



## **Rat Hepatic Macrophage (RHMa)**

Catalog Number: R5340

### **Cell Specification**

Macrophages are cells differentiated from circulating bone marrow-derived monocytes. Their main function is the removal of cellular debris and invading pathogens [1]. 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 [2]. 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 [3-6]. Macrophages isolated from fresh rat liver tissue serve as an excellent model for studying their functions under normal physiological and pathological conditions. Macrophages can be identified by specific expression of a number of proteins including F4/80 (mice)/EMR1 (human), CD14, CD11b, MAC-1/MAC-3 and CD68 by flow cytometry or immunocytochemical staining [7].

RHMa from ScienCell Research Laboratories are isolated from neonatal day 2 rat liver tissue. Cells are cryopreserved after purification 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.

### **Recommended Medium**

It is recommended to use Macrophage Medium (MaM, Cat. No. 1921) for the culturing of 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**

Transfer cells directly and immediately from dry ice to liquid nitrogen upon receiving and keep the cells in liquid nitrogen until cell culture is needed for experiments.

### **Shipping**

Dry ice.

## Reference

- [1]. Wynn TA and Barron L. (2010). *Semin Liver Dis.* 30:245-57.
- [2]. Liaskou E, Wilson DV and Oo YH. (2012). *Mediators Inflamm.* 2012: 949157.
- [3]. Ajakaiye M, Jacob A, Wu R, Zhou M, et al. (2011). *Biochem Biophys Res Commun.* 409: 406–411.
- [4]. Bieghs V, Verheyen F, van Gorp PJ, Hendrikx T, et al. (2012) *PLoS One.* 7: e34378.
- [5]. Tian Y, Jochum W, Georgiev P, Moritz W, et al. (2006). *Proc Natl Acad Sci U S A.* 103: 4598–4603.
- [6]. Seki E, De Minicis S, Inokuchi S, Taura K, et al. (2009). *Hepatology.* 50: 185–197.
- [7]. Gordon S and Taylor P. (2005). *Nature Reviews Immunology.* 5:953-964.

## Instruction for culturing cells

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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!

### Set up culture after receiving the cryopreserved vial:

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 tube with medium to recover the entire volume.
3. Place the vial in a 37°C water bath, hold and rotate the vial gently until the contents are completely thawed. Remove the vial from the water bath promptly, wipe it down with 70% ethanol and transfer it to the sterile field. Remove the cap carefully without touching the interior threads with fingers. Gently resuspend the contents of the vial using 1 ml eppendorf pipette.
4. Transfer the contents of the vial to a 15 ml centrifuge tube which contains 10 ml of macrophage medium. Centrifuge the tube at 1000 rpm for 5 minutes.
5. Discard the supernatant. Resuspend the cell pellet in macrophage medium and plate cells in flask or plate.
6. Return the culture vessel to the 37°C incubator.
7. For best result, 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 the residual DMSO and unattached cells, then every other day thereafter. A health culture will display polygonal shaped, sheets of contiguous cells and the cell number will be double after two to three days in culture.

**Maintenance of Culture:**

1. Refresh supplemented culture medium the next morning after establishing a culture from cryopreserved cells.
2. Change the medium every two to three days thereafter.

***It is not recommended that macrophages be subcultured beyond their initial plating.***

*Caution: Handling animal derived products is potentially biohazardous. Always wear gloves and safety glasses when working 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 and Polt S. (1988). J Tissue Culture Methods. 11:191-9.