CELLULAR ENGINEERING TECHNOLOGIES

Version Date: 1/11/25

Product Summary

Human Alzheimer's Presenilin-1 Mutation iPSCs

Catalog Number: CR1008-500

Product Overview	
Product Name	**Human Alzheimer's Presenilin-1 Mutation iPSCs**
Catalog #s	CR1008-500
Quantity	One vial (approx. 500,000 cells)
Product Form	Frozen
Cell Type	Disease Model iPSCs - Alzheimer's Presenilin-1 Mutation
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

Mutations in the PSEN1 gene, encoding presenilin-1 (PS1), are the most common cause of familial Alzheimer's disease (FAD). PS1 functions as the catalytic subunit of γ-secretase, an intramembranous protease that cleaves various type 1 transmembrane proteins, notably including the amyloid precursor protein (APP) and Notch. Following prior cleavage by β-secretase, processing of APP by γ-secretase generates β-amyloid (Aβ) peptides of varying lengths. Whereas Aβ40 accounts for ~90% of Aβ production, the minor Aβ42 product is more hydrophobic and is thought to nucleate Aβ aggregation, leading to amyloid plaque deposition in the AD brain[2].

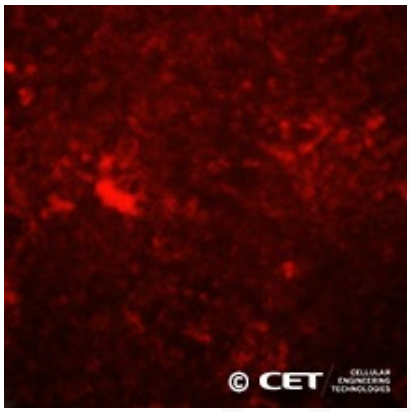
Primary donor fibroblast cells were collected from a 70-year-old male of Caucasian descent diagnosed with early-onset Alzheimer's disease (EOAD). Cells were reprogrammed to a pluripotent state using our patented method using non-integrating episomal DNA with our proprietary mix of transcription factors 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.

Cryopreserved 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.

Cell Image



Cell Characteristics	
Growth Properties	Adherent
Donor Age	70-year-old
Ethnicity	Caucasian

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

Gender	Male
Gene, Gene Mutation, Chromosomal Location	*PSEN1*, GC14P071108, (GC14P071108), Chr 14q24.2: 73.14 – 73.22 Mb

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]. 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 Alzheimer's Presenilin-1 Mutation iPS Cells 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).

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Storage and Stability		
	Storage Temperature	**Storage Time**
Human Alzheimer's Presenilin-1 Mutation iPSCs Cat. CR1008	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)*	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] Zhang S. et al. A presenilin-1 mutation causes Alzheimer's disease without affecting Notch signaling | Molecular Psychiatry 25, 603-613 (2020).

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