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IMMORTALIZED CELL LINES

IMMORTALIZED HUMAN UROTHELIAL CELLS

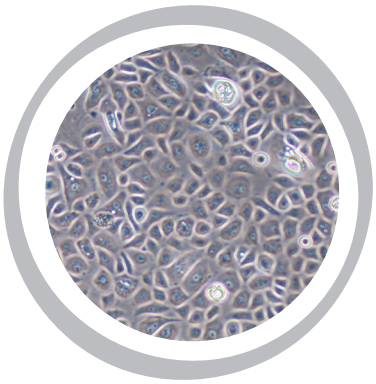
P10952-IM

Immortalized Human Urothelial Cells provided by Innoprot have been obtained by immortalizing primary human urothelial cells using SV40 and Bm-7e lentiviral vectors.

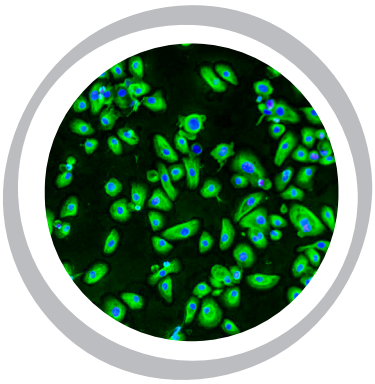
Urothelial cells line the inner surface of the urinary tract, including the renal pelvis, ureters, bladder, and proximal urethra. This transitional epithelium is uniquely specialized to withstand cyclic mechanical stretching and high concentrations of potentially harmful solutes, while preserving a tight barrier that protects underlying tissues from urine constituents. Urothelial cells play key roles in maintaining barrier integrity, regulating host defense mechanisms, and facilitating tissue repair.

Under pathological conditions such as urinary tract infections (UTIs), interstitial cystitis, or bladder cancer, urothelial dysfunction contributes to barrier breakdown, chronic inflammation, and altered cellular differentiation. These cells are responsive to mechanical and chemical stimuli through signaling pathways such as MAPK, NF-κB, and PI3K/AKT, making them a valuable in vitro model for studying inflammation, carcinogenesis, drug toxicity, and regenerative therapies.

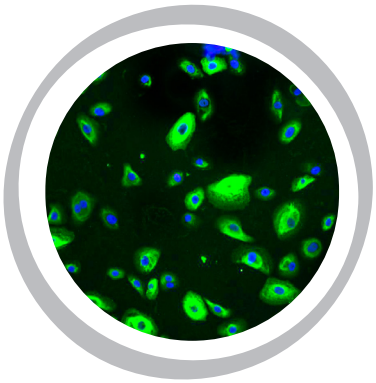
Product Type: Immortalized Human Urothelial Cells
Catalog Number: P10952-IM
Immortalization: SV40 Large T Antigen and Bm-7e. G418 resistant.
Number of cells: >1x10^6 cells (cryopreserved vials)
Storage: Liquid Nitrogen
Recommended Medium: Alveolar Epithelial Cell Medium (Ref: P60102)
Product Characterization: Expression of constitutive phenotypic epithelial markers (Citokeratin-18 and 19; Vimentin). Functional analysis: Urothelial Differentiation Assay (ZO-1/Occludin Expression); Transepithelial Electrical Resistance (TEER) Measurement and Epithelial Permeability Assay.



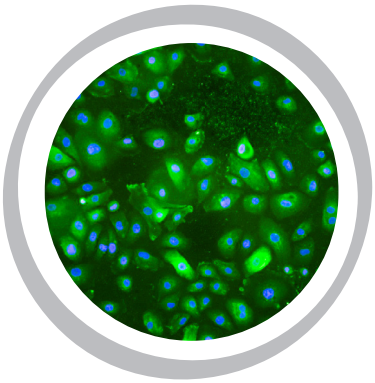
Phase-contrast



Vimentin



Citokeratin-18



Citokeratin-19

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It is not to be used for drug or diagnostic purposes, nor is it intended for human use. Innoprot products may not be resold, modified for resale, or used to manufacture commercial products without written approval of Innovative Technologies in Biological Systems, S.L.

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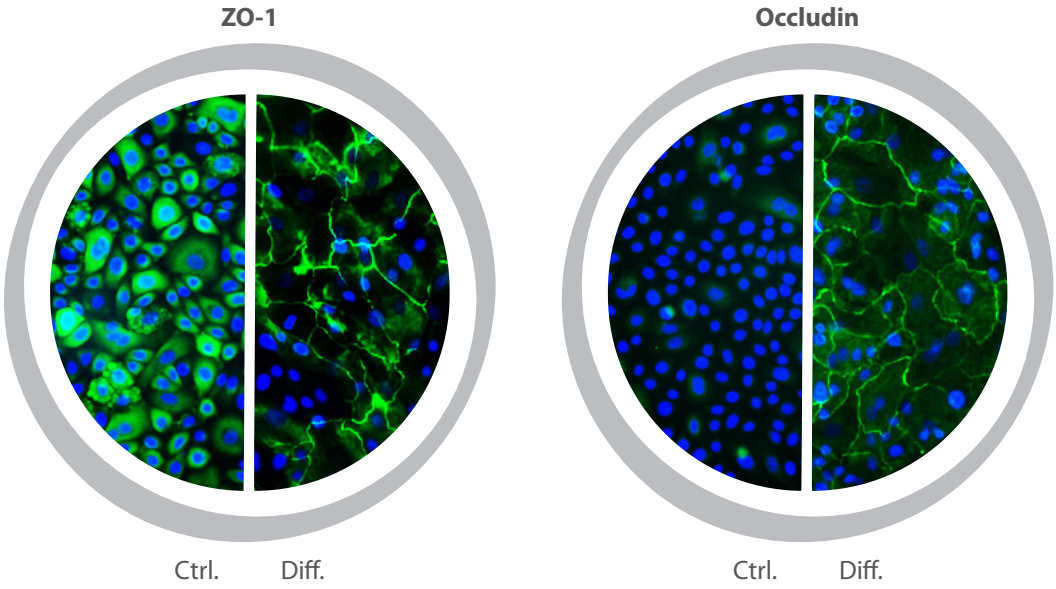
About Immortalized Human Urothelial Cells

The immortalized human urothelial cell line has been developed through genetic modification of primary human urothelial cells, employing a dual-lentiviral approach using SV40 large T antigen (SV40LT) and Bm7e vectors for immortalization. SV40LT, derived from the Simian Virus 40 Large T-antigen, disrupts cell cycle regulation to prevent senescence, while Bm7e contributes to enhanced proliferative capacity and long-term culture stability. This combined strategy enables the generation of a robust, immortalized cell line with extended lifespan and reproducible growth kinetics, suitable for a wide range of in vitro applications. Compared to primary urothelial cells, which typically undergo senescence after approximately five passages, the SV40LT and Bm7e-transduced cells remain viable and proliferative beyond 20 passages, making them highly suitable for long-term studies in epithelial biology, barrier function, drug screening, and urothelial disease modeling.

Functional Analysis

1. Urothelial Differentiation Assay (ZO-1/Occludin Expression)

Cells were seeded at 30,000 cells/well in 96-well plates and treated with a differentiation medium (homemade alveolar medium supplemented with 1.5 mM $CaCl_2$ and 10% FBS) for 3 and 7 days. Immunocytochemistry was performed using ZO-1 and occludin antibodies to assess tight junction formation, a hallmark of urothelial differentiation. Differentiated cells showed clear expression of ZO-1 by day 3, with enhanced tight junctions by day 7. In contrast, undifferentiated controls lacked expression of these markers.



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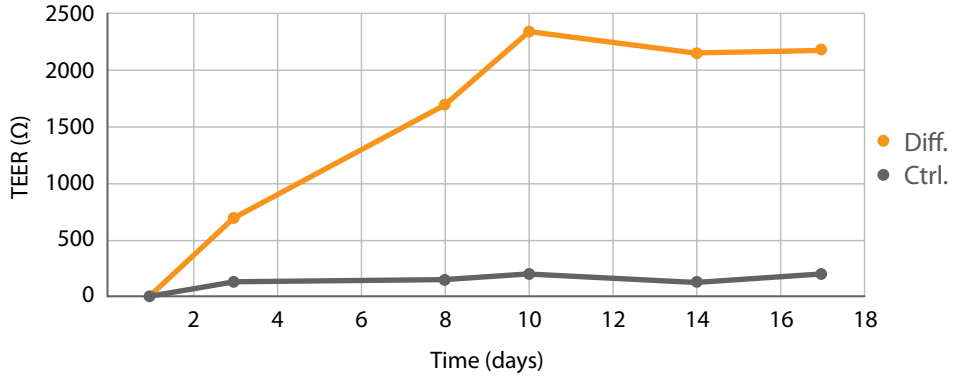
Functional Analysis

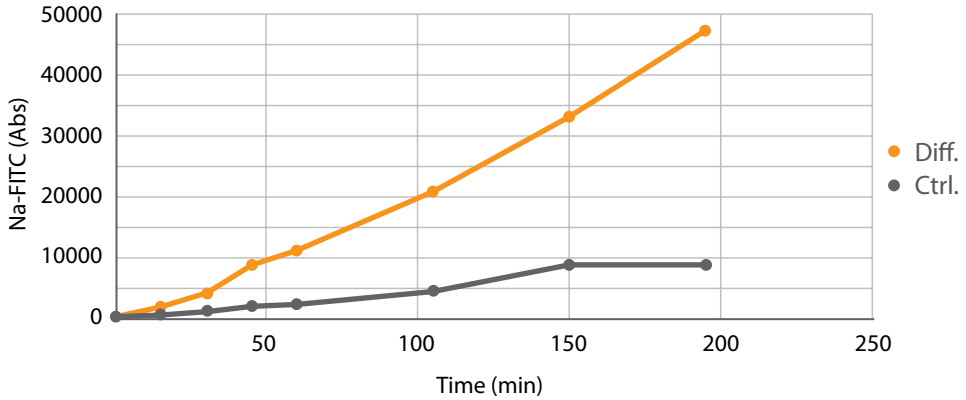
2. Transepithelial Electrical Resistance (TEER) Measurement

Cells were cultured on collagen-coated transwell inserts (150,000 cells/insert) and induced to differentiate using medium supplemented with 10% FBS. TEER values were measured at days 3, 8, 10, 14, 17, and 20. A progressive increase in TEER was observed in differentiated cultures, indicating epithelial maturation. Control cultures maintained low TEER throughout, consistent with an undifferentiated phenotype.



3. Epithelial Permeability Assay

On day 20, permeability to sodium fluorescein (Na-FITC, 376 Da) was assessed in cultures with TEER >200 Ω. Fluorescein was added to the apical compartment (10 µg/mL), and basolateral fluorescence was measured over 180 minutes. Differentiated cells restricted fluorescein passage, confirming tight junction integrity. Control cells showed higher permeability.



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Culturing conditions

1 IMMEDIATELY UPON DELIVERY

1.1 Remove the vial from the shipping container to check for freezing.

1.2 Transfer the frozen vial to liquid nitrogen until ready to thaw.

2 THAWING CELLS:

2.1 Prepare "Thawing medium" by combining 500 ml of basal medium, 25 ml of fetal bovine serum, 5 ml of Growth supplement and 5 ml of penicillin/streptomycin solution.

2.2 Prepare collagen I coating (final 50 µg/ml): dilute collagen I (1 mg/ml) 1:20 in 1× Collagen Solvent. Add 100 µl/cm^2, incubate 60 min at room temperature, aspirate completely, and allow the surface to air-dry in the laminar flow hood. Before use, it is recommended to rinse with PBS to remove residual acidic solvent.

2.3 Thaw cells rapidly in a 37ºC water bath; avoid allowing the sample to warm to 37ºC. Cryovials should be cool to the touch when removed.

2.4 Remove the vial, wipe it dry, and transfer it to a sterile field.

2.5 Rinse the vial with 70% ethanol, then wipe to remove excess. Open the vial and resuspend its contents using a 1 ml Eppendorf pipette.

2.6 Dispense the contents into a 25 cm^2 culture flask with warm complete media (FBS percentage can be increased up to 10% for better culture establishment).

2.7 Place the flask in the incubator.

2.8 For optimal results, avoid disturbing the culture for 16 hours after initiation. Change the growth medium the next day to remove unattached cells, then every other day thereafter.

3 MAINTENANCE OF THE CULTURE:

3.1 Change medium 48 hours after establishing a subculture.

3.2 Subculture when cells are over 90% confluent.

4 SUBCULTURING:

Remove medium, rinse with 0.05% trypsin-EDTA solution. Add 1 to 2 mL of trypsin-EDTA solution and allow the flask to sit until cells detach. Add fresh culture medium, aspirate, and dispense into new culture flasks.

Recommended subcultivation ratio of 1:2 to 1:6.

Medium Renewal: 2 to 3 times per week.

Reagents for cryopreservation: Cryostor S10.

Quality Control / Biosafety

The cells test negative for HIV-1, HBV, HCV, mycoplasma, bacteria, yeast and fungi.

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