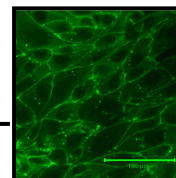


Clonetics™ Human Renal Epithelial Culture Model



Introduction

Clonetics™ Renal Epithelial Culture Model recreates human renal tubular function in an *in vitro* environment. The model consists of primary epithelial cells that were isolated from human renal cortices and seeded on extracellular-matrix-coated polyester membrane inserts, which allow for bi-compartmental (apical and basal) exposure to culture medium. Isolation and culture parameters (e.g., culture medium, matrix coating protocols, cell seeding density) have been optimized for the development of a functional renal tubular epithelial monolayer as assessed through specific markers.

Cell System Components

- 12 polyester inserts in 24 well plates seeded with renal epithelial cells, sealed with agarose and medium.
- One Renal Epithelial Cell BulletKit™ - 500 ml

Characterization of Cells

The apical brush border enzyme γ -glutamyl transpeptidase (γ -GTP), specific to proximal tubule epithelium, has been shown to be consistently and stably expressed in these cultures through at least 14 days in culture. Apical chamber expression of γ -GTP is typically greater than 10-fold higher than expression in the basal chamber, demonstrating the presence both of physiologically accurate polarity and of epithelial barrier properties in the culture model. Consistent ammoniogenesis, critical in renal acid-base homeostasis, has also been confirmed through at least 14 days in culture and typically is concentrated in the apical chamber, mimicking the excretion of ammonia in urine. Uptake of rhodamine-123 from the basal chamber is commonly several times higher than uptake from the apical chamber, illustrating that an active directional organic ion transport mechanism is present. This culture model therefore provides a polarized, functional renal tubular epithelium in an *in vitro* environment. In conjunction with specific functional assays such as those described here, the system provides a physiologically relevant platform technology through which human kidney's response to nephrotoxins and other xenobiotics can be probed for all phases of renal ADME/Tox. Test lots have

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been additionally screened and stain positive for the following cell-cell junction proteins: ZO-1, E-cadherin, Occludin, α -, β -, γ -Catenins and Desmoplakin.

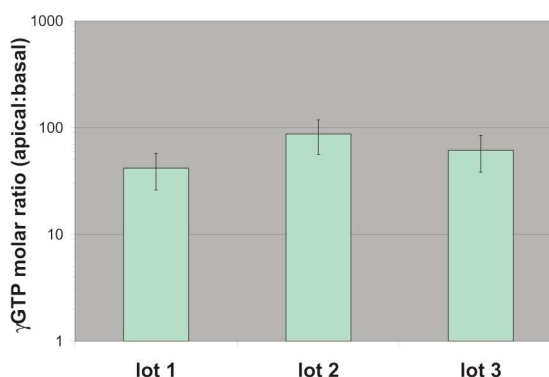


Figure 3: γ -GTP is Expressed Primarily on apical surface. Ratios of apical-to-basal expression of γ -GTP were averaged for three lots of Clonetics™ Renal Cortical Epithelial Cells in two separate experiments (triplicate wells for each lot).

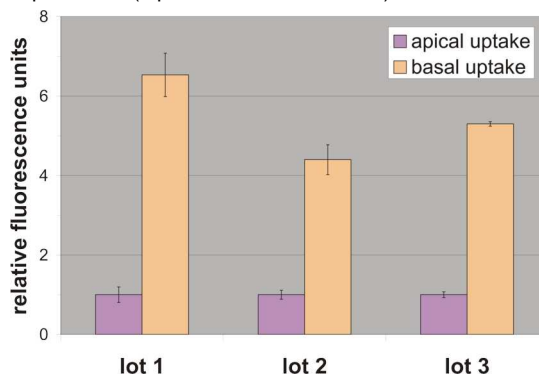


Figure 5: Basolateral Uptake of Organic Cations. Uptake of rhodamine 123 was assessed from both apical and basal culture medium. Fluorescence of extracted rhodamine 123 was normalized to apical concentration in each experiment. Results are from three lots of Clonetics™ Renal Cortical Epithelial Cells (triplicate wells for each lot) in two separate experiments.

Quality Control

All renal culture models exhibit greater than 5-fold apical: basal ratio of gamma glutamyl transpeptidase expression. Inserts are inspected to ensure confluence prior to shipping. For detailed information concerning QC testing, please contact Scientific Support.

Lonza

All cells are performance assayed and test negative for HIV-1, mycoplasma, Hepatitis-B, Hepatitis-C, bacteria, yeast and fungi. Clonetics™ Media are formulated for optimal growth of specific types of normal human cells. COA's for media products are available upon request.

CULTURES HAVE A FINITE LIFESPAN IN VITRO. Lonza warrants its cells only if Clonetics™ Media are used, and the recommended protocols are followed.

THESE PRODUCTS ARE FOR RESEARCH USE ONLY. Not approved for human or veterinary use, for application to humans or animals, or for use in clinical or in vitro procedures.

Ordering Information

CMS-2000 12 polyester inserts in a 24 wellplate seeded with renal epithelial cells, sealed with agarose and medium.

Renal Epithelial Growth Medium (Must be purchased separately):

| | | |
|---------|-----------------------|---|
| CC-3190 | REGM™ BulletKit™ | Kit which contains a 500 ml bottle of REBM™, (CC-3191) and REGM™ SingleQuots™ (CC-4127). |
| CC-3191 | REBM™ | Renal Epithelial Basal Medium (no growth factors) (500 ml) |
| CC-4127 | REGM™ SingleQuots™ | Supplements and growth factors (hydrocortisone, hEGF, FBS, epinephrine, insulin, triiodothyronine, transferrin and gentamicin/amphotericin-B) |

WARNING: CLONETICS™ AND POIETICS™ PRODUCTS CONTAIN HUMAN SOURCE MATERIAL, TREAT AS POTENTIALLY INFECTIOUS. Each donor is tested and found non-reactive by an FDA approved method for the presence of HIV-1, Hepatitis B Virus and Hepatitis C Virus. Where donor testing is not possible, cell products are tested for the presence of viral nucleic acid from HIV, Hepatitis B Virus, and Hepatitis C Virus. Testing can not offer complete assurance that HIV-1, Hepatitis B Virus, and Hepatitis C Virus are absent. All human sourced products should be handled at the Biological Safety Level 2 to minimize exposure of potentially infectious products, as recommended in the CDC-NIH Manual, [Biosafety in Microbiological and Biomedical Laboratories](#), 5th Edition. If you require further information, please contact your site Safety Officer or Scientific Support.

When placing an order or for technical service, please refer to the product numbers and descriptions listed above. For a complete listing of all Clonetics™ Products, refer to the Lonza website or the current catalog. To obtain a catalog, additional information or technical service you may contact Lonza web, e-mail, telephone, fax or mail.