

General Instructions for Culturing

Rat Macrophages (RMcp)

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 (R8818-10a)

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

B. PRE-PLATED CELLS (R8819-)

1. Examine under a microscope to check if all the cells are attached to the bottom of the culture vessel. If not, notify CAI or your distributor immediately.
2. Decontaminate the exterior of the culture vessel with 70% alcohol.
3. Place the sealed culture vessel in a 37°C, 5% CO₂ humidified incubator for 2 hours as shipped.
4. In a sterile Biological Safety Cabinet, remove the seal of the culture vessel very slowly and carefully.
5. Remove the Transport Medium by aspiration. Add fresh Growth Medium: 5 ml for a T-25 flask, 15 ml for a T-75 flask, 1 ml for each well of a 24-well plate, and 100 µl for each well of a 96-well plate.
6. Place the culture vessel in a 37°C, 5% CO₂ humidified incubator with loosened cap (of a flask) to allow gas exchange.
7. Change medium every other day.

C. CULTURE MEDIUM (R620-100)

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 protective lab gear (lab coat, gloves, safety glasses, etc.) when working with cell cultures.
 - c. Handle all cell culture work in a sterile hood.

III. CULTURING RMcp

A. PREPARING FOR SEEDING RMcp

1. Take the Macrophage Culture Medium from the refrigerator. Decontaminate the bottle with 70% alcohol in a sterile hood.
2. Transfer 12 ml of Macrophage Culture Medium to a 50ml tube and equilibrate the medium in a 37°C, 5% CO₂ humidified incubator for 1 hour.

B. THAWING AND PLATING RMcp

1. Remove the cryopreserved vial of RMcp 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 for 1 minute.
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 2 times with a 2 ml pipette. Be careful not to pipette too vigorously as to cause foaming.

8. Transfer the cell suspension from the vial into a 50 ml tube. Dropwise add 12 ml of equilibrated Macrophage Culture Medium to the cells while swirling the tube to mix. Rinse the cryovial to recover all of the content. Collect the medium to the tube.
9. Gently mix the cell suspension in the 50 ml tube by pipetting and aliquot 1 ml into each well of a 24-well plate. A seeding density of 40,000 cells per cm² or above is recommended.
10. Put the lid back to the 24-well plate and rock gently to evenly distribute the cells.
11. Place the 24-well plate in a 37°C, 5% CO₂ humidified incubator. For best results, do not disturb the culture for 24 hours after inoculation.
12. Change to fresh Macrophage Culture Medium after 24 hours or overnight to remove all traces of DMSO.
13. Change Macrophage Culture Medium every other day.
14. Subculturing is not recommended for macrophage cultures due to their limited proliferation capacity *in vitro*.