CELLULAR ENGINEERING TECHNOLOGIES

Version Date: 3/2/24

Product Information Sheet

Human Foreskin Fibroblast iPS Cells

Catalog Number: CR1001-500

Product Overview	
Product Name	**Human Foreskin Fibroblast iPS Cells**
Catalog #s	CR1001-500
Quantity	1 vial (approx. 500,000 cells)
Product Form	Frozen
Cell Type	Human Induced Pluripotent Stem Cells
Reagents Needed	Penicillin/Streptomycin/Amphotericin B solution or Penicillin/Streptomycin solution, 100x (not included)[1]

Product Description

Fibroblast cells were isolated from human neonatal foreskin and subsequently reprogrammed with our patented episomal, virus-free method using a proprietary mix of vectors, excluding *L-Myc*, *c-Myc*, and *Lin28* transcription factors.

The cell line was validated for pluripotency based on colony morphology, alkaline phosphatase staining, and expression of SSEA-4. Cells are free of Mycoplasma and exhibit classical iPS colony morphology and growth characteristics.

Fibroblast cells can be differentiated into neural and epithelial progenitor cells for further use in cell therapy, regenerative medicine, wound healing, and tissue engineering research applications.

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.

Product Image

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 5mL of antibiotic/antimycotic solution (not included) in a 37°C water bath until ice in the tubes is no longer visible. Never defrost the iPS Growth 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.
Feeding	CET recommends that cells should be fed with fresh, complete media every 24 hours, and old media should be discarded before complete media is added.

CELLULAR ENGINEERING TECHNOLOGIES, INC.
2500 Crosspark Road, Suite E110 Coralville, IA 52241
T: (319) 665-3000 F: (319) 665-3003
Email: support@celleng-tech.com
www.celleng-tech.com

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 Foreskin Fibroblast 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% CO_2 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 Foreskin Fibroblast iPS 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 Foreskin Fibroblast 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)*	4°C	14 days
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 -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.

CELLULAR ENGINEERING TECHNOLOGIES, INC.
2500 Crosspark Road, Suite E110 Coralville, IA 52241
T: (319) 665-3000 F: (319) 665-3003
Email: support@celleng-tech.com
www.celleng-tech.com