Enzyme Digestion of Hydrogels for Recovery of Encapsulated Cells

This protocol is for recovering cells that have been encapsulated in HyStem®-C, HyStem®-HP and HyStem® hydrogels and grown in tissue-culture inserts. It can be easily adapted for other gel formats. Note that HyStem hydrogels do not contain Gelin-S® and thus do not strictly need collagenase to be digested.

Tips

Be cautious about mechanically breaking up the hydrogel prior to digestion because this can lower cell viability significantly.
If the 1:10 dilution of 1x collagenase/hyaluronidase is not satisfactory, try a 1:5 dilution with digestion overnight.

Required Materials

One HyStem, HyStem-C, or HyStem-HP hydrogel kit
StemCell Technologies 10x collagenase/hyaluronidase (Cat # 07912)

Procedure

1. Dilute the 10X collagenase/hyaluronidase solution 1:10 in the cell culture media (with no FBS) used to cultivate your cells.

Note: Do not use undiluted enzyme since this results in low cell viability.

2. If you are using media that contains FBS, make sure to wash the hydrogels with FBS-free media before starting the digestion process.
At a minimum, wash hydrogels two times for one hour to clear FBS.

3. Remove the tissue culture insert from the 24-well culture plate. Place upside down in a Petri dish.

4. Run a 200 µL pipette tip around the edge of the membrane, cutting it loose from the insert.
The membrane will stay attached to the insert, but usually flips up out of the way.

5. Turn the insert right side up and, using the back of a 10 µL pipette tip, punch the hydrogel out of the insert into the Petri dish.

6. Place the hydrogel in a 15 mL conical.

7. Add 5 mL of the dilute collagenase/hyaluronidase solution to the hydrogel for each 100 µL of hydrogel.

8. Incubate by shaking gently overnight at 37 °C.

9. At the end of the incubation, there will still be some hydrogel left in the tube.

10. Centrifuge the conical at 1500 rpm for five minutes. Aspirate off enzymes in media.

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11. Wash cells in 5 mL PBS.

12. Centrifuge at 1500 rpm for five minutes. Aspirate off PBS.

13. Resuspend the cell pellet and remove all the PBS and cells.

Note: In the PBS you can see any remaining hydrogel.

14. When you remove the cells, leave behind any remaining hydrogel.

15. Wash the cells in media and centrifuge at 1500 rpm for five minutes.

16. Aspirate off all media but ~0.5 mL.

17. Resuspend the 0.5 mL of remaining media and cells in media.