CELL
APPLICATIONS, INC.

General Instructions for Culturing

Rat Hepatocytes (RH)
Adherent in EHS Matrix Extract

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. EHS MATRIX EXTRACT (126-2.75)

Store the EHS Matrix Extract at -20°C immediately upon arrival.

C. HEPATOCYTE ATTACHMENT MEDIUM (R711-100)

Store the Hepatocyte Attachment Medium at 4°C immediately upon arrival.

D. MAINTENANCE MEDIUM (R713-250)

Store the Maintenance Medium at 4°C 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. EHS Matrix Extract Preparation:
   Take the Rat Hepatocyte Maintenance Medium from the refrigerator. Decontaminate the bottle with 70% alcohol in a sterile hood.
   *Keep EHS Matrix Extract on ice throughout the preparation. Use chilled pipette tips, pipettes, plates and tubes for preparing EHS Matrix Extract.
   a. Thaw EHS Matrix Extract at 4°C overnight on ice.
   b. Use cooled pipette tips, mix the EHS Matrix Extract on ice to homogeneity.
   c. Prepare EHS Matrix Extract matrix by adding chilled Hepatocyte Maintenance Medium to EHS Matrix Extract on ice in a 1:1 ratio.
   d. Coat tissue culture surface evenly, which was kept on ice.
      Keep the EHS Matrix Extract to surface ratio at 1ml/10cm^2
      For example,
      1.25ml for each T-12.5 flask, (625 µL of Hepatocyte Maintenance Medium to 625 µL of EHS Matrix Extract.)
      210µl for each 24-well plate well, (105 µL of Hepatocyte Maintenance Medium to 105 µL of EHS Matrix Extract)
   e. Incubate at 37°C for one hour.
   f. Rinse EHS Matrix Extract with attachment medium.
   g. EHS Matrix Extract coated tissue culture surface is now ready for use.

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 Attachment Medium.
9. Centrifuge the cells at 220 x g for 5 minutes at 4°C to pellet the cells.
10. Aspirate the supernatant from the tube without disturbing the cell pellet.
11. Resuspend the cells in 3 ml of warm Rat Hepatocyte Attachment Medium by gently pipetting the cells 5 times with a 5 ml pipette.
12. Transfer 3 ml of RH suspension into the T-12.5 flask or seed 0.5 ml/well for each 24-well plate and place the T-12.5 flask or the 24-well plate in a 37°C, 5% CO_2 humidified incubator. Loosen the cap to allow gas exchange.
13. After 2-3 hours, aspirate off Hepatocyte Attachment Medium and add Hepatocyte Maintenance Medium.

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