HUMAN RENAL PROXIMAL TUBULE CELLS

Product No.	Description	Size
M03805	Male cryopreserved human renal proximal tubule cells	1 million cells
F03805	Female cryopreserved human renal proximal tubule cells	1 million cells
Z990001	INVITROGRO™ PT Medium	250 mL bottle
Z99000	TORPEDO™ Antibiotic Mix	5.5 mL

Product Description:

Human renal proximal tubule (HRPT) cells have been used in the disciplines of physiology, pharmacology, toxicology and cell biology. HRPT cells can be used for transport, apoptosis, toxicity and metabolism assays[1,2]. These cells have tested negative for mycoplasma. The donor serology is negative for HIV-1, HBV and HCV. The cells stained positive for gamma-glutamyltransferase activity[1,2].

Stability: Two years at –150 °C or less
Storage: ≤–150 °C

Procedure:

1. Place one TORPEDO™ Antibiotic Mix in a 37 °C water bath until thawed and then remove from water bath.
2. Complete INVITROGRO™ PT Medium by adding the TORPEDO™ Antibiotic Mix.
3. Warm complete INVITROGRO PT Medium to 37 °C.
   a. Following addition of TORPEDO Antibiotic Mix the shelf life for the complete medium is 7 days. Store at 4 °C when not in use.
4. Under aseptic conditions, dispense 4 mL of warm completed PT Medium into a sterile conical tube.
5. Carefully remove the vial of RPT cells from the shipping container or cryogenic freezer.
6. Immediately immerse the vial into a 37 °C water bath. Shake gently for 75–90 seconds until contents are partially thawed. Spray the vial with 70% ethanol and place it in a biosafety cabinet before opening.
7. Under aseptic conditions, transfer the contents of the vial to the conical tube containing warm completed PT Medium and rinse the vial with 1 mL of completed PT Medium to recover the most cells possible.
8. Determine the total cell count and the number of viable cells using the Trypan blue exclusion method.
   Recommendations for the cell count: Add 50 µL of Trypan blue to 100 µL of cells – this is a dilution factor of 1.5.
9. The post-thaw viability should be ≥70%, yielding ≥1.0 × 10^6 viable cells. Resuspend cells and inoculate culture vessels with pre-warmed completed PT Medium at an appropriate cell

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density for your application. BioIVT recommends the use of collagen-coated vessels for culturing these cells.

10. To achieve a 50% confluent monolayer in multiwell plates after one day of cell attachment, dilute the cells to 100,000 cells/mL. Add the appropriate volume of the diluted cells to each well of the plates according to the information below:

    6-well plate: 1.5 mL/well (requires a total volume of 9 mL per 6-well plate)
    12-well plate: 0.6 mL/well (requires a total volume of 7.2 mL per 12-well plate)
    24-well plate: 0.3 mL/well (requires a total volume of 7.2 mL per 24-well plate)
    96-well plate: 0.075 mL/well (requires a total volume of 7.2 mL per 96-well plate)

11. Completely replace the medium in the culture vessels 4 to 24 hours after inoculation to remove any cells that do not attach.
12. Replace medium in the cultures every other day beginning 2 days after the first medium replacement.

Human Renal Proximal Tubule Cell Seeding Chart

Seeding Density Day 1 (cells/cm²)	Target Day 2 (% confluence)	Target Day 3 (% confluence)	Target Day 4 (% confluence)
2×10^3	10–20	30–40	60-80
4×10^3	30	60	90
8×10^3	45–50	90	100
15×10^3	70	100	>100

References:

1. Trifillis, A. L. Isolation, culture, and characterization of human renal proximal tubule and collecting duct cells. *Exp. Nephrol.* **1999**, *7*, 353–359.
2. Cummings, B. S.; Lasker, J. M.; Lash, L. H. Expression of glutathione-dependent enzymes and cytochrome P450s in freshly isolated and primary cultures of proximal tubular cells from human kidney. *JPET* **2000**, *293*(2), 677–685.

Caution: Treat all products containing human and monkey-derived materials a potentially infectious, as no known test methods can offer assurance that products derived from human or monkey tissues will not transmit infectious agents.

All products are for research use only. Do not use in animals or humans. These products have not been approved for any diagnostic or clinical procedures.

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