



Mouse Hepatic Macrophages (MHMa)

Catalog #M5340

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 mouse liver. MHMa are cryopreserved after purification and delivered frozen. Each vial contains $> 1 \times 10^6$ 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, *RHMa 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, Hendriks 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 cells

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

Note: It is not recommended that macrophages be subcultured beyond their initial plating. Experiments should be well organized before thawing the cells.

Initiating the culture:

1. Macrophages are not expected to further expand in culture. It is recommended to use either cell culture-grade or bacterial-grade plastics for the culturing of macrophages since they easily attach to culture plastics.
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. Add complete medium to the culture vessel. 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. Carefully remove the cap without touching the interior threads.
5. Gently resuspend and dispense the contents of the vial into the equilibrated culture vessel. A seeding density of $\geq 10,000$ cells/cm² 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.

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. Change the growth medium the next day to remove residual DMSO and unattached cells. Once macrophages attach, the culture is ready for experiment.
9. If necessary, refresh media every other day thereafter; however, we do not recommend culturing macrophages for an extended period.

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.