3-D Cell Encapsulation in Hydrogels using TC Inserts

This protocol describes how to make HyStem®-C hydrogels in a 24-well plate format using tissue culture inserts. 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
24-well plate with tissue-culture inserts 8.0 µm pore size

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 two 24-well plates with tissue culture inserts by removing them from their sterile packaging.

4. Mix 1.0 mL of Glycosil® and 1.0 mL of Gelin-S®.

5. Add 0.2 mL cells to Glycosil + Gelin-S such that the proper cell density is reached when the 100 µL of the total solution volume of 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.

6. When you are ready to pour the hydrogels, add 0.5 mL of Extralink® to Glycosil + Gelin-S with cells. 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 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 into each insert.

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

8. Place the plates in the 37° C incubator with 5% CO2. Allow HyStem to gel for one hour.

9. Remove the plates from incubator.
Verify that the hydrogel is solid. If so, add 1.8 mL of media to each well.

10. Place in the 37° C incubator with 5% CO2.

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Changing Media

1. Move each tissue-culture insert to the adjacent empty well. Aspirate off the media.
2. Tap each insert carefully to remove the media above the gel in the insert. You can aspirate off the media; however, the gel can easily be removed by the vacuum as well, so this must be done gently and carefully.
3. Replace the insert into its normal well.
4. Slowly and carefully pipette 1.8 mL 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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