3-D Cell Encapsulation in Hydrogels using 96-Well Plates

This protocol describes how to encapsulate cells in HyStem®-C hydrogels in a 96-well plate format. This protocol can easily be adapted for use with HyStem®-HP and HyStem®hydrogel kits.

Required Materials
HyStem-C, HyStem-HP, or HyStem hydrogel kit
Sterile 96-well plate

Procedure:
1. Solubilize HyStem-C Hydrogel Kit components under aseptic conditions as directed by the instructions.

2. Prepare cells for use in 3-D cell culture, as per standard procedures.
Seeding density varies with cell type, but a typical range is 5,000 to 20,000 cells per insert.

3. Prepare a 96-well plate by removing it from its sterile packaging.

4. Mix equal volumes of Glycosil® and Gelin-S® (Typically 1.0 mL of each for small vials and 5.0 mL each for large vials).

Note: Leftover solutions can be frozen at -20° C under a nitrogen or argon head and are viable for ~2 weeks. However, gel property can still change due to slow autocrosslinking, we recommend always using freshly reconstituted material when possible.

5. Add cells to Glycosil + Gelin-S such that the proper cell density will be reached in the total solution volume, typically 13.5 mL (5.0 mL Glycosil + 5.0 mL Gelin-S + 2.5 mL Extralink® + 1.0 mL cells) or 2.7 mL (1.0 mL Glycosil + 1.0 mL Gelin-S + 0.5 mL Extralink® + 0.2 mL cells). Pipette up and down to mix. More or less cell volume will respectively prolong or shorten gelation.

Note: Media can impact gelation properties, so where possible it is advised to wash your cell pellet with PBS or dilute media.

6. When you are ready to pour the hydrogels, add Extralink to Glycosil + Gelin-S with cells.
Extralink volume should be one-fourth the volume of the Glycosil + Gelin-S mixture (typically 2.5 mL or 1.0 mL). Once the Extralink is added, you have < 20 minutes before the hydrogel forms.

Note: The gelation time is very dependent upon the pH of the HyStem-C solution with the cells. The higher the pH, the faster the gelation time. Different media will have different effects on the final pH and gelation time.

7. Quickly pipette 100 µL of HyStem-C into each insert.

Note: Do not add media at this point, since this will dilute the hydrogel and prevent it from gelling.

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8. Place the plates in the 37° C incubator with 5% CO_2.
Allow HyStem-C to gel for one hour.

9. Remove the plates from incubator.
Verify that the hydrogel is solid. If so, add 100 µL of media to each well.

10. Place in the 37° C incubator with 5% CO_2.

Changing Media

Carefully aspirate off the media. The hydrogel can easily be removed by the vacuum as well, so this must be done gently and carefully.

Pipette 100 µL media into each well. Try to avoid disrupting the gel.
Return the plate to the 37°C incubator with 5% CO_2.

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