CELL
APPLICATIONS, INC.

General Instructions for Culturing

Human Dendritic Cells-Peripheral Blood (HDC-PB)

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. CRYOPRESERVED VIALS (6908-05a)

Store the cryovials in a liquid nitrogen storage tank immediately upon arrival.

B. CULTURE MEDIUM (623-50)

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

II. PREPARATION FOR CULTURING

1. Make sure the Class II Biological Safety Cabinet, with HEPA filtered laminar airflow, is in proper working condition.
2. Clean the Biological Safety Cabinet with 70% alcohol to ensure it is sterile.
3. Turn the Biological Safety Cabinet blower on for 10 min. before cell culture work.
4. Make sure all serological pipettes, pipette tips and reagent solutions are sterile.
5. Follow the standard sterilization technique and safety rules:
   a. Do not pipette with mouth.
   b. Always wear gloves and safety glasses when working with human cells even though all the strains have been tested negative for HIV, Hepatitis B and Hepatitis C.
   c. Handle all cell culture work in a sterile hood.

III. CULTURING HDC-PB

A. PREPARING CELL CULTURE FLASKS FOR CULTURING HDC-PB

1. Take the Culture Medium from the refrigerator. Decontaminate the bottle with 70% alcohol in a sterile hood.
2. Pipette 9 ml of Culture Medium to a 15 ml conical tube.
3. Pipette 4 ml of Culture Medium* to a T-25 flask.

* Keep the medium to surface area ratio at 1ml per 5 cm^2.
For example,
5 ml for a T-25 flask or a 60 mm tissue culture dish.
15 ml for a T-75 flask or a 100 mm tissue culture dish.

B. THAWING AND PLATING HDC-PB

1. Remove the cryopreserved vial of CD34-BM from the liquid nitrogen storage tank and bury the cryovial in dry ice using proper protection for your eyes and hands.
2. Turn the vial cap a quarter turn to release any liquid nitrogen that may be trapped in the threads, then re-tighten the cap.
3. Thaw the cells quickly by placing the lower half of the vial in a 37°C water bath for 90 seconds. Make sure that water does not reach the cap seal and thawing progress no more than 90 seconds.
4. Take the vial out of the water bath and wipe dry.
5. Decontaminate the vial exterior with 70% alcohol in a sterile Biological Safety Cabinet.
6. Remove the vial cap carefully. Do not touch the rim of the cap or the vial.
7. Resuspend the cells in the vial by gently pipetting the cells 5 times with a 2 ml pipette. Be careful not to pipette too vigorously as to cause foaming.
8. Transfer the cell suspension to the 15 ml conical tube prepared in Section IIIA Step 2.
9. Centrifuge at 400 x g for 10 minutes to pellet the cells.
10. Aspirate the supernatant from the tube without disturbing the cell pellet.
11. Flick the tip of the conical tube with your finger to loosen the cell pellet.
12. Resuspend the HDC-PB in 1 ml of Culture Medium by gently pipetting the cells.
13. Transfer 1 ml of HDC-PB to the T-25 prepared in Section IIIA Step 3.
14. Incubate HDC-PB in a 37°C, 5% CO_2 humidified incubator.
15. HDC-PB can be cultured for up to 7 days with media change at day 3 or 4.

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