Rat Pulmonary Alveolar Macrophages (RPAMa)

Catalog #R3040

Cell Specification

Pulmonary alveolar macrophages (PAMa) are located in the alveoli of the lungs and protect the respiratory tract against respiratory pathogens. PAMa are the main phagocytes of the innate immune system and they originate from the yolk sac. Functionally, PAMa are tasked with maintaining pulmonary homeostasis and protecting the body from invading pathogens [1]. Importantly, they also control the response to pathogens and epithelial damage, use phagocytosis to remove apoptotic cells, and remove waste material from the epithelium [1]. PAMa have two functional states known as the M1 phenotype and the M2 phenotype. The function of M1 macrophages is to recruit immune cells into the lung. Conversely, M2 macrophages secrete factors which reduce inflammation and stimulate repair in the epithelium. In order to identify pathogens and injury sites, PAMa have pattern recognition receptors, such as toll-like recepetors [1]. Additionally, PAMa are known to express angiotensin-converting enzyme 2 (ACE2), making them a potential alternative route for infection from pathogens such as SARS-CoV-2 [2]. The infection of alveolar macrophages by SARS-CoV-2 may also promote the cytokine storm which occurs in severe COVID-19 patients [3]. Rat PAMa (RPAMa) can be used to study pulmonary disease and better understand the role these cells play in lung disease tolerance.

RPAMa from ScienCell Research Laboratories are isolated from rat lung. RPAMa are cryopreserved at P0 and delivered frozen. Each vial contains >1 x 10^6 cells in 1 ml volume. RPAMa are characterized by morphology and immunofluorescence with antibodies specific to F4/80. RPAMa are negative for mycoplasma, bacteria, yeast, and fungi. RPAMa are guaranteed to further culture under the conditions provided by ScienCell Research Laboratories, *however, RPAMa are not recommended for expanding or long-term cultures since the cells do not proliferate in regular culture*.

Recommended Medium

It is recommended to use Macrophage Medium (MaM, Cat. #1921) for culturing RPAMa *in vitro.*

Product Use

RPAMa 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.

References

[1] Allard B, Panariti A, Martin J. (2018) “Alveolar macrophages in the resolution of inflammation, tissue repair, and tolerance to infection.” Front Immunol. 9: 1777.

[2] Abassi Z, Knaney Y, Karram T, Heyman S. (2020) "The lung macrophage in SARS-CoV-2 infection: A friend or a foe? Front Immunol 11: 1312.
[3] Wang C, Xie J, Zhao L, Fei X, Zhang H, Tan Y, Nie X, Zhou L, Liu Z, Ren Y, Yuan L, Zhang Y, Zhang J, Liang L, Chen X, Liu X, Wang P, Han X, Weng X, Chen Y, Yu T, Zhang X, Cai J, Chen R, Shi Z, Bian X. (2020) "Alveolar macrophage dysfunction and cytokine storm in the pathogenesis of two severe COVID-19." EBioMedicine 57: 102833.

Instructions for culturing cells

Caution: Cryopreserved primary cells are very delicate. Thaw the vial in a 37ºC water bath and return the cells to culture as quickly as possible with minimal handling! Do not centrifuge the cells after thawing as this can damage the cells.

Note: ScienCell primary cells must be cultured in a 37ºC, 5% CO_2 incubator. Cells are only warranted if ScienCell media and reagents are used and the recommended protocols are followed. *Experiments should be well organized before thawing RPAMa. It is recommended that RPAMa are used for experiments as quickly as possible after thawing the cells.* ***RPAMa cannot be subcultured or passaged, as the cells do not proliferate.***

Initiating the culture:

1. Prepare a poly-L-lysine-coated culture plate (2 μg/cm^2 is recommended). For example, add 2 ml of sterile water to one well of a 6-well plate and then add 20μl of poly-L-lysine stock solution (1 mg/ml, Cat. #0403). Leave the plate in a 37ºC 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 tubes with medium to recover the entire volume.
3. Rinse the poly-L-lysine-coated culture vessel twice with sterile water and then add the volume of complete medium recommended in Table 1 or Table 2. Leave the vessel in the sterile field and proceed to thaw the cryopreserved cells.
4. Place the frozen vial in a 37ºC 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.

   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. Carefully remove the cap without touching the interior threads and gently resuspend the cell suspension. A seeding density of 10,000-15,000 cells/cm^2 is recommended depending on your experiments. We recommend following Table 1 for seeding RPAMa onto 6-well, 12-well, or 24-well plates.

Table 1

Recommended cell suspension volume per vial using a 6-well, 12-well, or 24 well format

Well format	Surface area/well (approx. values)	Volume of media/well	Volume of cell suspension from vial/well	# of wells/vial
6-well	9.6 cm^2	3.0 ml	150 µl	6 wells
12-well	3.9 cm^2	2.0 ml	60 µl	15 wells
24-well	1.9 cm^2	1.0 ml	30 µl	30 wells

7. 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.
8. Return the culture vessel to the incubator.
9. 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. Once the macrophages have attached and spread, the cells can be used for experiments.

   ***Note**: Cells may take a few days to spread.*
10. Use cells promptly for experiments.

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 S. (1988) "Guidelines to avoid personal contamination by infective agents in research laboratories that use human tissues." *J Tissue Cult Methods*. 11: 191-9.