Rev. 0

Mouse Hepatic Mesothelial Cells (MHMeC)

Catalog #M5350

Cell Specification

Mesothelial cells line the surfaces of internal organs and cavities and form an epithelial monolayer [1, 2]. Mesothelial cells have characteristics of both epithelial cells and mesenchymal cells and can undergo mesothelial-mesenchymal transition to become myofibroblasts upon injury [1,2]. Due to their intermediate phenotype, mesothelial cells express markers of both epithelial and mesenchymal cells, but also express the mesothelial cell marker mesothelin. In the liver, hepatic mesothelial cells (HMeC) can transition to become hepatic stellate cells and myofibroblasts suggesting that mesothelial cells may contribute to the development of liver fibrosis. [3, 4]. Signaling via TGFβ1 has been shown to induce the transition from mesothelial cell to mesenchymal cell types [3]. Mouse HMeC (MHMeC) can be used to better understand the molecular mechanisms of mesothelial-mesenchymal transition, which may aid in the development of treatments for liver disease.

MHMeC from ScienCell Research Laboratories are isolated from postnatal day 2 or postnatal day 8 CD-1 mouse liver. MHMeC are cryopreserved at passage one and delivered frozen. Each vial contains >5 x 10^5 cells in 1 ml volume. MHMeC are characterized by immunofluorescence with antibodies specific to mesothelin. MHMeC are negative for mycoplasma, bacteria, yeast, and fungi. MHMeC are guaranteed to further culture under the conditions provided by ScienCell Research Laboratories*; however, MHMeC are not recommended for long-term cultures due to limited expansion capacity and senescence after subculturing*.

Recommended Medium

It is recommended to use Mesothelial Cell Medium (MesCM, Cat. #5351) for culturing MHMeC *in vitro*.

Product Use

MHMeC 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] Koopmans T, Rinkevich Y. (2018) "Mesothelial to mesenchyme transition as a major developmental and pathological player in trunk organs and their cavities." Commun Biol 170:1-14.

[2] Lua H, Asahina K. (2016) "The role of mesothelial cells in liver development, injury, and regeneration." *Gut Liver* 10(2): 166-176.

[3] Li Y, Wang J, Asahina K. (2013) "Mesothelial cells give rise to hepatic stellate cells and myofibroblasts via mesothelial-mesenchymal transition in liver injury." *Proc Natl Acad Sci* 110:2324-2329.

[4] Lua I, Li Y, Pappoe L, Asahina K. (2015) "Myofibroblastic conversion and regeneration of mesothelial cells in peritoneal and liver fibrosis." *Am J Pathol* 185:3258-3273.

Rev. 0

Instructions for culturing cells

Caution: Cryopreserved 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!

Note: MHMeC are very sensitive cells and they are not expected to proliferate many times in culture. Experiments should be well organized before thawing the cells. It is recommended that MHMeC are used for experiments at earliest passage after initial plating with minimal expansion.

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 vessel (2 µg/cm^2, T-75 flask is recommended). To obtain a 2 µg/cm^2 poly-$_L$-lysine-coated culture vessel, add 10 ml of sterile water to a T-75 flask and then add 15 µl of poly-$_L$-lysine stock solution (10 mg/ml, Cat. #0413). Leave the vessel in a 37ºC incubator overnight (or for a minimum of one hour).
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. Rinse the poly-$_L$-lysine-coated vessel twice with sterile water and then add 20 ml of complete medium. Leave the vessel 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.
5. Carefully remove the cap without touching the interior threads. Gently resuspend and dispense the contents of the vial into the equilibrated, poly-$_L$-lysine-coated culture vessel. A seeding density of 7,000-9,000 cells/cm^2 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. It is also important that cells are plated in poly-$_L$-lysine-coated culture vessels to promote cell attachment.
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. Do not disturb the culture for at least 16 hours after the culture has been initiated. Refresh culture medium the next day to remove residual DMSO and unattached cells.

Maintaining the culture:

1. Refresh supplemented culture medium the next morning after establishing a culture from cryopreserved cells.

2. Change the medium every three days thereafter, until the culture is approximately 70% confluent.
3. Once the culture reaches 70% confluency, change medium every two days until the culture is approximately 90% confluent.
4. Use directly 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.