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cell based solutions

IMMORTALIZED CELL LINES

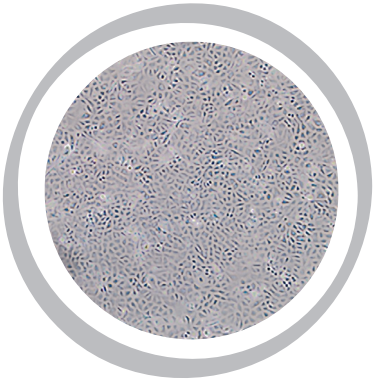
# P10851-IM

Keratinocytes are the predominant cell type in the epidermis, comprising approximately 85% of living epidermal cells. As the primary constituents of stratified squamous epithelium, keratinocytes originate in the basal layer of the epidermis, where they proliferate before undergoing a tightly regulated differentiation process. During their migration towards the surface, they progressively accumulate keratin and eventually undergo programmed cell death, forming the protective outermost layer of the skin.

Beyond their structural role, keratinocytes are key players in skin homeostasis, wound healing, carcinogenesis, and gene therapy approaches targeting the skin. They actively participate in innate immunity by expressing adhesion molecules and cytokines, contributing to epidermal defense and inflammatory responses.

## IMMORTALIZED HUMAN
# EPIDERMAL KERATINOCYTES

**Product Type:** Immortalized Human Epidermal Keratinocytes
**Catalog Number:** P10851-IM
**Immortalization:** SV40 Large T Antigen. G418 resistant
**Number of cells:** >1x10$^6$ cells (cryopreserved vials)
**Storage:** Liquid Nitrogen
**Recommended Medium:** Keratinocyte Medium (Reference: P60154)
**Product Characterization:** Immunofluorescence for CK-18, CK-19, Occludin and ZO-1



Phase-contrast

## About Immortalized Human Epidermal Keratinocytes

The Immortalized Human Epidermal Keratinocytes line has been developed through genetic modification of primary culture of human epidermal keratinocytes, employing the SV40LT protein as the immortalization method. The SV40LT protein, derived from the Simian Virus 40 Large T-antigen, has been introduced to confer immortality to the cells, allowing for an extended lifespan compared to primary cells that typically undergo senescence after a limited number of passages. The use of SV40LT for immortalization is a common technique in cell biology and allows for the establishment of cell lines with a more stable and prolonged growth capacity. This modification enables researchers to conduct long-term experiments and studies that require consistent cellular behavior over an extended period. Primary cells exhibit senescence following the 5th passage, whereas the SV40LT-transduced cells demonstrate a prolonged viability, extending beyond 20 passages.

***THIS PRODUCT IS FOR RESEARCH PURPOSES ONLY***
*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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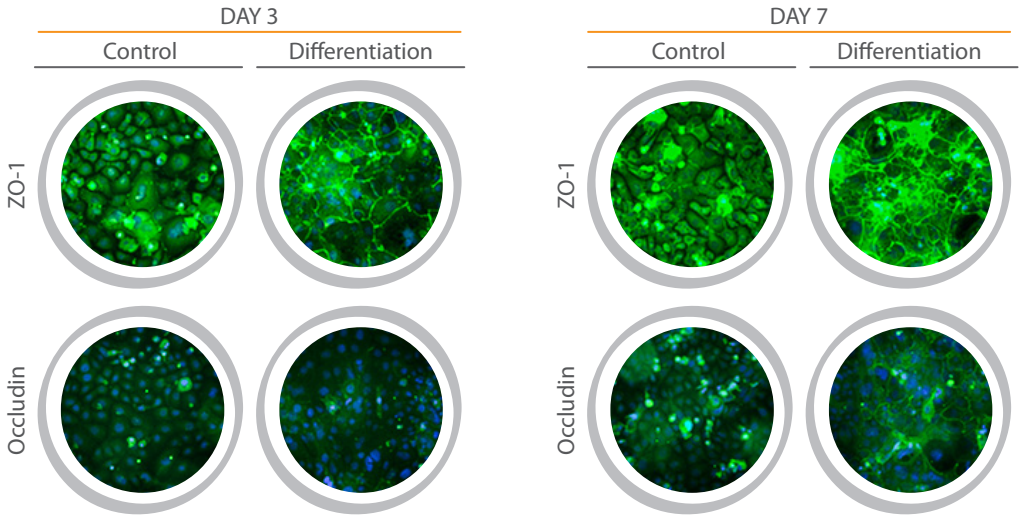
## Functional Analysis: Reconstruction of Primary Human Epidermis

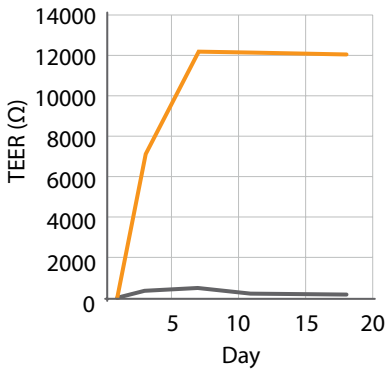
### 1. Immunohistochemical Analysis in Differentiated Cultures

Keratinocytes (30,000 cells/well, 96-well plate) are seeded in keratinocyte medium. The next day, the medium is replaced, and half of the wells receive differentiation medium (keratinocyte medium + 1.5 mM $CaCl_2$) for 3 or 7 days. Cells are then fixed with 4% paraformaldehyde and analyzed via immunohistochemistry for ZO-1 and occludin, markers of differentiated cells. Differentiation medium enhances maturation, forming a structured epithelium with increased intercellular junctions. By day 3, ZO-1 is detected, and by day 7, both ZO-1 and occludin are present.




### 2. Measurement of Transepithelial Electrical Resistance (TEER) in Differentiated Cultures

Keratinocytes (150,000 cells/insert, 24-well plate) are cultured on collagen-coated inserts with keratinocyte medium in both compartments. After 2 days, differentiation medium is added, and the apical medium is removed to establish an air-liquid interface. Medium is refreshed twice weekly. TEER (Ω) is measured on days 3, 7, 11, and 18. An increase in TEER is associated with a differentiated epithelial phenotype and the formation of a mature epithelium. By day 3, some wells reach maximal differentiation, consistent with published data, while undifferentiated controls show low TEER values. By day 7, all air-liquid interface cultures are fully differentiated, maintaining this state for at least 18 days. (Orange line: liquid interface, Blue line: air-liquid interface).

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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, 5 ml of Growth supplement and 5 ml of penicillin/streptomycin solution.

2.2 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.

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

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

2.5 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).

2.6 Place the flask in the incubator.

2.7 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 80% confluent.

### 4 SUBCULTURING:

Aspirate the culture medium completely from the flask, rinse the cell layer with PBS without $Ca^{2+}$/$Mg^{2+}$ to remove residual serum, and add enough Triplex solution (typically 1–2 mL for a T25 flask) to cover the cells. Incubate at 37 °C until cells detach, gently tapping the flask if needed. Inactivate Triplex by adding fresh culture medium to achieve at least a 1:5 dilution, then transfer the cell suspension to a centrifuge tube and centrifuge 5 minutes at 300 × g. Finally, aspirate the supernatant, resuspend the cell pellet in fresh medium, and seed into new culture vessels.

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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