

**Product Information Sheet**  
**Human HaCaT Keratinocyte Cells**  
Catalog Number: CR1017-500

Product Overview	
Product Name	Human HaCaT Keratinocyte Cells
Catalog #s	CR1017-500
Quantity	1 vial (approx. 500,000 cells)
Product Form	Frozen
Cell Type	Human Keratinocytes
Reagents Needed	Customer choice of high-grade or fully defined Fetal Bovine Serum (FBS) (not included) Penicillin/Streptomycin/Amphotericin B solution or Penicillin/Streptomycin solution, 100x (not included) <sup>1</sup>

Product Description
<p>This cell line was derived from an in-vitro spontaneously transformed keratinocyte cell from the histologically normal skin of a 62-year-old male Caucasian donor. The morphology is a keratinocyte; these cells typically grow as an adherent monolayer when grown in standard media.</p> <p>Keratinocyte cells are widely used in iPSC reprogramming workflows, cell therapy development, and the study of viral infections, oncogenesis, wound healing, and skin grafting applications. These cells have a high differentiation capacity.</p> <p>These cells exhibit robust growth characteristics in cell culture with fast doubling times and consistent morphology.</p> <p>Vial contains approximately 500,000 cells. Shipped with dry ice.</p> <p><small>Note: This product is designed and tested to function with Cellular Engineering Technologies Inc. ("CET") product MR1013 Human Keratinocyte Growth Media (not included). Although investigators are welcome to use this product with other media formulations, CET cannot and will not guarantee this product's performance. Additionally, such use of third-party media with this product will void CET's warranty should they not function as indicated. Please refer to CET's Terms &amp; Conditions, available at <a href="http://www.celleng-tech.com">www.celleng-tech.com</a>.</small></p>



Media Formulation Instructions (for MR1013 Human HaCaT Keratinocyte Growth Media <u>not</u> included)	
Defrosting / Preparation	Defrost 50mL of FBS (not included) and 5mL of antibiotic/antimycotic solution (not included) in a 37°C water bath until ice in the tubes is no longer visible. Immediately disinfect the tubes and the bottle containing this base media with 70% isopropanol (not included).
Mixing	Working in a laminar flow hood, remove 5mL of Human Keratinocyte Expansion Media (MR1013) (not included) from the bottle and discard. This and all other procedures must be done in a sterile manner. Add 50mL of FBS to the base media. Add 5mL of the antibiotic/antimycotic solution to the base media <sup>1</sup> . Cap the bottle containing the mixed liquid solution and gently swirl a few times. This formulated media is now considered complete media and ready to use with cells.

FOR RESEARCH APPLICATIONS ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

### Cell Thawing and Plating Instructions

<b>Thawing</b>	Remove the Human HaCaT Keratinocyte Cells vial from dry ice or a storage unit. Defrost the vial of cells in a 37°C water bath with constant, moderate agitation until ice in the ampoule is barely visible. DO NOT OVERTHAW. Immediately disinfect with 70% isopropanol (not included).
<b>Plating</b>	Working in a laminar flow hood, open the vial and transfer the contents to a sterile 15 mL tube. Very slowly, add approximately 10 mL of complete media (see Media Formulation Instructions), pre-warmed to 37°C before use. Centrifuge suspended cells at 200 x g for 10 minutes. Decant the medium and gently resuspend the pellet in the appropriate amount of complete media necessary to achieve a plating density of 20,000 cells/cm <sup>2</sup> of surface area. After 24 hours, aspirate media from the flask or dish, rinse with 1X Dulbecco's Phosphate Buffered Saline (not included), and replenish with fresh complete media, pre-warmed to 37°C before use.
<b>Observation/ Expansion</b>	It is normal for HaCaT keratinocyte cells to grow slowly initially for one-week post-thaw. It is also normal for some cells to be shed during media changes. Subculture cells at a 1:3 split ratio using 0.25% Trypsin/EDTA (not included).

### Storage and Stability

	Storage Temperature	Storage Time
<b>Human HaCaT Keratinocyte Cells</b>	-80°C (preferably in the vapor phase of a liquid nitrogen storage unit)	12 months
Human HaCaT Keratinocyte Growth Media (not included)	4°C	3 months
complete media <i>(see Media Formulation Instructions)</i>	2-8°C	Not applicable
<i>Avoid repeated freeze-thaw cycles for cells. Avoid repeated exposure to room temperature and light for media.</i>		

<sup>1</sup> These solutions should be portioned in 5mL aliquots, stored at -20C and never frozen/thawed. Although antimycotics are not necessary, CET highly recommends their usage for long-term cell culture. Antibiotics and antimycotics should not be used as an alternative to proper aseptic techniques.