CELLULAR ENGINEERING TECHNOLOGIES

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Product Summary

Human Foreskin Fibroblast Induced Pluripotent Stem Cells (iPSCs)

Catalog Number: CR1001-500

Product Overview	
Product Name	**Human Foreskin Fibroblast iPSCs**
Catalog #s	CR1001-500
Quantity	1 vial (approx. 500,000 cells)
Product Form	Frozen
Cell Type	Human Induced Pluripotent Stem Cells
Reagents Needed	– **Antibiotic** - Penicillin/Streptomycin/Amphotericin B solution or Penicillin/Streptomycin solution, 100x (not included)[1] – **Basement membrane matrix** suitable for adherent cells – based on customer preference, we recommend using Geltrex™ hESC-Qualified Ready-to-Use, Reduced Growth Factor Basement Membrane Matrix manufactured by Thermo Fisher Cat. A1569601) – 70% isopropanol solution – **ROCK Inhibitor** Y-27632 (Dihydrochloride) – based on customer preference – **Cell disassociation reagent** – based on customer preference, we recommend using Gibco™ Versene Solution (Cat. 15040066) or STEMCELL Technologies Gentle Cell Disassociation Reagent (Cat. 100-0485)

Product Description

Fibroblast cells were isolated from the human neonatal foreskin and 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.cet.bio.

Product Image

Media Formulation Instructions (for MR1001-K Human iPSC Growth Media Kit not included)	
Defrosting the iPSC Growth Supplement	1. Defrost the iPSC 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 iPSC Growth Supplement in a 37°C water bath. It is normal for the iPSC Growth Supplement to appear hazy or have suspended solutes. Gently mix by inversion. 2. Immediately disinfect the tubes and the bottle containing the iPSC Growth Base Media with 70% isopropanol (not included).
Mixing	1. Working in a laminar flow hood, remove 12mL of iPSC Growth Base Media (not included with cells) from the bottle and discard. This and all other procedures must be done in a sterile manner. 2. Add the complete contents of the iPSC Growth Supplement to the iPSC Growth Base Media. Add 5mL of the antibiotic/antimycotic solution to the iPSC Growth Base Media[1].

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

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	3. 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	
Cell thawing	1. Before thawing the cells, substrate-coated dishes should be prepared accordingly. 2. Thirty (30) minutes before thawing the iPS cells, the coating solution on the plates must be entirely replaced with complete media (see Media Formulation Instructions) containing five (5) uM ROCK Inhibitor Y-27632 (not included) and equilibrated to room temperature. 3. Remove the Human Foreskin Fibroblast iPSCs vial from the dry ice or a storage unit. 4. 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. 5. Immediately disinfect with 70% isopropanol (not included).
Cell plating	1. Working in a laminar flow hood, open the vial and transfer the contents to a sterile fifteen (15) mL tube. 2. Very slowly, add approximately nine (9) mL of complete media (see Media Formulation Instructions) containing five (5) uM ROCK Inhibitor Y-27632, pre-warmed to 37°C before use. 3. Centrifuge suspended cells at 200 x g for 10 minutes. 4. Decant the medium and gently resuspend the pellet in 6 mL of complete media containing 5 uM ROCK Inhibitor Y-27632. Do this gently to avoid shearing the colonies. 5. Gently pipette the resuspended cells onto the previously coated dishes. One vial of iPSCs contains enough colonies to seed six (6) wells of a standard six (6)-well tissue culture plate or three (3)- one-hundred (100) mm tissue culture dishes. 6. 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. 7. After 24 hours, aspirate media from the dish and replenish with fresh complete media (WITHOUT 5uM ROCK Inhibitor Y-27632), pre-warmed to 37°C before use. 8. Repeat media changes every 24 hours.
Observation and expansion	– The cells should attach over 24 hours. It is normal for these cells to grow slowly initially for one week after thawing and for some colonies to be shed during media changes. – Subculture cells at a 1:6 split ratio using cell disassociation reagent (not included).
Feeding	– CET recommends feeding cells fresh, completing media every 24 hours, and discarding old media before adding new media.

Storage and Stability		
	Storage Temperature	**Storage Time**
Human Foreskin Fibroblast iPSCs Cat. CR1001	Upon arrival, place the cells at a temperature below -130°C, preferably in liquid nitrogen vapor, until ready for use	12 months
Human iPSC Growth Media Kit (not included) Cat. MR1003-K		
iPSC Growth Base Media	4°C	3 months
iPSC Growth Supplement	-20°C	Not applicable (use entire contents)
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.		

Publications and Product Citations

Development of a High-Efficacy Reprogramming Method for Generating Human Induced Pluripotent Stem (iPS) Cells from Pathologic and Senescent Somatic Cells

Tanaka, N et al. | International Journal of Molecular Sciences 2020 SEP
Department of Cardiology, Saitama Medical University
Department of Preventive Medicine and Public Health, **Keio University School of Medicine**.

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Fibroblasts-dependent invasion of podoplanin-positive cancer stem cells in squamous cell carcinoma Miyashita, T et al. | Journal of Cellular Physiology 2020 FEB Department of Integrated Biosciences, Laboratory of Cancer Biology, **The University of Tokyo**.
Spatiotemporal characteristics of fibroblasts-dependent cancer cell invasion Miyashita, T et al. | Journal of Cancer Research and Clinical Oncology 2018 NOV Department of Integrated Biosciences, Laboratory of Cancer Biology, **The University of Tokyo**.
Cell Injury-Induced Release of Fibroblast Growth Factor 2: Relevance to Intracerebral Mesenchymal Stromal Cell Transplantations Aizman, I et al. | Stem Cells and Development 2015 JUL Departments of Research and Production Development, **SanBio, Inc**.
Human dendritic cells transfected with a human papilloma virus-18 construct display decreased mobility and upregulated cytokine production Khaiboullina, SF et al. | International Journal of Oncology 2013 NOV Department of Biochemistry and Molecular Biology, **University of Nevada** Human Physiology, **Vrije Universiteit Brussel** Department of Genetics, Institute of Fundamental Medicine and Biology, **Kazan (Volga Region) Federal University**.

[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.

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