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Technical Data Sheet: hTERT HCEC

ATCC® Number	CRL-4067™
Organism	*Homo sapiens*
Tissue/Disease Source	Eye; Cornea
Product Description	hTERT HCEC is a clonal cell line immortalized by stably expressing hTERT and CDK4R24C genes in primary human corneal epithelial cells. hTERT HCEC retains important corneal epithelial cell characteristics and functions such as expressing keratin 14 (KRT14), having migration ability, and can be used for in vitro ocular toxicity studies.
Application	This immortalized cell line is useful for studying eye diseases, high-throughput screening, and toxicology studies.

Cell Morphology

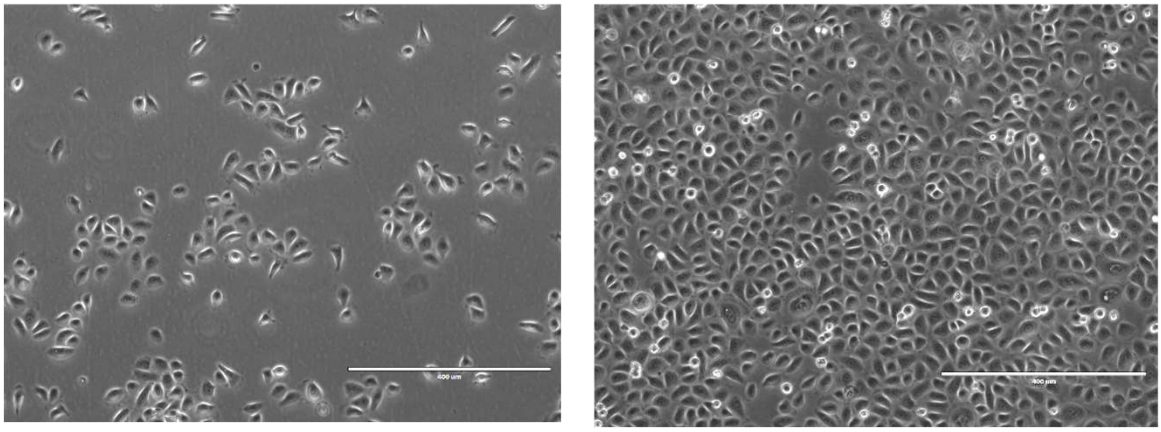

Figure 1. Cell morphology of hTERT HCEC. Cells were maintained in ATCC recommended culture conditions. Cell morphology in low (left) and high (right) densities were observed under microscopy and images were captured using a digital camera.

Marker expression

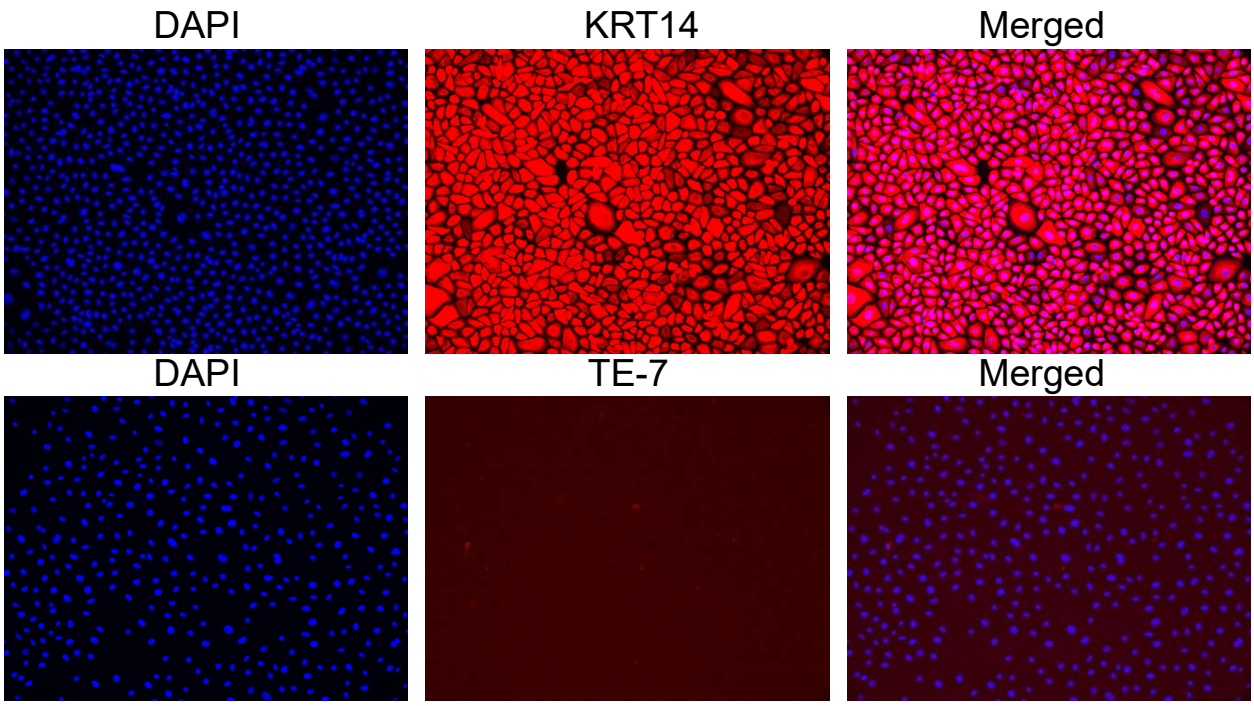

Figure 2: The hTERT HCEC cell line is positive for KRT14 and negative for TE-7. Cells were Fixed with 4% PFA and stained with Mouse Anti-Human KRT14 or Mouse Anti-Human TE-7 antibodies and then DAPI . Images were taken using a fluorescence microscope.

Wound healing assay

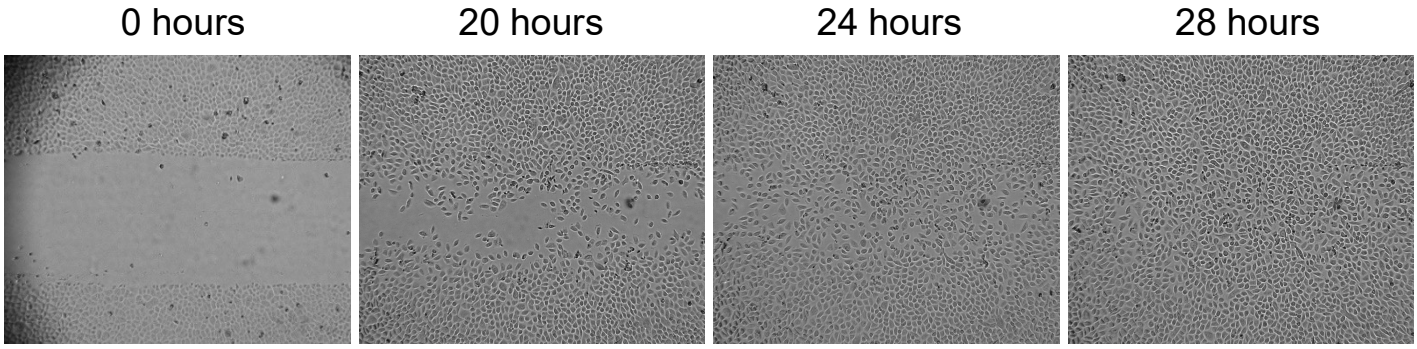

Figure 3 : hTERT HCEC in vitro wound healing assay. Representative images from wound healing assay at different time points (0, 20, 24, and 28 hours) during wound healing assay. Cells were plated into a 96 well plate and grown until confluent, then scratch wounds were made using an Incucyte Woundmaker Tool. Time-lapse phase contrast Images were taken with a BioTek BioSpa Live Cell Analysis System every 2 hours.

Short time exposure (STE) ocular toxicity test

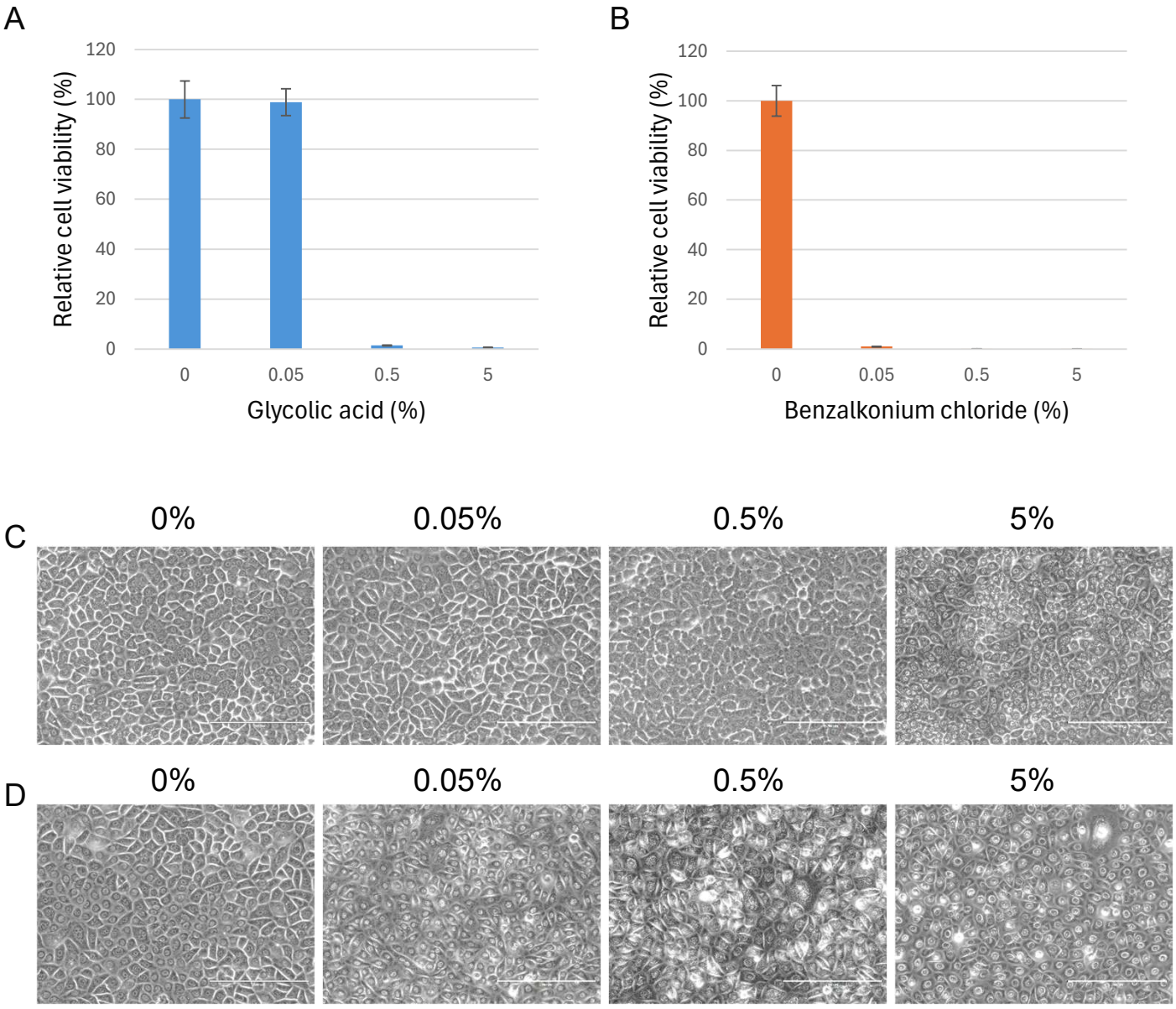

Figure 4. Toxicity of glycolic acid and benzalkonium chloride on hTERT HCEC cells. The cells were seeded in 96 well plates and grown for 24 hours, then the fully confluent cells were exposed for 5 minutes to 0, 0.05, 0.5, or 5% of glycolic acid in corneal epithelial cell basal medium (A, C) or 0, 0.05, 0.5, or 5% of benzalkonium chloride in corneal epithelial cell basal medium (B, D). The cell viability was assessed using CellTiter-Glo assay (A, B). Representative images of the cells treated with glycolic acid (C) or benzalkonium chloride (D) without adding CellTiter-Glo reagent.

Table 1: Prediction model of the STE test method[1]

Cell Viability at 5%	Cell Viability at 0.05%	UN GHS Classification
> 70%	> 70%	No Category
≤ 70%	>70%	No stand-alone prediction can be made
≤70%	≤70%	Category 1

[1] Short Time Exposure (STE) Test, NIH. Available at: https://ntp.niehs.nih.gov/go/41456

No Category: Chemicals which do not require classification for eye irritation or serious eye damage.

No stand-alone prediction can be made: The result of the STE test should be considered in the context of an IATA for classification purposes.

Category 1: Chemicals which induce serious eye damage.