Lonza Walkersville, Inc.
www.lonza.com
scientific.support@lonza.com
Scientific Support: 800-521-0390
Document # IN-CMS-2000-3 11/10
Walkersville, MD 21793-0127 USA

Clonetics™ Human Renal Epithelial Culture Model

Instructions for Use

Safety Statements

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.

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-I, 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.

Product Description

The Clonetics™ Human Renal Epithelial Culture Model (CMS-2000) is designed to re-create human renal tubular function in an *in vitro* environment. It consists of primary human renal cortical epithelial cells (passage 1) seeded on extracellular-matrix-coated polyester membranes. Isolation and culture parameters have been optimized for the development of a functional renal tubular epithelial monolayer as assessed through specific markers.

Unpacking and Storage Instructions

The apical wells of these culture plates contain a gel-based medium sealant to maintain cell viability and epithelial integrity through the shipping process. This medium becomes removable at 37℃. The following protocol provides instructions for gel removal and preparation for culture and should be initiated immediately upon receipt.

Day of receipt

1. Add 0.1 ml pre-warmed culture medium to each upper/apical well. Add 0.6 ml culture medium to each lower/basal well.
2. Place culture plate in 37℃ humidified CO_2 incubator for at least 2 hrs.
3. Gently aspirate the solution from each lower and upper well. Be careful not to touch the membranes with aspirating pipet. *Note: Some gel may still remain after this step. If removal is difficult, do not try to aspirate all of the gel!*
4. Slowly add 0.1 ml culture medium to the upper well (pipet medium on walls of well, not directly onto cells). Add 0.6 ml culture medium to the lower well.
5. Place in the incubator overnight to equilibrate.

Day after receipt

1. Gently aspirate the solution from each lower and upper well. Be careful not to the touch membranes.
2. Add 0.1 ml of pre-warmed culture medium to each upper well. Add 0.6 ml of pre-warmed culture medium to each lower well.
3. If residual gel is observed in the wells, steps 1-2 may be repeated.

The plates are now ready for use.

Note: Inserts can be easily picked up during aspiration. For best results, use lowest possible vacuum aspiration to remove medium without disturbing the insert. A small (200uL) size pipet tip can be placed on the end of the aspirator. A small-bore aspirating pipet can be used to remove the medium. If necessary, use sterile forceps to hold insert in place during aspiration.

Medium

This product is cultured should be cultured Renal Epithelial Growth Medium (REGM™) for optimal overall performance. For certain applications, including some organic ion transport studies, withholding GA-1000 or FBS from the culture medium may improve functional performance.

Culture medium should be exchanged every two days: 0.1 mL upper/apical well, 0.6 mL lower/basal chamber. Extreme care should be taken to avoid contact with membrane during aspiration.

Medium Preparation

For a BulletKit™, perform the following steps:

1. Decontaminate the external surfaces of all supplement vials and the medium bottle with ethanol or isopropanol.
2. Aseptically open each supplement vial and add the entire amount to the basal medium with a pipette.
3. Rinse each cryovial with the medium. It may not be possible to recover the entire volume listed for each cryovial. Small losses, even up to 10%, should not affect the cell growth characteristics of the supplemented medium.
4. Transfer the label provided with each kit to the basal medium bottle being supplemented. Use it to record the date and amount of each supplement added. We recommend that you place the completed label over the basal medium label (Avoid covering the basal medium lot # and expiration date) to avoid confusion or possible double supplementation.
5. Record the new expiration date on the label based on the shelf life.

Note: If there is concern that sterility was compromised during the supplementation process, the entire newly prepared growth medium may be refiltered with a 0.2µm filter to assure sterility. Routine refiltration is not recommended.

Quality Control

All renal culture models exhibit greater than 5-fold apical: basal ratio of gamma glutamyl transpeptidase expression.

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

Order Information

CMS-2000	12 polyester inserts in a 24 well plate, pre-seeded with renal epithelial cells.

Related Products

Renal Epithelial Growth Media (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)