CELL
APPLICATIONS, INC.

General Instructions for Culturing

Rat Hepatocytes (RH)
In Suspension

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 (R780-30)

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

B. MAINTENANCE MEDIUM (R713-250)

Store the Maintenance 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 animal cells even though all the rodents were provided and inspected by Harlan Sprague Dawley, Inc.
   c. Handle all cell culture work in a sterile hood.

III. PLATING RH

A. PREPARING CELL CULTURE FLASKS FOR CULTURING RH

1. Take the Rat Hepatocyte Maintenance Medium from the refrigerator. Decontaminate the bottle with 70% alcohol in a sterile hood.
2. Pipette 10 ml of Rat Hepatocyte Maintenance Medium into a 50 ml conical tube, warm, and equilibrate the medium in the incubator with the cap loosened.
3. Pipette 5 ml of Rat Hepatocyte Maintenance Medium into a T-75 flask, warm, and equilibrate the medium in the incubator with cap loosened.

B. THAWING AND PLATING RH

1. Remove the cryopreserved vial of RH 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. Pipette the cell suspension from the vial into a sterile conical tube containing 30 ml of cold Rat Hepatocyte Maintenance Medium.
9. Centrifuge the cells at 220 x g for 5 minutes at room temperature to pellet the cells.
10. Aspirate the supernatant from the tube without disturbing the cell pellet.
11. Resuspend the cells in 10 ml of Rat Hepatocyte Maintenance Medium (prewarmed and equilibrated in Section III Step A2) by gently pipetting the cells 5 times with a 5 ml pipette.
12. Transfer 10 ml of RH suspension into the T-75 flask that contains 5 ml of Rat Hepatocyte Maintenance Medium. Place the flask in a 37°C, 5% CO_2 humidified incubator. Loosen the cap to allow gas exchange.
    For seeding 96 well plate, pipette 75 µl of RH suspension to each well and place the plate in a 37°C, 5% CO_2 humidified incubator.

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