugation. If this occurs, perform the following procedure to completely remove the gel.



1. Discard the supernatant, and carefully remove about 2/3 of cloudy gel from above the spheroid pellet.
2. Add TrypLE™ Express Enzyme (1X) (Thermo Fisher 12604021) or 0.05% Trypsin-EDTA solution to the centrifuge tube. Recommended volume of cell dissociation reagent should be double the maximum plating volume for each well (Table 1) (i.e., 200 µL/well for 96-well microplate or 1000 µL/well for 24-well plate). Pipette gently several times after reagent addition.
3. Incubate at 37°C for 5 min., then add equal volume of culture medium to neutralize dissociation reagent.
4. Gently pipet mix to dissociate gel.
5. Centrifuge at 200 to 300 g for 5 min.
6. Discard supernatant and resuspend spheroids in complete culture medium. Spheroids can now be used for further analysis or re-encapsulated in Synthegel Spheroid Matrix hydrogel or another Synthegel 3D Matrix kit for downstream uses.

NOTES:

- For Synthegel Spheroid Matrix gel with a final concentration of 2% W/V. If a cloudy gel is observed after using TrypLE Express Enzyme (1X), a 0.05% Trypsin-EDTA solution can be used for spheroids harvesting following Steps 2 to 4 outlined above.
- Small amounts of gel residue will not interfere with PCR or immunostaining.

5. **Spheroids size distribution and separation:** Cell strainer with pores of 40 µm, 70 µm and 100 µm or flow cytometry can be used to sort cells based on size for downstream applications.

Table 4. The recommended conical centrifuge tube size vs. well plate size for gel dilution*.

	6-well	12-well	24-well	48-well	96-well
Gel plating volume	2,000 µL	1,000 µL	500 µL	250 µL	100 µL
Final volume of diluted gel and cell mixture from one well (20-25 folds)	40 - 50 mL	20 - 25 mL	10 - 15 mL	5 - 7.5 mL	2 - 3 mL
Suggested conical tube size	50 mL	50 mL	15 mL	15 mL	2 or 15 mL

*The conical tube sizes suggested here are for harvesting one well of cultured cell per plate. If more than one well is harvested, tube size should be sized accordingly. For example, a 50 mL tube can be used if 3 wells of 24-well plate cultured cells are harvested at the same time (3 x 15 mL).

Single cell recovery

Spheroid break-up: To break-up spheroids into single or small cluster cells, follow the conventional 2D culture protocol by adding enzyme solution or other dissociation reagent, or following procedures described below.

1. To break up the spheroids, add Trypsin-EDTA solution (i.e., 0.25% Trypsin + 0.02% EDTA) to the cell pellet at recommended Trypsin-EDTA solution volume equal to the gel plating volume (Table 1) (i.e., 100 µL/well for 96-well microplate or 500 µL/well for 24-well plate).
2. Pipet mix gently to resuspend the spheroid pellet in dissociation reagent. Incubate at 37°C (5% CO_2) for 10 min.
3. After incubation, gently pipette the cell solution up and down to mechanically break up the spheroids. Observe the spheroid size under a microscope to determine if extended incubation times are needed. If the majority (≥90%) of the cells are single cells, then proceed to the next step, otherwise incubate an additional 5 to 10 min. at 37°C (5% CO_2) until the majority are single cells.
4. Add an equal volume of culture medium or Trypsin neutralization solution to the conical tube, and centrifuge at 100 to 200 g for 5 min. (depending on cell type).
5. Carefully remove supernatant and discard. Resuspend cell pellet in complete culture medium for enumeration or passage.

NOTE: The Enzyme concentration and incubation time for spheroids disruption depends on the cell type, spheroid size, and quantity. Certain cell lines need longer incubation time with selected dissociation reagent to break up spheroids (50 to 100 µm) into single cells (i.e., 20 to 30 min. is required for 4t1, PANC 1, and HepG2 spheroids). The procedure is for reference only. Dissociation reagent and times will need to be determined empirically for each cell type, spheroid size, and quantity.

E. Cell Cryopreservation

1. After enumeration, centrifuge solution at 100 to 200 g for 5 min. (depending on cell type).
2. Carefully remove supernatant and discard. Resuspend cell pellet in freezing medium or complete growth medium with 5% to 10% DMSO to a final concentration of 1×10^6 to 1×10^7 cells/mL depending on cell type. Aliquot into cryopreservation vials and incubate at room temperature for 15 min. to allow diffusion of cryoprotectant into cells.

NOTE: Do not add DMSO directly to cells in complete growth medium. Prepare solution of complete cell growth medium with 5% to 10% DMSO prior to resuspending cell pellet.

3. Slowly freeze cells at 1°C/min. by using a programmable cooler, or a Corning CoolCell® module (Corning 432000), and place it in a -80°C freezer for at least 24 hours.
4. Quickly transfer the vial to liquid nitrogen or -130°C freezer for long-term storage. Do not store cells at -80°C for extended periods of time as it negatively impacts cell viability.

F. Immunostaining

Staining can be performed from spheroids harvested from Synthegel Spheroid Matrix or can be stained directly within the hydrogel. The Recommended procedure for staining cells directly in the hydrogel is as follows (i.e., hiPSC cells).

All reagents and procedures are done at room temperature unless otherwise noted.

1. Carefully remove the medium on top of the hydrogel in cultured wells.
2. Carefully rinse each well of hydrogel surface once with DPBS (without Ca^{2+} and Mg^{2+}). Rinse volume should be equal to initial media volume (Table 2) (i.e., 300 mL/well for 96-well microplate or 1.5 mL/well for 24-well plate).
3. Carefully remove DPBS overlay.
4. Fix cells by adding 10% neutral buffered formalin and incubate for 30 to 40 min. Volume of buffered formalin should be equal to initial media volume (Table 2) (i.e., 300 mL/well for 96-well microplate or 1.5 mL/well for 24-well plate).
5. Carefully remove buffered formalin overlay.
6. Wash hydrogel twice with DPBS (without Ca^{2+} and Mg^{2+}) for 15 min. each.
7. Prepare washing buffer and blocking solution.
   a) Washing buffer: DPBS (with Ca^{2+} and Mg^{2+}) + 0.2% V/V Triton X-100 + 0.1% V/V cold water fish gelatin. Mix by pipetting thoroughly or by vortexing gently.
   b) Blocking solution: washing buffer + 10% V/V serum from the same source animal as secondary antibody. Mix by pipetting thoroughly or by vortexing gently.

NOTE: Blocking solution should be prepared just prior to application to the sample.

8. Carefully remove DPBS overlay.
9. Wash twice with washing buffer for 10 min. each. Volume of washing buffer should be equal to initial media volume (Table 2) (i.e., 300 mL/well for 96-well microplate or 1.5 mL/well for 24-well plate).

NOTE: If the sample is not blocked and stained immediately, it can be stored after the addition of washing buffer. Stored sample plate should be sealed with parafilm, to minimize evaporation and stored at 4°C until ready for immunostaining. Remove plate from 4°C and allow to reach room temperature before proceeding to blocking step.

10. Blocking (Day 1)
    - Carefully remove washing buffer overlay, and add blocking solution to the fixed samples. Volumes of blocking solution should be 100% to 150% of maximum plating volume/well (Table 2) (i.e., 500 to 750 µL/well for 24-well plates or 100 to 150 µL/well for 96-well microplates).
    - Incubate overnight or >12 hrs.
11. Primary Antibody (1Ab) (Day 2)
    - Prepare primary antibody solution by diluting the primary antibody into washing buffer. Primary antibody concentrations for 3D staining should be the same or higher than what is used for 2D culture staining (i.e., Goat Oct 3/4 antibody (N-19), with final concentration of 3 µg/mL, was used for hiPSC staining directly in 3D Synthegel 3D hiPSC Matrix)[1].
    - Carefully remove blocking buffer.
    - Add primary antibody solution to the gel. Volumes of blocking solution should be twice the maximum plating volume/well (Table 2) (i.e., 1000 µL/well for 24-well plates or 200 µL/well for 96-well microplates).
    - Incubate overnight or >12 hrs.

    NOTES:

    - Volume of primary antibody solution should be enough to completely soak the gel.
    - Blocking buffer should be added to one well as a negative control to assess background staining.
12. Rinse (Day 3)
    - Carefully remove primary antibody solution or blocking buffer from wells.
    - Wash four times with washing buffer for 2 hours each. Volume of washing buffer should be equal to initial media volume (Table 2) (i.e., 300 mL/well for 96-well microplate or 1.5 mL/well for 24-well plate).
13. Secondary antibody (2Ab) (Day 3)
    - Prepare secondary antibody solution by diluting the secondary antibody into washing buffer. Secondary antibody concentration for 3D staining should be the same or higher than that used for 2D culture staining. Use the concentration recommended by vendor or determined empirically (i.e., Rabbit anti-goat IgG (H+L) secondary antibody Alexa Fluor™ 488 , with a final concentration of 5 µg/mL, was used for hiPSC staining directly in Synthegel 3D hiPSC Matrix)[9].
    - Wrap the plate with aluminum foil to prevent bleaching of fluorescent dyes.
    - Incubate overnight or > 2 hrs.
14. Rinse and Imaging (Day 4)
    - Carefully remove secondary antibody solution from wells.
    - Wash a minimum of 6 times with washing buffer for 1 hr. each. Volume of washing buffer should be equal to initial media volume (Table 2) (i.e., 300 mL/well for 96-well microplate or 1.5 mL/well for 24-well plate).
    - After final wash, replace washing buffer with Glycerin for imaging.
    - Proceed with imaging.

    NOTES:

    - To achieve strong and specific signals, it is critical to thoroughly block non-specific binding of antibodies and allow the diffusion of antibodies through to the hydrogel matrix (Synthegel 3D Matrix). To facilitate these requirements, extended blocking and incubation times as well as multiple washes are required.
    - The background signal, in 3D systems, is influenced by cells located in different planate positions. Therefore, post-processing of images may be needed to reduce background brightness.

Tips for handling hydrogels in plates

1. Tilt the plate at a slight angle (approx. 15-20°) when working pipetting.
2. Be sure to expel air from the pipet prior to placing into liquid.
3. Immerse pipet tip into the gel, while not touching the well bottom, when disrupting hydrogel.
4. Slowly aspirate fluids from above the hydrogel to prevent accidental removal of cells.
5. lowly dispense fluids along the top wall of the well for to prevent disruption of hydrogel.
6. Avoid air bubbles during pipetting.

NOTE: Results reported here are under standard cell culture condition (37°C and 5% CO_2) and for reference only. Use appropriate cell medium, growth supplements, and growth conditions appropriate for each cell type.

Table 5. Reference: 3D Spheroid Culture in Corning® Synthegel™ Spheroid Matrix.

Cells	Corning Synthegel Spheroid Matrix Hydrogel Concentration	Gelation Time (min.)	Cell Seeding Density (cell/mL)	Spheroid Size (µm)	Proliferation (folds)	Cell Viability (%)
MCF-7 (ATCC)	0.2% - 0.3%	60	2.8×10^5	30 - 80	4	90
Hela cell	0.5% - 1%	30	8×10^4	50 - 100	5 - 6	90 - 95
Head Neck cell	0.5% - 1%	30	4×10^4	50 - 200	15 - 20	90 - 95
A549 (ATCC)	0.5% - 2%	30	5×10^4 to 1.5×10^5	40 - 100	6 - 7	90
LET1	0.5% - 2%	30	5×10^4 to 1.5×10^5	40 - 100	6 - 7	90
HepG2 (ATCC)	0.5% - 2%	30	4×10^4 to 1×10^5	50 - 200	10	90 - 95
PANC 1 (ATCC)	0.5%	30	2×10^4 to 5×10^4	30 - 80	10 - 15	90 - 95
4t1 (ATCC)	0.5%	30	4×10^4 to 1×10^5	30 - 60	10 - 20	90 - 95
FHC (ATCC)	0.3% - 0.5%	30 - 60	1×10^6 to 3×10^6	20 - 40	*	90 - 95
AD MSC (ATCC)	0.5% - 1%	30	6×10^6	40 - 80	*	90

*No proliferation of cells were seen but spheroids of 20 µm to 100 µm spontaneously formed from adjacent cells.

References

1. Nethercott HE, et al. Immunocytochemical analysis of human pluripotent stem cells. Human Pluripotent Stem Cells: Methods and Protocols. 2011:201-20.

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