Rev. 1

Mouse Hepatic Macrophages (MHMa)

Catalog #M5340-57

Cell Specification

Macrophages are cells differentiated from circulating bone marrow-derived monocytes. The main function of macrophages is to remove cellular debris and destroy invading pathogens. Mouse Hepatic Macrophages (MHMa), which are also known as Kupffer cells, reside within the lumen of liver sinusoids. MHMa protect the liver by responding to pathogens and metastatic cells, while tolerating harmless self and foreign antigens, which enter via blood flow through the portal vein and hepatic artery [1]. Recent studies have shown that hepatic macrophages play an important role in fibrosis, liver inflammation, fatty liver disease, and liver transplantation [2-4]. MHMa are an excellent model for studying macrophage functions under normal physiological and pathological conditions.

MHMa from ScienCell Research Laboratories are isolated from postnatal day 2 or postnatal day 8 C57BL/6 mouse liver. MHMa are cryopreserved at P0 and delivered frozen. Each vial contains > 1 x 10^6 cells in 1 ml volume. MHMa are characterized by immunofluorescence with antibody to F4/80. MHMa are negative for mycoplasma, bacteria, yeast, and fungi. MHMa are guaranteed to further culture in the conditions provided by ScienCell Research Laboratories; however, *MHMa 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 MHMa *in vitro*.

Product Use

MHMa 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] Liaskou E, Wilson DV, Oo YH. (2012) "Innate immune cells in liver inflammation." *Mediators Inflamm*. 2012: 949157.

[2] Bieghs V, Verheyen F, van Gorp PJ, Hendrikx T, Wouters K, Lütjohann D, Gijbels MJ, Febbraio M, Binder CJ, Hofker MH, Shiri-Sverdlov R. (2012) "Internalization of modified lipids by CD36 and SR-A leads to hepatic inflammation and lysosomal cholesterol storage in Kupffer cells." *PLoS One*. 7: e34378.

[3] Tian Y, Jochum W, Georgiev P, Moritz W, Graf R, Clavien PA. (2006) "Kupffer cell-dependent TNF-alpha signaling mediates injury in the arterialized small-for-size liver transplantation in the mouse." *Proc Natl Acad Sci U S A*. 103: 4598-603.

[4] Seki E, de Minicis S, Inokuchi S, Taura K, Miyai K, van Rooijen N, Schwabe RF, Brenner DA. (2009) "CCR2 promotes hepatic fibrosis in mice." *Hepatology*. 50: 185-97.

Instructions for culturing primary 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: *Experiments should be well organized before thawing MHMa. It is recommended that MHMa are used for experiments as quickly as possible after thawing the cells.* ***MHMa cannot be subcultured or passaged, as the cells do not proliferate.***

Initiating the culture:

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.

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 (MaM, Cat. #1921). Thaw MaGS (Cat. #1972), FBS (Cat. #0025) and P/S solution (Cat. #0503) at 37ºC. Gently tilt the MaGS tube several times to ensure the contents are completely dissolved before adding to the medium. Decontaminate the external surfaces of medium bottle and medium supplement tubes with 70% ethanol and transfer them to a sterile field. In a sterile field, remove the cap, being careful not to touch the interior threads with fingers. Add MaGS, FBS and P/S solution to the medium and mix well.
3. Rinse the poly-$_L$-lysine-coated vessel twice with sterile water and then add the volume of complete medium recommended in Table 1 or Table 2. Leave the plate(s) 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.

   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.*
5. Carefully remove the cap without touching the interior threads and gently resuspend the cell suspension. A seeding density of 10,000-20,000 cells/cm^2 is recommended depending on your experiments. We recommend following Table 1 for seeding MHMa onto 6-well, 12-well, or 24-well plates. For seeding MHMa on 60 mm plates, use Table 2.

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

Table 2
Recommended cell suspension volume per vial using 60 mm plates

Plate Format	Surface area/plate (approx. values)	Volume of cell suspension from vial/plate	# of plates/vial	Volume of media (ml)/plate
60 mm	21 cm^2	300 µl	3	3.0 ml

6. Pipet the correct volume of cell suspension into each well of an equilibrated, poly-L-lysine-coated culture plate containing complete medium. Replace the lid of the culture plate and gently rock the plate to distribute the cells evenly.
7. Return the culture plate to the incubator.
8. For best results, do not disturb the culture for at least 16 hours after the culture has been initiated. Change the culture medium the next morning after establishing a culture from cryopreserved cells to remove residual DMSO and unattached cells. Once macrophages attach, the culture is ready for experiments.
9. Use cells promptly for experiments.

Note: We do not recommend cryopreservation of primary cells by the end user. Refreezing cells may damage them and affect cell performance. ScienCell does not guarantee primary cells cryopreserved by the end user.

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.