

## General Instructions for Culturing

### Rat Microglia (RMcg)

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 (R8816-10n)

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

##### B. PRE-PLATED CELLS (R8817-)

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% CO<sub>2</sub> 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% CO<sub>2</sub> humidified incubator with loosened cap (of a flask) to allow gas exchange.
7. Change medium every other day.

##### C. CULTURE MEDIUM (R619-100)

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 biological safety cabinet.

#### III. CULTURING RMcg

##### A. PREPARING FOR SEEDING RMcg

1. Take the Rat Macrophage Culture Medium from the refrigerator. Decontaminate the bottle with 70% alcohol in a sterile biological safety cabinet.
2. Transfer 12 ml of Rat Macrophage Culture Medium to a 50ml tube and equilibrate the medium in a 37°C, 5% CO<sub>2</sub> humidified incubator for 1 hour. Refer to Table 1 for recommended volume.

##### B. THAWING AND PLATING RMcg

1. Remove the cryopreserved vial of RMcg 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 pre-wetted, 1 ml aerosol pipette tip set at 950 ul. 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 Rat 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 appropriate volume into each well of the desired culture vessel. A seeding density of 40,000 cells per cm<sup>2</sup> or above is recommended.

Culture vessel	Surface area	Plating medium	Cell number	Culture medium
6-well	10 cm <sup>2</sup>	5 ml	400,000	5 ml
12-well	4 cm <sup>2</sup>	2 ml	160,000	2 ml
24-well	2 cm <sup>2</sup>	1 ml	80,000	1 ml
48-well	1 cm <sup>2</sup>	0.5 ml	40,000	0.5 ml
96-well	0.3 cm <sup>2</sup>	0.15 ml	12,000	0.15 ml

Table 1 Recommended coating and seeding conditions.

10. Put the lid back to the culture vessel and rock gently to evenly distribute the cells.
11. Place the culture vessel in a 37°C, 5% CO<sub>2</sub> 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. Refer to Table 1 for recommended volume.
13. Change Macrophage Culture Medium every other day.
14. Microglia cultures are ready for experiment after cells attach to the culture vessel. Subculturing is not recommended for microglia cultures due to their limited proliferation capacity *in vitro*. Microglia generally can be kept in culture for about one week.