# Mouse Renal Tubular Epithelial Cells (MRTEpiC)

Catalog Number: M4100-57

## Cell Specification

Renal tubular epithelial cells (RTEpiC) play a crucial role in renal function. They reabsorb nearly all of the glucose and amino acids in the glomerular filtrate, while allowing other substances of no nutritional value to be excreted in the urine. They are also a major site of injury in a variety of congenital, metabolic, and inflammatory diseases. RTEpiC can produce inflammatory mediators such as cytokines and chemokines and actively participate in acute inflammatory processes by affecting and directing leukocyte chemotaxis via the production of IL-8 [1, 2]. RTEpiC express IL-2R alpha and MHC class II antigens during inflammation after renal transplantation or in crescentic glomerulonephritis, indicating the capacity to participate in the pathogenesis of immune renal injury [3]. To be able to study the relationship between tubular cells and a variety of renal diseases, the MTEpiC culture provides a useful *in vitro* model.

MRTEpiC from ScienCell Research Laboratories are isolated from neonate day 2 C57BL/6 mouse kidneys. MRTEpiC are cryopreserved at passage one and delivered frozen. Each vial contains >5 x $10^5$ cells in 1 ml volume. MRTEpiC are characterized by immunofluorescent method with antibodies to cytokeratin-18, -19 and vimentin. MRTEpiC are negative for mycoplasma, bacteria, yeast and fungi. MRTEpiC are guaranteed to further expand for 5 population doublings in the condition provided by ScienCell Research Laboratories.

## Recommended Medium

It is recommended to use Epithelial Cell Medium-animal (EpiCM-a, Cat. No. 4131) for expanding MRTEpiC *in vitro*.

## Product Use

MRTEpiC are for research use only. It is not approved for human or animal use, or for application in *in vitro* diagnostic procedures.

## Storage

Directly and immediately transfer cells 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] van Kooten, C., van der Linde, X., Woltman, A. M., van Es, L. A. and Daha, M. R. (1999) Synergistic effect of interleukin-1 and CD40L on the activation of human renal tubular epithelial cells. *Kidney Int* 56(1):41-51.

[2]Schmouder, R. L., Strieter, R. M., Wiggins, R. C., Chensue, S. W. and Kunkel, S. L. (1992 ) In vitro and in vivo interleukin-8 production in human renal cortical epithelia. *Kidney Int* 41(1):191-8.

[3] Wuthrich, R. P., Glimcher, L. H., Yui, M. A., Jevnikar, A. M., Dumas, S. E. and Kelley, V. E. (1990) MHC class II, antigen presentation and tumor necrosis factor in renal tubular epithelial cells. *Kidney Int* 37(2):783-92.

# Instruction for culturing cells

Caution: Cryopreserved cells are very delicate. Thaw the vial in a 37$^o$C waterbath 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 then add 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$^o$C incubator).
2. Prepare complete medium: decontaminate the external surfaces of medium and medium supplements with 70% ethanol and transfer them to sterile field. Aseptically open each supplement tube and add them 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 go to thaw the cells.
4. Place the vial in a 37$^o$C waterbath, hold and rotate the vial gently until the contents are completely thawed. Remove the vial from the waterbath immediately, wipe it dry, rinse the vial with 70% ethanol and transfer it to a sterile field. Remove the cap, being careful not to touch the interior threads with fingers. Using 1 ml eppendorf pipette gently re-suspend the contents of the vial.
5. Dispense the contents of the vial into the equilibrated, poly-L-lysine coated flask. 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 DMSO residue in the culture. It is also important that cells are plated in poly-L-lysine coated culture vessels that promote cell attachment.*
6. Replace the cap or cover of flask, and gently rock the vessel to distribute the cells evenly. Loosen caps if necessary to permit gas exchange.
7. Return the culture vessels to the incubator.
8. 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 healthy culture will display cobblestone or spindle shaped morphology, nongranular cytoplasm and the cell number will be doubled after two to three days in culture.

**Maintenance of Culture:**

1. Change the medium to fresh supplemented medium the next morning after establishing a culture from cryopreserved cells. For subsequent subcultures, change medium 48 hours after establishing the subculture.
2. Change the medium every other day thereafter, until the culture is approximately 50% confluent.
3. Once the culture reaches 50% confluence, change medium every day until the culture is approximately 80% confluent.

**Subculture:**

1. Subculture the cells when they are over 90% confluent.
2. Prepare Poly-L-Lysine coated flasks (2 μg/$cm^2$) one day before subculture.
3. Warm medium, trypsin/EDTA solution (T/E, cat. no. 0103), trypsin neutralization solution (TNS, cat. no. 0113), and DPBS to **room temperature**. We do not recommend warming the reagents and medium at 37°C waterbath prior to use.
4. Rinse the cells with DPBS.
5. Add 10 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 cells are covered by trypsin/EDTA solution; incubate the flask at 37°C incubator for 1 to 2 minutes or until cells are completely rounded up (monitored with inverted microscope). During incubation, prepare a 50 ml conical centrifuge tube with 5 ml of fetal bovine serum (FBS, cat. no. 0500); transfer trypsin/EDTA solution from the flask to the 50 ml centrifuge tube (a few percent of cells may detached); continue incubate the flask at 37°C for 1 minutes (no solution in the flask at this moment); at the end of trypsinization, one hand hold one side of flask and the other hand gently tap the other side of the flask to detach cells from attachment; check the flask under inverted microscope to make sure all cells are detached, add 5 ml of trypsin neutralization solution to the flask and transfer detached cells to the 50 ml centrifuge tube; add another 5 ml of TNS to harvest the residue cells and transfer it to the 50 ml centrifuge tube. Examine the flask under inverted microscope to make sure the cell harvesting is successful by looking at the number of cells left behind. There should be less than 5%.
   *Note: Use ScienCell Research Laboratories' trypsin/EDTA solution that is optimized to minimize the killing of the cells by over trypsinization.*
6. Centrifuge the 50 ml centrifuge tube (harvested cell suspension) at 1000 rpm (*Beckman Coulter* Allegra 6R centrifuge or similar) for 5 min; re-suspend cells in growth medium.
7. Count cells 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 animal origin as the minimum precaution against contamination [1].*

[1]. Grizzle, W. E., and Polt, S. S. (1988) Guidelines to avoid personal contamination by infective agents in research laboratories that use human tissues. J Tissue Culture Methods. 11(4).