



株式会社 ケーエーシー

Human Renal Proximal Tubule Epithelial Cell Care Manual

INSTRUCTION MANUAL ZBM0061.07

SHIPPING CONDITIONS

Human Renal Proximal Convoluted Tubule Epithelial Cells, Cryopreserved

Orders are delivered via Federal Express courier. All USA and Canada orders are shipped via Federal Express Priority service and are usually received the next day. Non North American International orders are usually received in 2-4 days. Primary human cells can be sensitive to extended times at dry ice temperatures. If your transit time will exceed 3 days, use of a dry vapor shipper is required. Please inquire if alternate couriers are needed.

All orders should be processed immediately upon shipment receipt.

STORAGE CONDITIONS

Media: Store at +4°C. **Expiration date 30 days from ship date.** DO NOT FREEZE

Cells: Store in vapor phase nitrogen (-150°C to -190°C) IMMEDIATELY UPON RECEIPT. Any other use negates the warranty.

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

ORDERING INFORMATION AND TECHNICAL SERVICES

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THIS MANUAL IS SUITABLE FOR USE WITH THE FOLLOWING PRODUCTS:

RPCT-F	CRYOPRESERVED HUMAN RENAL PROXIMAL TUBULE EPITHELIAL CELLS, (500,000 CELLS/VIAL)
RPCT-1	RENAL PROXIMAL CONVOLUTED TUBULE PLATING MEDIUM, 500 mL
RPCTFM-1-100	RENAL PROXIMAL CONVOLUTED TUBULE CRYOPRESERVATION MEDIUM, 100 mL

<u>CONTENTS</u>	<u>PAGE #</u>
Limited Product Warranty	3
Precautions	3
Introduction	3-4
Quality Control	4
Catalog Items	4
Media Compositions	5
Plating and Expansion Procedures	6-7
Cryopreservation Procedure	8
Troubleshooting Guide	9
Frequently Asked Questions	9
Pathogen Testing	10

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 the performance of cells only if Zen-Bio media are used and the recommended storage conditions and protocols are followed without amendment or substitution. ZenBio, Inc. cryopreserved cells are assured to be viable when stored as recommended and thawed according to Zen-Bio protocols and using the recommended protocol.

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.

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 cultureware used in this protocol should be sterile.

Human renal proximal convoluted tubule epithelial cell viability depends greatly on the use of suitable media, reagents, and sterile plastic wear. If these parameters are not carefully observed, cell growth may be slower than expected.

INTRODUCTION

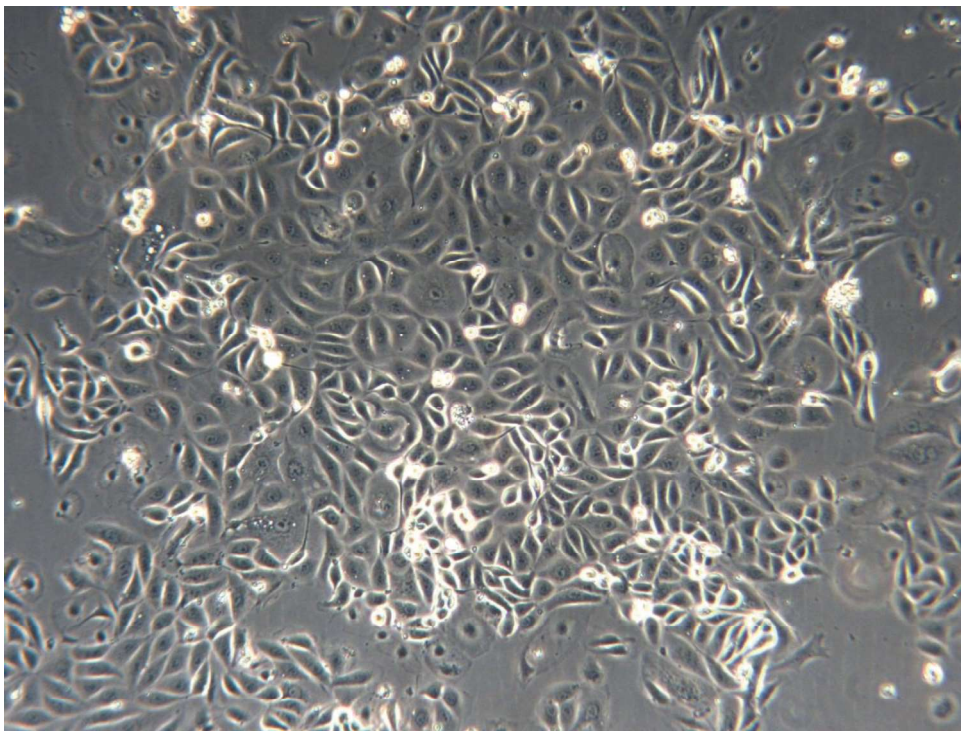
Renal proximal tubule cells play an important role in renal function. They reabsorb nearly all of the glucose and amino acids in the glomerular filtrate, while allowing other substances of no nutritional value to be excreted. They are also a major site of injury in a variety of congenital, metabolic, 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.

ZenBio, Inc. Human Renal Proximal Convoluted Tubule epithelial cells (RPCT) are collected from unfractionated kidney cells from a consented adult donor with normal kidney morphology. The kidney cells are obtained via the gift of organ donation from donor tissue that is not suitable for organ transplantation. Each donor has confirmed documentation on file allowing for research use of any non-transplantable organs or tissues.

QUALITY CONTROL

The cells are assessed for viability, alkaline phosphatase staining and cell surface markers via flow cytometry screening for CD13 and CD10.

Human renal proximal convoluted tubules viability depends greatly on the use of ZenBio recommended storage conditions, media, reagents, and suitable sterile plastic wear. If these parameters are not carefully observed cell responsiveness in assays may be lower than expected.



Morphology after 5 days in culture (flat curved shape morphology)

CATALOG ITEMS

- ❖ **Cryopreservation Medium for Renal Epithelial Cells**
 - Cat # RPCTFM-1-100
 - Store -20°C
- ❖ **Renal Proximal Convoluted Tubule Plating Medium**
 - Cat # RPCT-1
 - Store 6°C to 8°C **DO NOT FREEZE**
- ❖ **Cryopreserved Human Renal Proximal Convoluted Tubule Epithelial Cells**
 - Cat # RPCT-F
 - Cryopreserved vial containing 500,000 renal proximal convoluted tubule epithelial cells per vial (**store in vapor phase liquid nitrogen IMMEDIATELY upon receipt**) **any other storage negates the warranty**

MEDIA COMPOSITIONS

<u>Renal Proximal Convoluted Tubule Plating Medium</u> <u>(Cat# RPCT-1)</u>	<u>Storage and Expiration Date</u>
DMEM, 4.5 g/L (25 mmol/L) D-glucose MCDB153 Human Epidermal Growth Factor (hEGF) Human Insulin, recombinant Human apo-Transferrin Bovine Serum Albumin (BSA), Fatty Acid Free O-Phosphorylethanolamine Ethanolamine Hydrocortisone Calcium Chloride, anhydrous Epinephrine Bovine Pituitary Extract (BPE; NZ Origin) Penicillin Streptomycin Amphotericin B	<ul style="list-style-type: none"> • Store at 4°C • The expiration date is 30 days from the ship date. • Medium is provided ready to use and prepared fresh prior to shipment. <p style="text-align: center;"><u>DO NOT FREEZE</u></p>

<u>Renal Proximal Convoluted Tubule Cryopreservation</u> <u>Medium (cat# RPCTFM-1-100)</u>	<u>Storage and Expiration Date</u>
Fetal Bovine Serum (FBS; US Origin) Dimethyl sulfoxide (DMSO)	<ul style="list-style-type: none"> • Store at -20°C upon arrival until ready for use or the expiration date on bottle. Cryopreservation medium has an expiration date 1 year from the manufacture date when stored frozen. • The media will expire 45 days after the thaw date.

PLATING AND EXPANSION PROCEDURES

HUMAN RENAL PROXIMAL CONVOLUTED TUBULE EPITHELIAL CELLS

Note: *Thawed cells are fragile. Handle gently and quickly to maintain viability.*

Note: *Primary human cell viability is greatly dependent on the use of appropriate sterile tissue culture treated cultureware Zen-Bio recommends the use of ZenBio brand poly-L-lysine coated cultureware. See FAQ for details.*

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. Before taking the vial of cells out of liquid nitrogen storage, warm the Renal Proximal Convoluted Tubule Plating Medium (cat # RPCT-1) to 37°C.
4. 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, leaving the vial in for less than 1 minute, leaving a little bit of ice in the vial. Rinse the outside of the vials with 70% ethanol before taking them to the culture hood.
5. Upon thawing, transfer the cells to a poly-L-lysine coated T-75 flask with an appropriate amount of warm RPCT-1 media.

Note: *Dilution and centrifugation of cells after thawing are not recommended. These actions are more harmful to the cells than the effect of residual DMSO in the culture media.*

6. Replace the cap or lid of the culture vessel and gently rock the vessel to distribute the cells evenly. Return the culture vessel to the incubator.
7. The cells should be plated at 5,000 cells/cm² on poly-L-lysine coated cultureware.
8. Place the plates in a 37°C, 5% CO₂, humidified incubator to allow the cells to attach. Replace medium after 16-24 hours to remove residual DMSO and unattached cells. Do not disturb the culture for at least 16 hours after plating.
9. Replace medium every 3 days after the first time until the cells reach 70% confluency. Once 70% confluent, change medium every other day until the culture is 90% confluent.
10. Harvest cells once they have reached 90% confluency. Renal proximal convoluted tubule cells should be sub confluent (less than or equal to 90% confluent) upon harvest.
11. The cells are ready for cryopreservation, use, or expansion (only if under passage 3).

OPTIONAL – RENAL PROXIMAL TUBULE EPITHELIAL CELL SUBCULTURE

Note: *Human Renal Proximal Tubule Epithelial cells should not be expanded after passage 3.*

1. Before handling plated cells, warm the Renal Proximal Convoluted Tubule Plating Medium (cat# RPCT-1) to 37°C. Locate sterile 0.25% trypsin/2.21 mM EDTA solution and Dulbecco's phosphate buffered saline without calcium or magnesium and allow to come to room temperature. Do not warm trypsin/EDTA solution.

2. Remove the plated human renal proximal tubule epithelial cells from the incubator and bring them into a biosafety cabinet, following all appropriate procedures to ensure sterility.
3. Aspirate the medium and wash the cells 2 times with sterile Ca^{2+} / Mg^{2+} free DPBS.
4. Add cold 0.25% trypsin/2.21 mM EDTA solution in 1 mL per T-75 flask (or 0.5 mL per T-25 flask), ensuring the bottom is coated. Incubate the cells at room temperature for 30-60 seconds, monitoring cell detachment under the microscope. A longer incubation in trypsin can damage the renal proximal tubule epithelial cells.
5. Inactivate the trypsin by adding RPCT-1 medium to the flask. Transfer the entire contents of the flask into a sterile 15 mL conical tube, rinsing the flask at least once with RPCT-1 medium to collect any remaining cells. Ensure cells were transferred by viewing empty flask under a microscope.
6. Centrifuge at 300 x g at 20°C for 5 minutes.
7. Aspirate off supernatant, being careful not to disturb the cell pellet, and gently resuspend the cell pellet in a small volume of warm RPCT-1 to count.
8. Perform a cell count using trypan blue and a hemocytometer.
9. After counting, bring the volume up with warm RPCT-1 medium to an appropriate amount for plating at 5,000 cells/cm² on poly-L-lysine coated cultureware.
10. Place the culture vessel(s) in a 37°C, 5% CO₂, humidified incubator to allow the cells to attach. Replace medium after 16-24 hours to remove unattached cells. Do not disturb the culture for at least 16 hours after plating.
11. Replace medium every 3 days after the first time until the cells reach 70% confluency. Once 70% confluent, change medium every other day until the culture is 90% confluent.
12. Harvest cells once they have reached 90% confluency. Renal proximal convoluted tubule cells should be sub confluent (less than or equal to 90% confluent) upon harvest.
13. The cells are ready for cryopreservation, use, or further expansion (only if under passage 3).

CRYOPRESERVATION PROCEDURE

1. Renal proximal convoluted tubule cells should be sub confluent (about 90% confluent) upon harvest for expansion or cryopreservation.
2. Aspirate medium and wash cells using sterile Phosphate Buffered Saline without magnesium or calcium (PBS) to remove all traces of serum (until there is no foaming of the medium).
3. Remove the PBS and release the cells from the cultureware bottom by adding 2 ml/T-75 flask of 0.25% trypsin/ 2.21 mM EDTA solution.
4. Incubate cells with trypsin solution for 5 minutes at 37°C.
5. Neutralize the trypsin using 0.1 ml Renal Proximal Convoluted Tubule Plating Medium (cat# RPCT-1) per cm² cultureware surface area (7.5 mL for T-75 flask). Check under a microscope to ensure all cells are removed.
6. Centrifuge at 300xg at 20°C for 5 minutes.
7. Aspirate the medium and suspend cells in a volume of RPCT-1 medium appropriate for counting the cells. Count using a hemocytometer.
8. Centrifuge at 300xg at 20°C for 5 minutes. Suspend in cold Renal Proximal Convoluted Tubule Cryopreservation Medium (cat# RPCTFM-1-100) at a concentration of 1 million cells/mL. Do not exceed a 6:1 ratio of cells (per million): volume cryopreservation medium (per mL). Remember to account for the volume of the cell pellet before adding the volume of cryopreservation medium necessary for cell suspension.
9. If using a controlled-rate freezer: Freeze by reducing the temperature by 1°C per minute until the temperature reaches -80°C. If using a cell cryopreservation container, prepare according to the manufacturer's instructions.
10. For best results we recommend transferring the vials to the vapor phase of a liquid nitrogen storage facility as soon as possible after the cells have reached -80°C.

TROUBLESHOOTING GUIDE

Observation	Possible Cause	Suggestions
Renal Proximal Convoluted Tubules do not attach.	<ol style="list-style-type: none"> 1. Cells stored at a suboptimal temperature. 2. Cells plated on uncoated cultureware. 	<ol style="list-style-type: none"> 1. Store cells in vapor phase liquid nitrogen IMMEDIATELY upon arrival. 2. Plate cells on Zen-Bio brand poly-L-lysine coated cultureware.

FREQUENTLY ASKED QUESTIONS

Can I passage these cells and what is the maximum passage?

Yes. The limit of expansion is passage 3 only.

What is the average doubling time of these cells?

Average doubling time varies for each lot but ranges from 54-96 hours.

Do you test for pathogens? Which ones?

Yes. See section titled Pathogen Testing for more information.

What donor information do I receive?

The donor's age, gender, and body mass index (BMI) are provided in the certificate of analysis that accompanies each lot of cells.

Are antibiotics included in the medium?

Yes, an antibiotics/antimycotic solution (penicillin, streptomycin, and amphotericin B) is included in the Renal Proximal Convoluted Tubule Plating Medium (cat# RPCT-1). However, there are no antibiotics in the Renal Proximal Convoluted Tubule Cryopreservation Medium (cat# RPCTFM-1-100).

What quality control measures are used to test the cells?

Renal proximal convoluted tubule cells are assessed for cell viability, accurate viable cells/vial, CD13 cell surface marker staining, alkaline phosphatase staining, and cell morphology.

Is there a specific type of cultureware that should be used?

Yes, only Zen-Bio Poly-L-Lysine Cultureware should be used (catalogue numbers below). Please inquire for custom format coating.

Item#	Item Description
PLL-6	Poly-L-Lysine Coated 6-well Plate, Pack of 5
PLL-96	Poly-L-Lysine Coated 96-well Plate, Pack of 5
PLL-75	Poly-L-Lysine Coated T-75 Flask, Vent Cap, Pack of 5

PATHOGEN TESTING

Samples from each donor are tested via PCR and found non-reactive to viral DNA from HIV and Hepatitis B and viral RNA from Hepatitis C. However, no known test can offer complete assurance that these viruses are not present. Since we cannot test all pathogens, always treat the culture as a potentially infectious reagent. We recommend using the US Centers for Disease Control (CDC) Universal Precautions for prevention of blood-borne pathogens as a minimum guideline for standards of practice. Our products are tested for mycoplasma contamination. Mycoplasma is not detected in our labs. 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-1 (Biosafety Level 1) or higher. Always wear gloves and work behind a protective screen when handling primary human cells.

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