CELL
APPLICATIONS, INC.

Instructions for Encapsulating Chondrocytes in Alginate Bead Kit

Catalog No: 072K-Alginate

I. Chondrogenesis Kit Components

Component	Cat. No.	Volume	Storage Temperature
Sodium Alginate Solution	072-25	25 ml	Room Temperature
Calcium Chloride Solution	071-100	100 ml	Room Temperature
Sodium Chloride Solution	074-100	100 ml	Room Temperature
Chondrocyte Differentiation Medium	411D-250	250 ml	4°C in the dark
Depolymerization Solution	073-50	50 ml	Room Temperature

II. Procedure:

A. Encapsulation of De-differentiated Chondrocytes in Alginate Beads (for 1 x 10^6 cells)

1. Use standard trypsinization techniques to detach Chondrocytes from the tissue culture ware.
2. Resuspend chondrocytes in Sodium Alginate Solution at 4-5 x 10^5 cells/ml (1 x 10^6 cells in 2 ml).
3. Mix thoroughly by pipetting up and down gently using sterile Pasteur pipette.
4. Transfer the suspended chondrocytes to a 10 ml syringe attached with a 22-gauge needle.
5. Express the suspended chondrocytes dropwise through the 22-gauge needle into 8-10 ml of Calcium Chloride Solution which was gently agitated during the addition of chondrocytes.
   *1 x 10^6 cells in 2-2.5 ml of Alginate Solution will polymerize into ~150 beads.
6. Allow the alginate beads to polymerize for 10 minutes in the $CaCl_2$ solution.
7. Decant $CaCl_2$ solution and wash the beads 5 times in 10 ml of Sodium Chloride Solution, followed by one wash in 4 ml of Chondrocyte Differentiation Medium.
8. Culture the encapsulated chondrocytes in Chondrocyte Differentiation Medium with a seeding density of 150 beads per 2 ml of Chondrocyte Differentiation Medium in a T-25 flask or 400±50 beads in T75 flask.
9. Incubate the cells at 37°C in a humidified CO2 incubator for 3 to 4 weeks for fully re-differentiation of Chondrocytes.
10. Change the medium twice a week by carefully removing the old medium with serological pipette (do not aspirate) without disturbing the beads.

B. Recovery of Re-differentiated Chondrocytes by Depolymerizing Alginate Beads (for 1 x 10^6 cells)

1. Remove Chondrocyte Differentiation Medium.
2. Wash the beads 2 times with Sodium Chloride Solution.
3. Add Depolymerizing Solution to the beads at 7.5 ml/ 1 x 10^6 chondrocytes in beads. (3 times volume of alginate beads)
4. Mix at room temperature for 20 to 30 minutes with gentle agitation until the beads have completely dissolved.
5. Pellet Re-differentiated Chondrocytes at 2000 rpm for 10 minutes.
6. Pipette out the supernatant, and save in a 10cm non-tissue culture dish. Do not aspirate.

7. Check the supernatant under the microscope to make sure that Re-differentiated Chondrocytes are not lost. If not all Re-differentiated Chondrocytes have been pelleted down, spin the supernatant again and collect pellet
8. Wash the Re-Differentiated Chondrocytes 2 times with Sodium Chloride Solution, and save the wash each time to make sure that all the Re-differentiated Chondrocytes are recovered.