



Rat Meningeal Cells (RMC) Catalog #R1400

Cell Specification

Meningeal cells surrounding the brain participate actively in the normal development of the central nervous system. They play important roles in stabilizing the extracellular matrix of the pial surface, organizing the radial glial scaffold, laminating the cerebellar cortex. Selective pharmacological destruction of the meningeal cells during a critical ontogenetic period leads to specific malformation of both the cerebellar cortex and dentate gyrus [1]. Grafts of meningeal cells, which are derived from meninges overlying the cerebral cortex in adult rat spinal cord lesion, promote axonal regrowth [2]. Additionally, *in vitro* studies showed that meningeal cells chemotactically orient the migration of immature neurons but not glial cells [3].

RMC from ScienCell Research Laboratories are isolated from rat leptomeningi. RMC are cryopreserved at passage one culture and delivered frozen. Each vial contains $>5 \times 10^5$ cells in 1 ml volume. RMC are characterized by immunofluorescence; they are positive for fibronectin and negative for GFAP, α -smooth muscle actin and Thy 1.1. RMC are negative for mycoplasma, bacteria, yeast and fungi. RMC are guaranteed to further expand for 5 population doublings under the conditions specified by ScienCell Research Laboratories.

Recommended Medium

It is recommended to use Fibroblast Medium (FM, Cat. #2301) for the culturing of RMC *in vitro*.

Product Use

RMC are for research use only. They are not approved for human or animal use, or for application in *in vitro* diagnostic procedures.

Storage

Upon receiving, directly and immediately transfer the cells from dry ice to liquid nitrogen and keep the cells in liquid nitrogen until they are needed for experiments.

Shipping

Dry ice.

Reference

- [1] Hartmann, D., Sievers, J. Pehlemann, F. W. and Berry, M. (1992) Destruction of meningeal cells over the medial cerebral hemisphere of newborn hamster prevents the formation of the infrapyramidal blade of the dentate gyrus. *J. Comparative Neurol.* 320:33-61.
- [2] Franzen, R., Martin, D., Daloze, A., Moonen, G. and Schoenen, J. (1999) Grafts of meningeal fibroblasts in adult rat spinal cord lesion promote axonal regrowth. *Neuroreport* 10:1551-1556.
- [3] Hartmann, D., Schulze, M. and Sievers, J. (1998) Meningeal cells stimulate and direct the migration of cerebellar external granule cells *in vitro*. *J. Neurocytol.* 27:395-409.

Instruction for culturing cells

Caution: Cryopreserved cells are very delicate. Thaw the vial in a 37°C water bath and return them to culture as quickly as possible with minimal handling!

Initiating the culture:

1. Prepare a poly-L-lysine coated flask (2 $\mu\text{g}/\text{cm}^2$, T-75 flask is recommended). Add 10 ml of sterile water to a T-75 flask and then add 15 μl of poly-L-lysine stock solution (10 mg/ml, Cat. #0413). Leave the vessel in a 37°C incubator overnight (or for a minimum of one hour).
2. Prepare complete medium. Decontaminate the external surfaces of medium bottle and medium supplement tubes with 70% ethanol and transfer them to a sterile field. Aseptically transfer supplement to the basal medium with a pipette. Rinse the supplement tube with medium to recover the entire volume.
3. Rinse the poly-L-lysine-coated vessel twice with sterile water and then add 15 ml of complete medium. Leave the vessel in the sterile field and proceed to thaw the cryopreserved cells.
4. Place the frozen vial in a 37°C water bath. Hold and rotate the vial gently until the contents completely thaw. Promptly remove the vial from the water bath, wipe it down with 70% ethanol, and transfer it to the sterile field.
5. Carefully remove the cap without touching the interior threads. Gently resuspend and dispense the contents of the vial into the equilibrated, poly-L-lysine-coated culture vessel. A seeding density of 5,000 cells/ cm^2 is recommended.

Note: Dilution and centrifugation of cells after thawing are not recommended since these actions are more harmful to the cells than the effect of residual DMSO in the culture. It is also important that cells are plated in poly-L-lysine-coated culture vessels to promote cell attachment.

6. Replace the cap or lid of the culture vessel and gently rock the vessel to distribute the cells evenly. Loosen cap, if necessary, to allow gas exchange.
7. Return the culture vessel to the incubator.
8. For best results, do not disturb the culture for at least 16 hours after the culture has been initiated. Refresh culture medium the next day to remove residual DMSO and unattached cells, then every other day thereafter.

Maintaining the culture:

1. Refresh supplemented culture medium the next morning after establishing a culture from cryopreserved cells.
2. Change the medium every three days thereafter, until the culture is approximately 70% confluent.
3. Once the culture reaches 70% confluency, change medium every other day until the culture is approximately 90% confluent.

Subculture:

1. Subculture when the culture reaches 90% confluency.
 2. Prepare poly-L-lysine-coated culture vessels ($2 \mu\text{g}/\text{cm}^2$) one day before subculture.
 3. Warm complete medium, trypsin/EDTA solution (T/E, Cat. #0103), T/E neutralization solution (TNS, Cat. #0113), and DPBS (Ca^{++} - and Mg^{++} -free, Cat. #0303) to **room temperature**. We do not recommend warming reagents and medium in a 37°C water bath prior to use.
 4. Rinse the cells with DPBS.
 5. Add 8 ml of DPBS and then 2 ml of T/E solution into flask (in the case of a T-75 flask). Gently rock the flask to ensure complete coverage of cells by T/E solution. Incubate the flask in a 37°C incubator for 1 to 2 minutes or until cells completely round up. Use a microscope to monitor the change in cell morphology.
 6. During incubation, prepare a 50 ml conical centrifuge tube with 5 ml of fetal bovine serum (FBS, Cat. #0500).
 7. Transfer T/E solution from the flask to the 50 ml centrifuge tube (a small percent of cells may detach) and continue to incubate the flask at 37°C for another 1 to 2 minutes (no solution in the flask at this moment).
 8. At the end of incubation, gently tap the side of the flask to dislodge cells from the surface. Check under a microscope to make sure that all cells detach.
 9. Add 5 ml of TNS solution to the flask and transfer detached cells to the 50 ml centrifuge tube. Rinse the flask with another 5 ml of TNS to collect the residual cells.
 10. Examine the flask under a microscope for a successful cell harvest by looking at the number of cells being left behind; there should be less than 5%.
- Note: Use ScienCell T/E solution that is optimized to minimize cell damages due to over trypsinization.*
11. Centrifuge the 50 ml centrifuge tube at 1000 rpm for 5 minutes. Resuspend cells in culture medium.
 12. Count and plate cells in a new poly-L-lysine-coated culture vessel with the recommended cell density.

Caution: Handling animal derived products is potentially biohazardous. Always wear gloves and safety glasses when working with these materials. Never mouth pipette. We recommend following the universal procedures for handling products of human origin as the minimum precaution against contamination [1].

[1] Grizzle WE, Polt SS. (1988) "Guidelines to avoid personal contamination by infective agents in research laboratories that use human tissues." *J Tissue Cult Methods*. 11: 191-9.