Corning® Synthegel™ 3D Matrix Kits

Frequently Asked Questions

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1. **What are the storage conditions and shelf life of Corning Synthegel 3D Matrix kits?**

   Synthegel 3D Matrix kits need to be refrigerated promptly upon arrival and are stable for 12 months when stored at 4°C.

2. **What is the storage condition and shelf life of Corning Synthegel 3D hiPSC Grow Mix, lyophilized powder?**

   The Grow Mix lyophilized powder should be stored at -20°C promptly upon arrival and is stable for 12 months when stored at -20°C. Once the powder is reconstituted the shelf life is 6 months when stored at -20°C. Users are encouraged to aliquot the reconstituted Grow Mix solution into smaller working volumes depending on their use to avoid frequent freeze/thaw cycles. Frequent freeze/thaw cycles are detrimental to functional performance.

3. **Can the hydrogel strength be altered by changing the peptide concentration?**

   Yes. The concentration of Corning Synthegel Matrices can be easily adjusted to either increase or reduce the gel shear strength in the range of 200 to 2,400 Pa (0.2% to 2%).

4. **What is the recommended cell seeding density and hydrogel concentration for 3D spheroid formation?**

   Cell seeding density and hydrogel concentration will depend on cell type and application, but for most standard cell lines (i.e., epithelial, T-cells, cancer cell lines, etc.), seeding densities are typically 0.4 to 2 x 10^5 cell/mL with hydrogel concentrations ranging from 0.2% to 0.5%. Primary cells, mesenchymal stem cells (MSCs), hepatocytes, etc. need to have higher seeding densities 2 to 6 x 10^6 cell/mL to form spheroids and maintain high cell viability. It is recommended that the cell seeding density and gel concentration be determined empirically for each cell type and application using the recommendations above as a starting point.

5. **How long can cells be maintained in Corning Synthegel 3D Matrices?**

   Spheroids formed within a Synthegel hydrogel can be maintained for up to 2 weeks with an optimized feeding strategy. For long-term spheroid cultures (up to 4 weeks) it is recommended that Synthegel 3D hiPSC Suspension Matrix be used.

6. **Are there recommendations for harvesting spheroids from Corning Synthegel 3D Matrices?**

   The recommended technique for successful spheroid harvesting is gel disruption followed by centrifugation.

   - Initial gel disruption, before gel dilution, is crucial to separating spheroids from Corning Synthegel 3D Matrices. Without removing the upper layer cell medium, mechanically disrupt the gel **THOROUGHLY** by pipetting the gel and medium mixture several times.
   - Use centrifugal force (700 to 800 x g for cancer cell lines, and 250-400 x g for stem cells (i.e., hiPSC)) when separating spheroids from Corning Synthegel 3D matrices.
   - Additional cycle of gel disruption/gel dilution/centrifugation will be necessary if a cloudy overlay is observed above cell pellets after centrifugation. This will help to separate the remaining spheroids from the hydrogel.
   - Finally, if cell isolation remains an issue, a 0.2% to 0.3% reduction in gel concentration is recommended. Cells secrete proteins while enveloped within Corning Synthegel 3D matrices resulting in a change to the gel strength, which can affect spheroid harvesting efficiency.

7. **How long should spheroid colonies be exposed to a dissociation reagent before dispersing into single cells?**

   The dissociation time for dispersing spheroids depends on several factors such as cell type, spheroid size, and quantity. A more detailed procedure for dispersing spheroid colonies is available in the Corning® Synthegel™ Spheroid Matrix Kit Guidelines for Use (CLS-AN-739DOC).

   When working with standard cancer cell lines the addition of Trypsin-EDTA solution (i.e., 0.25% Trypsin + 0.02% EDTA) to the spheroids pellet is sufficient to dissociate the spheroids into single cells and small cell clusters. The gel plating volume (i.e., 500 µL for 24-well plate), given in the Guidelines for Use for the various kits, should be referenced as the starting volume of dissociation reagent to be added to each well. Incubation times will fluctuate depending on cell type and spheroid size [i.e., 10 to 15 min. for Hela cells, Head and Neck cancer cells (50 to 100 µm) or 20 to 30 min. for 4t1, PANC 1, and HepG2 (50 to 150 µm)]. Following these guidelines will result in a ≥90% dissociation of spheroids into single cells or small cell clusters.

   NOTE: After 5 to 10 min. incubation in Trypsin-EDTA solution, pipette the cell solution up and down several times to mechanically break up the spheroids. Observe the spheroid size under a microscope to determine if incubation time should be extended. Repeat incubation and pipetting steps until spheroids are observed, under a microscope, to be of the desired size. The appropriate conditions needed to achieve desired results will need to be empirically determined for each cell type.

8. **Is the Corning Synthegel Spheroid Matrix kit appropriate to produce large quantities of physiological spheroids?**

   The Synthegel Spheroid Matrix kit can produce large quantities of physiological spheroids from dividing cells.

   Example: A single well of 24 well-plate, seeded at 5 x 10^4 with HepG2 cells, can produce ≥1 x 10^4 physiological spheroids (50 to 100 µm) in 6 to 7 days.

9. **What are the differences between the Corning Synthegel 3D hiPSC Matrix kit, and the Corning Synthegel Spheroid Matrix kit?**

   Synthegel Matrix kits are derivatives of each other. The Synthegel Spheroid Matrix kit is for producing physiologically relevant spheroids and can be used for bioprinting. The Synthegel 3D hiPSC Matrix kit is ideal for culturing 3D hiPSC for proliferation and spheroids production.

10. **Can spheroids or cells in Corning Synthegel Matrices be directly immunostained?**

    Yes. The same concentrations of fluorescent stain used for immunostaining 2D samples can be used in 3D. Refer to the related Guidelines for Use for the specific protocol.

    The gel itself will not be stained, but fluorescence from cells in different planar locations and background fluorescence from soluble proteins might be enhanced (Figure 1).

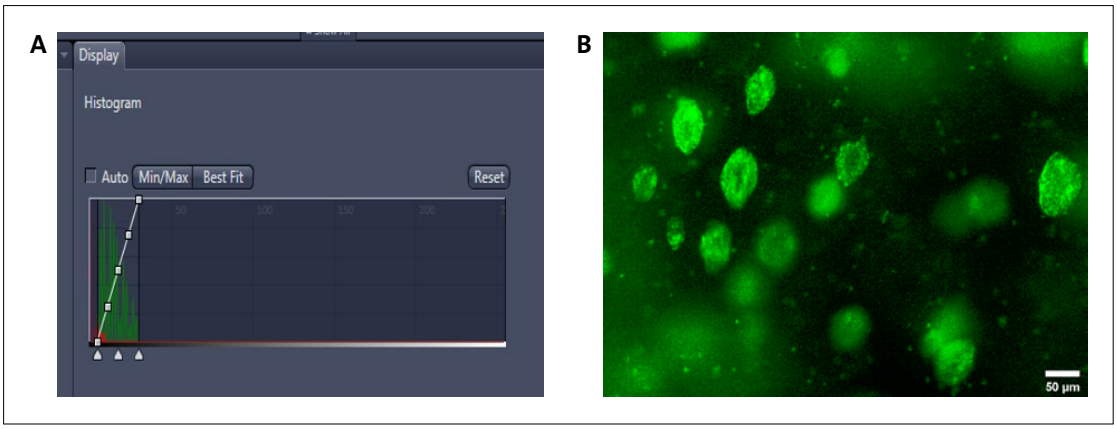

Figure 1. (A) Histogram of brightness, from Zeiss Microscope software, of hiPSC cultured in Corning Synthegel 3D hiPSC Matrix. Limiting brightness range to only green light, the background will be reduced (Alexa Fluor 488). (B) Fluorescent image of hiPSC cultured in Corning Synthegel 3D hiPSC Matrix immunostained with anti-Oct4 - Alexa Fluor 488.

For further information, please refer to these Guidelines for Use:

- Corning Synthegel 3D hiPSC Matrix Kit Guidelines for Use (CLS-AN-737DOC)
- Corning Synthegel hiPSC Suspension Matrix Kit Guidelines for Use (CLS-AN-738DOC)
- Corning Synthegel Spheroid Matrix Kit Guidelines for Use (CLS-AN-739DOC)

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