CELL
APPLICATIONS, INC.

General Instructions for Culturing

Human Periphery Blood CD14+ Monocyte Cells (HPBM)

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 (6906-50a)
Store the cryovials in a liquid nitrogen storage tank immediately upon arrival.

B. CULTURE MEDIUM (615-250)
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 HPBM

A. PREPARING CELL CULTURE FLASKS FOR CULTURING HPBM

1. Take the Blood Cell Culture Medium from the refrigerator.
2. Decontaminate the bottle with 70% alcohol in a sterile hood.
3. Pipette 9 ml of Blood Cell Culture Medium to a 15 ml conical tube.
4. Pipette 15 ml of Blood Cell Culture Medium* to a T-75 flask.

***** Use Corning and Grenier flasks for best results.

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

B. THAWING AND PLATING HPBM

1. Remove the cryopreserved vial of HPBM from the liquid nitrogen storage tank 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 and watch the vial closely during the thawing process.
4. Take the vial out of the water bath when only small amount of ice left in the vial. Do not let cells thaw completely.
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 5 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 HPBM in 5 ml of Blood Cell Culture Medium by gently pipetting the cells.
13. Transfer 5 ml of HPBM to the T-75 prepared in Section IIIA Step 3. HPBM should float in the Blood Cell Culture Medium
14. Incubate HPBM in a 37°C, 5% CO_2 humidified incubator.
15. HPBM can be cultured for 3-4 days.

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