CELL
APPLICATIONS, INC.

General Instructions for Culturing

Re-Differentiating Human Chondrocytes (HC-RD)

Be sure to wear face protection mask and gloves when retrieving cryovials from the liquid nitrogen storage tank. The dramatic temperature change from the tank to the room could cause any trapped liquid nitrogen in the cryovials to burst and cause injury.

Open all the packages immediately upon arrival and examine each component for shipping damage. Notify Cell Applications, Inc. or your distributor immediately if there is any problem.

I. STORAGE

A. CHONDROCYTES ENCAPSULATED in ALIGINATE BEADS (403RD-25, -75)
If you order a T-75 flask, Chondrocytes encapsulated in the alginate beads will be shipped in a T-25 flask for convenience. The flasks are shipped standing up.

1. Examine under a microscope to check if all the cells are viable in the alginate beads. If not, notify CAI or your distributor immediately.
2. Decontaminate the exterior of the flask with 70% alcohol.
3. Place the sealed flask in a 37°C, 5% CO_2 humidified incubator for 2 hours as shipped.
4. In a sterile Biological Safety Cabinet, open the cap of the flask very slowly and carefully.
5. If you ordered the T-75 flasks, transfer the suspended alginate beads from T-25 flask to a new T-75 flask by pipetting alginate beads gently using 25 ml pipet.
6. Remove the Transport Medium carefully without disturbing the beads. Use a serological pipet and a pipet-aid to remove the medium from the beads. (Some of the old medium left in the flask is all right. It is impossible to remove all the Transport Medium.)
7. Add fresh Chondrocyte Differentiation Medium: 10 ml for a T-25 flask and 40 ml for a T-75 flask.
8. Lay the flask flat and place in a 37°C, 5% CO_2 humidified incubator with loosened cap to allow gas exchange.

B. DIFFERENTIATION MEDIUM (411D-500)

Store the Growth Medium at 4°C in the dark immediately upon arrival.

C. DEPOLYMERIZATION SOLUTION (073-50)

Store at room temperature.

D. 0.155 M NaCl (074-100)

Store at room temperature.

II. CULTURING RD-HC

1. Change the medium twice a week by carefully removing the old medium with a serological pipet and pipet-aid without disturbing the beads.
2. Lay the flask flat and place in a 37°C, 5% CO_2 humidified incubator with loosened cap to allow gas exchange.
3. Fully differentiated Chondrocytes can be expected in 15 days after encapsulation.

III. RECOVERY OF RE-DIFFERENTIATED CHONDROCYTES BY DEPOLYMERIZING THE ALGINATE BEADS

1. Remove Chondrocyte Differentiation Medium.
2. Wash the beads 2 times with 0.155 M NaCl.
3. Add Depolymerization Solution to the beads: 15 ml for beads collected from T-25 flask and 40 ml for beads collected from T-75 flask.
4. Mix at room temperature for 20-30 minutes with gentle agitation until the beads have completely dissolved.
5. Pellet re-differentiated chondrocytes at 1000 rpm for 10 minutes.
6. Wash the cells 2 times with 0.155 M NaCl.

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