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IMMORTALIZED CELL LINES

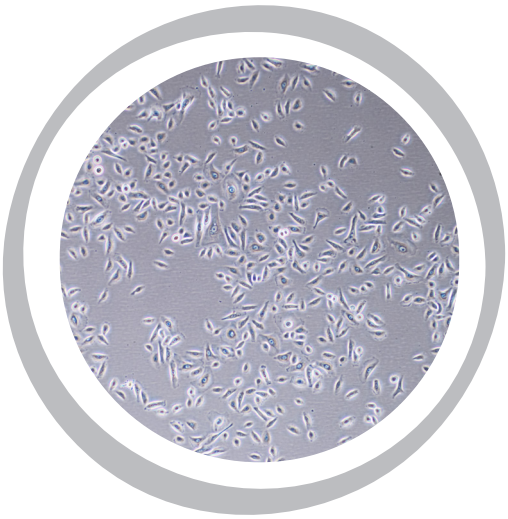
P10891-IM

Immortalized Human Mammary Epithelial Cells have been developed by transducing primary human mammary epithelial cells with SV40 large T antigen (SV40T) and Bm7e lentiviral vectors. SV40T enables bypass of cellular senescence by disrupting cell cycle checkpoints, while Bm7e contributes to enhancing proliferative capacity and maintaining long-term culture stability. This dual-vector strategy results in an immortalized epithelial cell line with extended lifespan and reproducible growth characteristics.

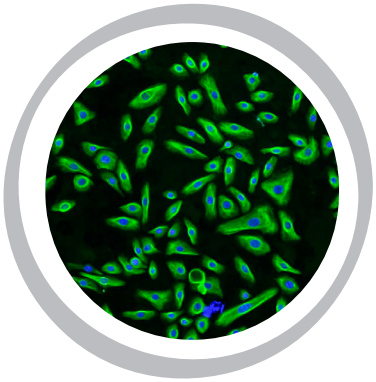
Human mammary epithelial cells form the inner lining of the mammary ducts and are central to tissue homeostasis, hormonal response, and epithelial integrity. Dysregulation of these cells plays a key role in breast development disorders and carcinogenesis. Immortalized cells retain epithelial morphology and express key markers such as cytokeratins and E-cadherin, making them a reliable in vitro model for studying epithelial biology, breast tissue physiology, and early events in tumorigenesis. Their stable phenotype and responsiveness to differentiation cues make these cells suitable for long-term experiments, mechanistic studies, and preclinical applications in breast cancer research and toxicology.

IMMORTALIZED HUMAN
MAMMARY EPITHELIAL CELLS

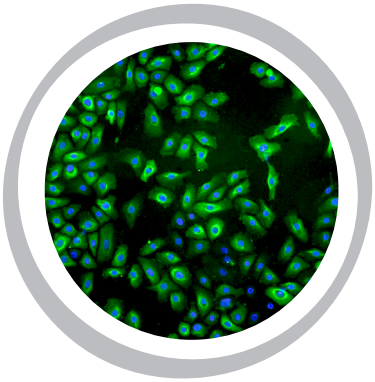
Product Type: Immortalized Human Mammary Epithelial Cells
Catalog Number: P10891-IM
Immortalization: SV40 Large T Antigen and Bm-7e. G418 resistant.
Number of cells: >1x10^6 cells (cryopreserved vials)
Storage: Liquid Nitrogen
Recommended Medium: Alveolar Epithelial Cell Medium (Ref: P60102)
Product Characterization: Expression of constitutive phenotypic epithelial markers (Citokeratin-18 and 19; Vimentin). Functional analysis: morphological evaluation, epithelial polarization, Transepithelial Electrical Resistance (TEER) measurement and mucin secretion assay.



Phase-contrast



Vimentin



Citokeratin-18

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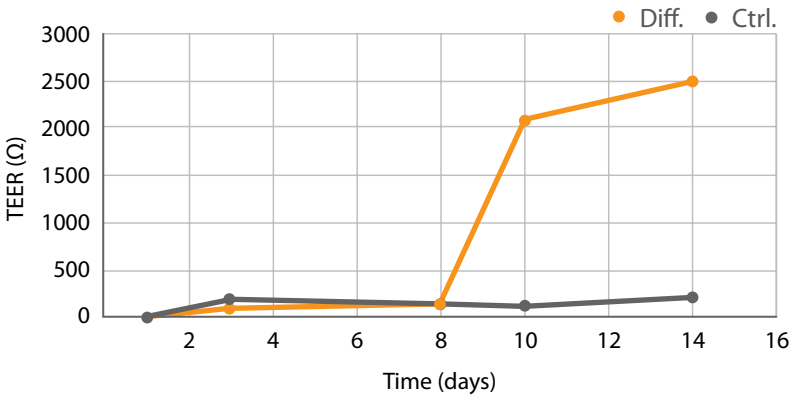
About Immortalized Human Mammary Epithelial Cells

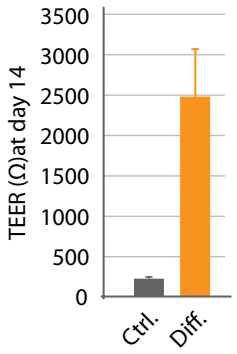
The immortalized human mammary epithelial cell line has been developed through genetic modification of primary human mammary epithelial cells, employing a dual-lentiviral approach using SV40 large T antigen (SV40LT) and Bm7e vectors for immortalization. SV40LT, derived from the Simian Virus 40 Large 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 mammary epithelial 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. Transepithelial Electrical Resistance (TEER) Measurement

Cells were seeded at 150,000 cells per insert (24-well format) on collagen-coated membranes and cultured in complete growth medium. After 48 hours, differentiation was induced using medium supplemented with 10% FBS. TEER was measured on days 3, 8, 10, and 14. Cells treated with differentiation medium exhibited a progressive increase in TEER, indicating the development of a tight epithelial barrier. A marked rise in resistance was observed from day 8 onward. In contrast, control cells maintained low TEER values throughout the assay, consistent with an undifferentiated phenotype. Calcium supplementation alone resulted in only a modest TEER increase (data not shown), reinforcing the role of serum in promoting epithelial integrity.





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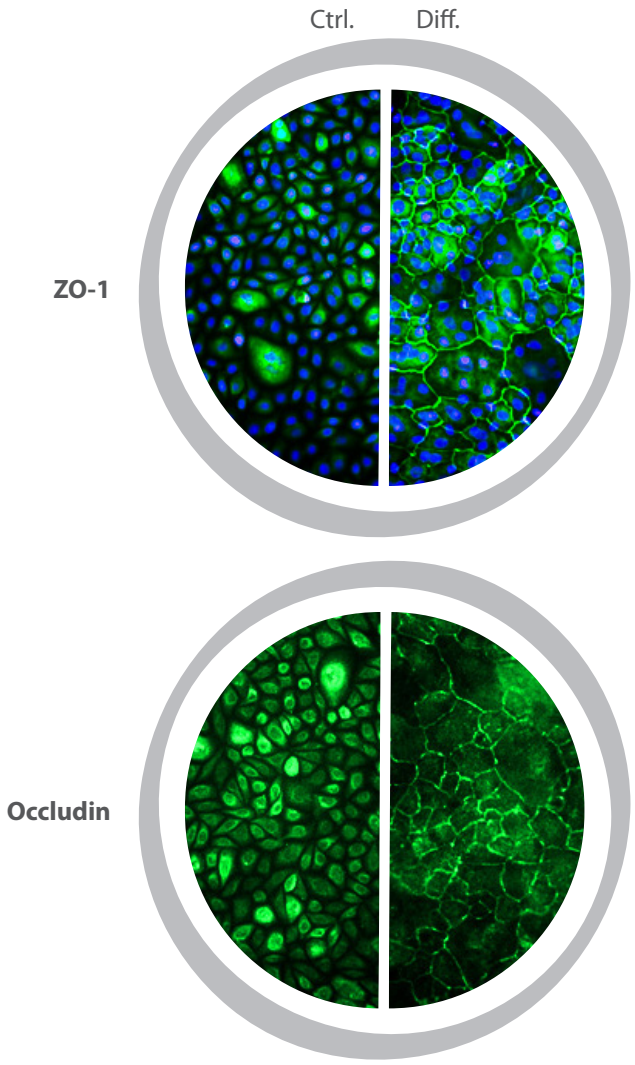
Functional Analysis

2. Morphological Evaluation and Epithelial Polarization

Cells were seeded at 30,000 cells per well in 96-well plates and cultured in standard epithelial growth medium. After 24 hours, half of the wells were switched to a differentiation medium consisting of alveolar basal medium supplemented with 1.5 mM $CaCl_2$ and 10% FBS. Cells were fixed at days 3 and 7 with 4% paraformaldehyde and processed for immunocytochemistry using tight junction markers ZO-1 and occludin. Differentiated cells showed increased expression of ZO-1 by day 3, and co-expression of ZO-1 and occludin by day 7, indicating the formation of a polarized, mature epithelium. Control cells cultured in low-calcium (60 µM) medium or in medium with calcium alone did not show comparable junctional marker expression, suggesting that serum is required to drive epithelial differentiation.



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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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