



## Rat Hepatic Macrophages (RHMa)

Catalog #R5340

### Cell Specification

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

RHMa from ScienCell Research Laboratories are isolated from postnatal day 2 or postnatal day 8 rat liver. RHMa are cryopreserved at P0 and delivered frozen. Each vial contains  $> 1 \times 10^6$  cells in 1 ml volume. RHMa are characterized by immunofluorescence with antibody to F4/80. RHMa are negative for mycoplasma, bacteria, yeast, and fungi. RHMa is guaranteed to further culture in the condition 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 RHMa *in vitro*.

### Product Use

RHMa 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] Ajakaiye MA, Jacob A, Wu R, Zhou M, Ji Y, Dong W, Wang Z, Qiang X, Chaung WW, Nicastro J, Coppa GF, Wang P. (2011) "Upregulation of Kupffer cell  $\alpha$ 2A-Adrenoceptors and downregulation of MKP-1 mediate hepatic injury in chronic alcohol exposure." *Biochem Biophys Res Commun.* 409: 406-11.
- [3] 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.
- [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**

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**Caution:** Cryopreserved primary 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! Do not centrifuge the cells after thawing as this can damage the cells.

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

### **Initiating the culture:**

**Note:** ScienCell primary cells must be cultured in a 37°C, 5% CO<sub>2</sub> 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<sup>2</sup> 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 37°C 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 37°C. 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 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.

*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<sup>2</sup> is recommended depending on your experiments. We recommend following Table 1 for seeding RHMa onto 6-well, 12-well, or 24-well plates. For seeding RHMa 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 <sup>2</sup>	3.0 ml	150 µl	6 wells
12-well	3.9 cm <sup>2</sup>	2.0 ml	60 µl	15 wells
24-well	1.9 cm <sup>2</sup>	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 <sup>2</sup>	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.