Mouse Splenic Macrophages (MSMa)

Catalog #M5550-57

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 Splenic Macrophages (MSMa) reside in the spleen and play an critical role in maintaining blood homeostasis [1]. There are three distinct populations of splenic macrophages including: red pulp macrophages, marginal zone macrophages, and marginal zone metallophillic macrophages [1, 2]. MSMa perform iron processing functions and aid in the capture of microbes and viruses in the circulatory system [1, 2]. Recent studies have shown that splenic macrophages contribute to stroke pathology and autoimmune diseases [3, 4]. MSMa are an excellent model for studying macrophage functions under normal physiological and pathological conditions.

MSMa from ScienCell Research Laboratories are isolated from adult C57BL/6 mouse spleen. MSMa are cryopreserved after purification and delivered frozen. Each vial contains > 1 x 10^6 cells in 1 ml volume. MSMa are characterized by immunofluorescence with antibody to F4/80. MSMa are negative for mycoplasma, bacteria, yeast, and fungi. MSMa are guaranteed to further culture in the conditions provided by ScienCell Research Laboratories; however, *MSMa 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 MSMa *in vitro*.

Product Use

MSMa 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] Borges da Silva H, Fonseca R, Pereira R, Cassado Ados A, Alvarez J, D'Imperio, Lima M. (2015) "Splenic macrophage subsets and their function during blood-borne infections." *Front Immunol.* 6:480.

[2] Gammella E, Buratti P, Cairo G, Recalcati S. (2014) "Macrophages: central regulators of iron balance." *Metallomics*. 6(8): 1336-1345.

[3] Liu Z, Chen C Li F, Shen J, Yang Y, Leak R, Ji X, Du H, Hu X. (2015) " Splenic responses in ischemic stroke: new insights into stroke pathology. *CNS Neurosci Ther*. 21(4): 320-326.

[4] McGaba T, Karlsson M. (2016) "Apoptotic cell responses in the splenic marginal zone: a paradigm for immunologic reactions to apoptotic antigens with implications for autoimmunity. *Immunol Rev.* 269(1): 26-43.

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: *Experiments should be well organized before thawing MSMa. It is recommended that MSMa are used for experiments as quickly as possible after thawing the cells. MSMa 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 37°C incubator overnight (or for a minimum of one hour).

2. Prepare complete medium (MaM, Cat. #1921). Thaw MaGS, FBS and P/S solution at 37°C. Gently tilt the tubes several times to ensure the contents are completely mixed before adding to the medium. Spray the medium bottle and tubes with 70% ethanol, and wipe to remove excess liquid. In a sterile field, remove the caps without touching 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^2 is recommended depending on your experiments. We recommend following Table 1 for seeding MSMa onto 6-well, 12-well, or 24-well plates. For seeding MSMa 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. Refresh the culture medium the next morning after establishing a culture from cryopreserved cells to remove residual DMSO and unattached cells.
9. 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 Culture Methods*. 11: 191-9.