

Product Information Sheet
Human HaCaT Keratinocyte Growth Media
Catalog Number: MR1013

Product Overview	
Product Name	Human HaCaT Keratinocyte Growth Media
Catalog #s	MR1013
Quantity	450 mL
Product Form	Liquid
Cell Type	Human HaCaT 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) ¹

Product Description
<p>Human HaCAT Keratinocyte Growth Media is designed to support robust keratinocyte growth and proliferation. When using this media, keratinocyte cells have been observed to retain cuboidal morphology and a 48-hour doubling time.</p> <p>Media is shipped with gel packs.</p> <p>Note: This product is designed and tested to function with Cellular Engineering Technologies Inc. ("CET") product CR1017-500 Human HaCaT Keratinocyte Cells (not included). Although investigators are welcome to use this product with other hepatocellular carcinoma cells, CET cannot and will not guarantee this product's performance. Additionally, using third-party cell lines with this product will void CET's warranty should they not function as indicated. Please refer to CET's Terms & Conditions, available on www.celleng-tech.com.</p>



Media Formulation Instructions	
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 the base media 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 ¹ . 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.

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Cell Thawing and Plating Instructions (for CR1017-500 Human HaCaT Keratinocyte Cells <u>not included</u>)	
Thawing	Remove the vial of Human HaCaT Keratinocyte Cells (CR1017-500) (not included) from dry ice. 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 the vial with 70% isopropanol (not included), ensuring no isopropanol enters the vial.
Plating	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 ² 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.
Observation/ Expansion	It is normal for HaCaT Keratinocyte cells to grow slowly initially for a period of 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
Human HaCaT Keratinocyte Growth Media	4°C	3 months
complete media (see Media Formulation Instructions)	2-8°C	Not applicable
<i>Avoid repeated exposure to room temperature and light.</i>		

¹ 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.