Mouse Renal Mesangial Cells (MRMC)

Catalog Number: M4200

Cell Specification

Renal mesangial cells are perivascular cells located within the central portion of the glomerular tuft between capillary loops, constituting 30-40% of the total glomerular cell population [1]. Mesangial cells synthesize, assemble and control turnover of the mesangial matrix [2]. They also regulate the intraglomerular capillary flow and ultrafiltration surface via mesangial cell contraction and release of various growth factors and vasoactive agents [3, 4]. By pinocytosis and phagocytosis, mesangial cells remove local accumulation of macromolecules in the mesangial space [5, 6]. Dysregulation of such housekeeping functions results in release of proinflammatory mediators and leukocyte infiltration, which subsequently affects glomerular permselectivity to cause proteinuria. Furthermore, mesangial cell proliferation and matrix overproduction have been observed in a wide variety of glomerular diseases, such as IgA nephropathy, mesangioproliferative glomerulonephritis, lupus nephritis, glomerulosclerosis and diabetic nephropathy [7, 8]. Such critical involvements suggest renal mesangial cells as an ideal model for studying mesangial injury and glomerular functions under both physiological and pathophysiological conditions.

MRMC from ScienCell Research Laboratories are isolated from neonatal day 2 mouse renal tissue. MRMC are cryopreserved after purification and delivered frozen. Each vial contains >5 x 10^5 cells in 1 ml volume. MRMC are characterized by immunofluorescence with antibodies specific to fibronectin, Thy-1, and smooth muscle actin. MRMC are negative for mycoplasma, bacteria, yeast and fungi. MRMC are guaranteed to further expand for 5 population doublings in the condition provided by ScienCell Research Laboratories.

Recommended Medium

It is recommended to use Mesangial Cell Medium (MCM, Cat. No. 4201) for the culturing of MRMC *in vitro*.

Product Use

MRMC 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] Olivetti G, Anversa P, Rigamonti W, et al. (1977) J Cell Biol. 75(2 Pt 1):573-85.
[2] Couchman JR, Beavan LA and McCarthy KJ. (1994) Kidney Int. 45:328-35.
[3] Riser BL, Cortes P, Heilig C, et al. (1996) Am J Pathol. 148:1915-23.
[4] Gruden G, Thomas S, Burt D, et al. (1999) J Am Soc Nephrol 10:730-37.
[5] Wanner C, Greiber S, Kramer-Guth A, et al. (1997) Kidney Int Suppl 63:S102-6.
[6] Gomez-Guerrero C, Suzuki Y and Egido J. (2002) Kidney Int 62:715-7.
[7] Schlöndorff D and Banas B. (2009) J Am Soc Nephrol. 20:1179-87.
[8] Abboud HE. (2012) Exp Cell Res. 318:979-85.

Instruction for culturing cells

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

Set up culture after receiving the order:

1. Prepare a poly-$_L$-lysine coated flask (2 μg/cm^2, T-75 flask is recommended). Add 10 ml of sterile water to a T-75 flask and 15 μl of poly-$_L$-lysine stock solution (10 mg/ml, ScienCell cat. no. 0413). Leave the flask in incubator overnight (minimum one hour at 37°C incubator).
2. Prepare complete medium: sterilize the external surfaces of medium and medium supplements with 70% ethanol and transfer them to sterile field. Aseptically open each supplement tube and transfer the contents to the basal medium with a pipette. Rinse each tube with medium to recover the entire volume.
3. Rinse the poly-$_L$-lysine coated flask with sterile water twice and add 20 ml of complete medium to the flask. Leave the flask in the hood and proceed to thaw the cells.
4. 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 dry, sterilize it with 70% ethanol and transfer it to a sterile field. Remove the cap, being careful not to touch the interior threads with fingers. Using a 1 ml eppendorf pipette gently resuspend the contents of the vial.
5. Dispense the contents of the vial into the poly-$_L$-lysine coated culture vessels. A seeding density of 5,000 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 flask that promotes cell attachment and growth.

6. Replace the cap or lid, 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 result, do not disturb the culture for at least 16 hours after the culture has been initiated. Refresh culture medium the next day to remove the residual DMSO and unattached cells, then every other day thereafter.

Maintenance of Culture:

1. Refresh culture medium the next morning after establishing a culture from cryopreserved cells.
2. Change medium every three days thereafter, until the culture is approximately 70% confluent.
3. Once the culture reaches 70% confluence, change medium every other day until the culture is approximately 90% confluent.

Subculture:

1. Subculture the cells when they are over 90% confluent.
2. Prepare poly-$_L$-lysine coated cell culture flasks (2 μg/cm^2).
3. Warm medium, trypsin/EDTA solution (T/E, ScienCell Cat. No. 0103), trypsin neutralization solution (TNS, ScienCell Cat. No. 0113), and DPBS (Ca^{++} and Mg^{++} free, ScienCell cat. no. 0303) to **room temperature**. We do not recommend warming the reagents and medium in 37^{o}C water bath prior use.
4. Rinse the cells with DPBS.
5. Add 8 ml of DPBS first and then 2 ml of trypsin/EDTA solution into flask (in the case of T-75 flask). Gently rock the flask to make sure that all cells are covered by trypsin/EDTA solution. Incubate the flask at 37^{o}C incubator for 1 to 2 minutes or until cells completely round up (monitored with microscope).
6. During incubation, prepare a 50 ml conical centrifuge tube with 5 ml of fetal bovine serum (FBS, ScienCell Cat. No. 0500).
7. Transfer trypsin/EDTA solution from the flask to the 50 ml centrifuge tube (a few percent of cells may detached) and continue to incubate the flask at 37^{o}C for 1 or 2 minutes more (no solution in the flask at this moment).
8. At the end of incubation, gently tap the side of the flask to dislodge cells from the surface. Check under microscope to make sure that all cells detach.
9. Add 5 ml of trypsin neutralization solution to the flask and transfer detached cells to the 50 ml centrifuge tube. Rinse the flask with another 5 ml of trypsin neutralization solution to collect the residual cells.
10. Examine under microscope for a successful cell harvest by looking at the number of cells being left behind. There should be less than 5%.

Note: Use ScienCell Research Laboratories trypsin/EDTA solution that is optimized to minimize over trypsinization-induced cell damages.

11. Centrifuge the 50 ml centrifuge tube at 1000 rpm for 5 min. Resuspend cells in culture medium.
12. Count and plate cells in a new, poly-$_{L}$-lysine coated flask with cell density as recommended.

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.