CELLULAR ENGINEERING TECHNOLOGIES

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Product Information Sheet

Human Alpha 1 Anti-Trypsin Deficiency iPSCs

Catalog Number: CR1014-500

Product Overview	
Product Name	**Human Alpha 1 Anti-Trypsin Deficiency iPS Cells**
Catalog #s	CR1014-500
Quantity	One vial (approx. 500,000 cells)
Product Form	Frozen
Cell Type	Disease Model iPSCs - Human Alpha 1 Anti-Trypsin Deficiency
Reagents Needed	Penicillin/Streptomycin/Amphotericin B solution or Penicillin/Streptomycin solution, 100x (not included)[1]

Product Description

Alpha-1 antitrypsin deficiency (A1AD or AATD) is a genetic disorder that may result in lung or liver disease due to a mutation in the SERPINA1 gene that results in shortness of breath, wheezing, or an increased risk of lung infection[2]. Serpin peptidase inhibitor, clade A, member 1 (SERPINA1) is the gene that encodes the protein alpha-1 antitrypsin (A1AT). A1AT is a glycoprotein mainly produced in the liver by hepatocytes. In a healthy lung, it functions as an inhibitor against neutrophil elastase, a neutral serine protease that controls lung elastolytic activity, which stimulates mucus secretion and CXCL8 release from epithelial cells that perpetuate the inflammatory state.

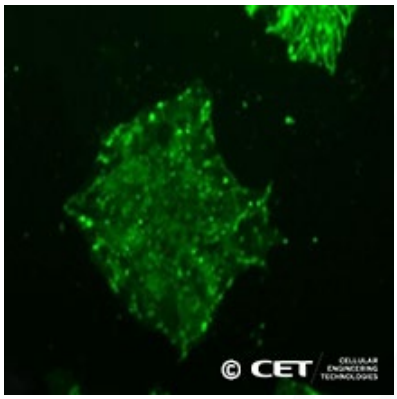
The cell line was derived from a 57-year-old female donor with two copies of the SERPINA1 gene that produces an abnormal type of Alpha-1 protein. Cells were reprogrammed to a pluripotent state using our patented method using non-integrating episomal DNA with our proprietary mix of transcription factors and small molecule chemistry. This delivers the safest clinical starting point with the lowest chance of insertional mutagenesis while delivering consistency, reprogramming efficiency, and flexibility.

We reprogram starting cells without the transcription factors *Myc* and *Lin28*, which are linked to neoplastic formation. This effectively lowers the clinical risk profiles of downstream differentiated cells.

Vial contains approximately 500,000 cells. Shipped with dry ice.

Note: This product is designed and tested to function with Cellular Engineering Technologies Inc. ("CET") product MR1001 Human iPS Cell 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 & Conditions, available at www.celleng-tech.com.

Cell Image



Cell Characteristics	
Growth Properties	Adherent
Donor Age	57-year-old
Ethnicity	Caucasian
Gender	Female
Gene, Chromosomal Location	SERPINA1, Chr 14: 94.38 – 94.39 Mb

Media Formulation Instructions (for MR1001 Human iPS Cell Growth Media not included)	
Defrosting / Preparation	Defrost the iPS Growth Supplement at 4°C (the day before the media is prepared) and 5 mL of antibiotic/antimycotic solution (not included) in a 37°C water bath until the ice in the tubes is no longer visible. Never defrost the iPS Growth

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	Supplement in a 37°C water bath. It is normal for the iPS Growth Supplement to appear hazy or have suspended solutes. Gently mix by inversion. Immediately disinfect the tubes and the bottle containing the iPS Base Media with 70% isopropanol (not included).
Mixing	Working in a laminar flow hood, remove 12mL of iPS Base Media (not included with cells) from the bottle and discard. This and all other procedures must be done in a sterile manner. Add the complete contents of the iPS Growth Supplement to the iPS Base Media. Add 5mL of the antibiotic/antimycotic solution to the iPS 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.

Cell Thawing and Plating Instructions	
Thawing	Before thawing the cells, substrate-coated dishes should be prepared accordingly. 30 minutes before thawing the iPS cells, the coating solution on the plates must be fully replaced with complete media (see Media Formulation Instructions) containing 5 uM Y-27632 (not included) and equilibrated to room temperature. Remove the Human Alpha 1 Anti-Trypsin Deficiency iPS 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).
Plating	Working in a laminar flow hood, open the vial and transfer the contents to a sterile 15 mL tube. Very slowly, add approximately 9 mL of complete media (see Media Formulation Instructions) containing 5 uM Y-27632, 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 6 mL of complete media containing 5 uM Y-27632. Do this gently to avoid shearing the colonies. Gently pipette the resuspended cells onto the previously coated dishes. One vial of iPS cells contains enough colonies to seed 6 wells of a standard 6-well tissue culture plate or 3-100 mm tissue culture dishes. Distribute the colonies evenly and gently rock the plate back and forth. Place the dish in an incubator at 37°C, 5% CO2, and 95% humidity. After 24 hours, aspirate media from the dish and replenish with fresh complete media (WITHOUT 5uM Y-27632), pre-warmed to 37°C before use. Repeat media changes every 24 hours.
Observation/ Expansion	The cells should attach over a period of 24 hours. It is normal for these cells to grow slowly initially for one-week post-thaw and for some colonies to be shed during media changes. Subculture cells at a 1:6 split ratio using Versene (not included).

Storage and Stability		
	Storage Temperature	**Storage Time**
Human Alpha 1 Anti-Trypsin Deficiency iPS Cells	-80°C (preferably in the vapor phase of a liquid nitrogen storage unit)	12 months
Human iPS Cell Growth Media (not included)	4°C	3 months
complete media *(see Media Formulation Instructions)*	2-8°C	Not applicable
Avoid repeated freeze-thaw cycles for cells. Avoid repeated exposure to room temperature and light for media.		

[1] These solutions should be portioned in 5mL aliquots, stored at -20°C, and never frozen/thawed. Although antimycotics are unnecessary, CET highly recommends their usage for long-term cell culture. Antibiotics and antimycotics should not be used as an alternative to proper aseptic techniques.
[2] Genetics Home Reference. January 2013. Retrieved 12 December 2017.

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