Renal Proximal Convoluted Tubules Care Manual

INSTRUCTION MANUAL   ZBM0061.01

SHIPPING CONDITIONS

Human Renal Proximal Convoluted Tubule Cells, cryopreserved

Orders are delivered via Federal Express courier.

Must be processed immediately upon shipment receipt.

STORAGE CONDITIONS

Media: Store at +4°C; DO NOT FREEZE. Store as indicated IMMEDIATELY UPON ARRIVAL

Cryopreserved cells: Renal Proximal Convoluted Tubule cells are to be stored in vapor phase nitrogen (-150°C to -190°C) IMMEDIATELY UPON RECEIPT.

All Zen-Bio Inc products are for research use only. Not approved for human or veterinary use or for use in diagnostic or clinical procedures.

PRECAUTIONS

This product is for research use only. It is not intended for human, veterinary, or in vitro diagnostic use. Proper precautions and biological containment should be taken when handling cells of human origin, due to their potential biohazardous nature. Always wear gloves and work behind a protective screen when handling primary human cells. All media, supplements, and tissue culture ware used in this protocol should be sterile.

ORDERING INFORMATION AND TECHNICAL SERVICES

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Electronic mail (e-mail) information@zenbio.com
World Wide Web http://www.zenbio.com
LIMITED PRODUCT WARRANTY

This warranty limits our liability to replacement of this product. No other warranties of any kind, expressed or implied, including without limitation implied warranties of merchantability or fitness for a particular purpose, are provided by Zen-Bio, Inc. Zen-Bio, Inc. shall have no liability for any direct, indirect, consequential, or incidental damages arising out of the use, the results of use, or the inability to use this product.

Zen-Bio, Inc warrants its cells only if Zen-Bio media are used and the recommended protocols are followed. Cryopreserved human renal proximal convoluted tubule cells are assured to be viable when thawed according to Zen-Bio protocols.

Contact ZenBio, Inc. within no more than 24 hours after receipt of products for all claims regarding shipment damage, incorrect ordering or other delivery issues. Delivery claims received after 7 days of receipt of products are not subject to replacement or refund.

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To comply with U.S. Food and Drug Administration (FDA) regulations, these products are not for use in Clinical Diagnostic or Therapeutic Procedures.

By your acceptance of these products, you are acknowledging that these products will be:

1. Treated as potentially contaminated biological specimens even if accompanying serological reports are negative;
2. Handled by establishing or following appropriate safety control procedures to ensure the safety of using these products.

Human renal proximal convoluted tubule cells

The human Renal Proximal Convoluted Tubule epithelial cells (RPCT) are collected from unfractionated kidney cells from a consented adult donor with normal kidney morphology via the gift of organ donation. Human renal proximal convoluted tubules viability depends greatly on the use of suitable media, reagents, and sterile plastic wear. If these parameters are not carefully observed cell responsiveness in assays may be lower than expected.
INTRODUCTION

Renal proximal tubule cells play an important 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. They are also a major site of injury in a variety of congenital, metabolic, and inflammatory diseases with polycystic kidney disease being one of the more important diseases. Proximal tubule cells 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. Proximal tubule cells express IL-2R alpha and MHC class II antigens during inflammation after renal transplantation or in crescentic glomerulonephritis indicating the capacity to participate in pathogenesis of immune renal injury.

MATERIALS PROVIDED FOR EACH CATALOG ITEM

- Cryopreserved human renal proximal convoluted tubules
  - Frozen vial containing 500,000 renal proximal convoluted tubule cells (store in vapor phase liquid nitrogen IMMEDIATELY upon receipt)
  - 50 ml RPCT-1 support medium
### MEDIA COMPOSITIONS

<table>
<thead>
<tr>
<th>Renal Proximal Convoluted Tubule Plating Medium (cat # RPCT-1)</th>
<th>Renal Proximal Convoluted Tubule Maintenance Medium (cat # RPCT-2)</th>
<th>Renal Proximal Convoluted Tubule Cryopreservation Medium (cat# RPCTFM-1-100)</th>
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<tbody>
<tr>
<td>RPMI-1640</td>
<td>RPMI-1640</td>
<td>RPMI-1640</td>
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<tr>
<td>3,3',5 Triiodothyronine (T3)</td>
<td>3,3',5 Triiodothyronine (T3)</td>
<td>3,3',5 Triiodothyronine (T3)</td>
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<tr>
<td>Recombinant Human Epidermal Growth Factor (rEGF)</td>
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<td>Recombinant Human Epidermal Growth Factor (rEGF)</td>
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<tr>
<td>Human Insulin</td>
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<tr>
<td>Apo-Transferrin</td>
<td>Apo-Transferrin</td>
<td>Apo-Transferrin</td>
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<tr>
<td>Hydrocortisone</td>
<td>Hydrocortisone</td>
<td>Hydrocortisone</td>
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<td>Epinephrine</td>
<td>Epinephrine</td>
<td>Epinephrine</td>
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<tr>
<td>Fetal Bovine Serum</td>
<td>Penicillin</td>
<td>DMSO</td>
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<td>Penicillin</td>
<td>Streptomycin</td>
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<td>Streptomycin</td>
<td>Amphotericin B</td>
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**NOTE:**

RPCT-1 and RPCT-2 are provided ready to use and prepared fresh prior to shipment. The expiration date of RPCT-1 and RPCT-2 is 30 days from the ship date.

DO NOT FREEZE. Store 2-8°C only. Please schedule your orders accordingly.

Cryopreservation medium has an expiration date 1 year from the manufacture date.
THAWING AND PLATING CRYOPRESERVED RENAL PROXIMAL CONVOLUTED TUBULE EPITHELIAL CELLS

NOTE: THAWED CELLS ARE FRAGILE. HANDLE GENTLY AND QUICKLY TO MAINTAIN VIABILITY. ZEN-BIO RECOMMENDS THE USE OF BIOCOAT® BRAND CULTUREWARE FROM BECTON-DICKINSON.

1. Cryovials should be stored in liquid nitrogen immediately upon arrival.
2. Remove the medium from the packaging material and place on ice or at 4°C. If you have media previously prepared or ordered, keep it on ice until ready to thaw the cells.
3. Remove vial of cells from liquid nitrogen and place immediately into a 37°C water bath and gently agitate while in bath. Be careful not to submerge the cap of the vial into water. Remove the vials from water bath after most of the content has thawed. Rinse the vials with 70% ethanol before taking them to the culture hood.
4. Upon thawing, transfer the cells to a sterile conical bottom centrifuge tube containing 15 ml of Plating Medium (cat # RPCT-1). Rinse the vial using 1-2 ml medium and add the contents to the same tube.
5. Centrifuge at 1200 RPM / 20°C / 5 minutes.
6. Gently resuspend the cell pellet in a small volume of RPCT-1 Plating Medium.
7. Perform a cell count using trypan blue and a hemacytometer.
8. Warm the media to 37°C prior to plating.
9. After counting, resuspend the cells to 1 million cells/ml into warm RPCT-1 Plating Medium.
10. Plate the cells at 6,000 cells/cm² on collagen coated culture ware.

Note: Zen-Bio recommends the use of BioCoat® brand cultureware from Becton-Dickinson.

11. Place the plates in a 37°C, 5% CO₂, humidified incubator to allow the cells to attach for 6-8 hours.
12. Observe the cells for adherence. If adherence is not complete, place the cells back in the incubator for a few hours. Once the cells are attached, aspirate the initial plating medium from the cells and replace with warm and fresh RPCT-2 Maintenance medium.

TROUBLESHOOTING GUIDE

<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible causes</th>
<th>Suggestions</th>
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FREQUENTLY ASKED QUESTIONS

1. Can I passage these cells and what is the maximum passage? We do not recommend passaging the cells.

2. What is the average doubling time of these cells? Average doubling time varies for each lot but ranges from 54-96 hours.

3. Do you test for pathogens? Which ones? Yes. Samples from each donor are tested via PCR to confirm non-reactivity for HIV-1, HIV-2, HTLV I, HTLV II, syphilis, CMV, hepatitis B and hepatitis C. However, since we cannot test all pathogens, please treat the culture as a potentially infectious agent at Biosafety Level 2 or higher.

4. What donor information do I receive? The donor’s age, gender, and BMI are provided in the certificate of analysis that accompanies each lot of cells.

5. Are antibiotics included in the medium? Yes, an antibiotics/antimycotic solution (penicillin, streptomycin, and amphotericin B) is included in the media RPCT-1 and RPCT-2.

6. What quality control measures are used to test the cells? Renal proximal convoluted tubule cells are assessed via cell viability, accurate viable Cells/vial, CD13 cell surface marker staining, alkaline phosphatase staining and cell morphology.

PATHOGEN TESTING

Samples from each donor are tested via PCR to confirm non-reactivity for HIV-1, HIV-2, HTLV I, HTLV II, syphilis, CMV, hepatitis B and hepatitis C. However, no known test can offer complete assurance that the cells are pathogen free. Our products are tested and are free from mycoplasma contamination. Proper precautions and biological containment should be taken when handling cells of human origin, due to their potential biohazardous nature. All human based products should be handled at a BSL-2 (Biosafety Level 2) or higher. **Always wear gloves and work behind a protective screen when handling primary human cells.**