Human iPSC Line, SCTi003-A-2, APP K670N/M671L (Swedish Mutation)

Human pluripotent stem cell line, frozen, CRISPR-edited from SCTi003-A, for familial Alzheimer's disease research

Catalog #200-0991 ~1 x 10^6 cells/vial

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Product Description

Genome-edited Human iPSC Line, SCTi003-A-2, APP K670N/M671L (Swedish Mutation), referred to as SCTi003-A-2, was generated by CRISPR/Cas9 technology, which introduced the amyloid precursor protein (APP) K670N/M671L double mutation (also known as the Swedish mutation, c.2026A>G & c.2032T>A) into the endogenous APP locus. The Swedish mutation is a well-characterized familial Alzheimer's disease mutation associated with increased β-secretase cleavage of APP, leading to an elevated production of amyloid-β peptides and early-onset Alzheimer's pathology (Sasaguri et al.; Thordardottir & Graff).

This product's parental line, Healthy Control Human iPSC Line, Female, SCTi003-A, is a well-characterized control line derived from peripheral blood mononuclear cells (PBMCs) from a 48-year-old donor. Targeted gene modifications were confirmed by Sanger sequencing. Post-editing, extensive quality control procedures were undertaken in the manufacturing process for SCTi003-A-2 to ensure optimal product performance and reproducibility. SCTi003-A-2 is karyotypically stable, expresses markers of the undifferentiated state, and remains capable of directed differentiation into all three germ layers, including neural lineage cells relevant for Alzheimer's disease research. This genome-edited hiPSC line, along with its parental hiPSC line, provides a robust, isogenic disease modeling tool for studying amyloidogenic processing, studying Alzheimer's disease mechanisms, and evaluating therapeutic interventions targeting the APP pathway.

SCTi003-A-2 is manufactured with mTeSR™ Plus (Catalog #100-0276) and is compatible with STEMdiff™ cell culture media products, allowing for standardized high-quality maintenance and differentiation to various cell types, such as neurons, astrocytes, and microglia.

Cells were obtained using Institutional Review Board (IRB)-approved consent forms and protocols.

Stability and Storage

Cells are frozen in a cryopreservation medium containing dimethyl sulfoxide (DMSO). Product stable at -135°C or colder for 12 months from date of receipt. Thawed samples must be used immediately.

Precautions

Cell Screening: hiPSC master cell banks are screened for AAV2, BK virus, Epstein-Barr Virus, Hepatitis A, Hepatitis B, Hepatitis C, Herpes Simplex 1 and 2, Herpes Virus Type 6, Herpes Virus Type 7, Herpes Virus Type 8, HIV-1, HIV-2, HPV-16, HPV-18, Human Adenovirus, Human Cytomegalovirus, Human Foamy Virus, Human T-Lymphotropic Virus, John Cunningham Virus, LCMV, Parvovirus B19, Sarbecovirus (SARS Virus), Seoul Virus, Corynebacterium Bovis, and Mycoplasma (Human Comprehensive CLEAR Panel) by PCR. As testing cannot completely guarantee that the donor was virus-free, THIS PRODUCT SHOULD BE TREATED AS POTENTIALLY INFECTIOUS and only used following appropriate handling precautions such as those described in biological safety level 2.

Storage of frozen cell products in the vapor phase of a liquid nitrogen storage tank is recommended. Storage in the liquid phase can result in cross-contamination if the vial breaks or is not sealed properly. Storage in the liquid phase also increases the potential for liquid nitrogen to penetrate the vial and cause it to explode when removed from storage. Use of a face shield is required as a safety precaution when transferring cells from one container to another. When handling this product, do not use sharps such as needles and syringes.

STEMCELL cannot guarantee the biological function or any other properties associated with performance of cells in a researcher's individual assay or culture systems. STEMCELL assures the cells will meet the specifications only when assessed immediately after thawing by our test methods.

FOR IN VITRO RESEARCH USE ONLY. NOT APPROVED FOR DIAGNOSTIC, THERAPEUTIC, OR CLINICAL APPLICATIONS.

Donor Information

For donor information, refer to the Product Information Sheet (PIS) for the parental hiPSC line, Healthy Control Human iPSC Line, Female, SCTi003-A (Document #10000014504), available at www.stemcell.com, or contact us to request a copy.

Directions for Use

SCTi003-A-2 was manufactured using mTeSR™ Plus, and the recommended thawing medium is mTeSR™ Plus. Thawed cells should be seeded into tissue culture-treated cultureware pre-coated with Corning® Matrigel® hESC-Qualified Matrix. For instructions on preparing complete mTeSR™ Plus and coated cultureware, refer to the Technical Manual: Maintenance of Human Pluripotent Stem Cells in mTeSR™ Plus (Document #10000007757), available at www.stemcell.com, or contact us to request a copy.

NOTE: The following instructions are for seeding cells into coated 6-well plates. If using other cultureware, adjust volumes accordingly.

1. Have all tubes, warmed mTeSR™ Plus (15 - 25°C), and coated cultureware ready before starting the protocol to ensure that the thawing procedure is completed as quickly as possible.

   NOTE: Do not warm mTeSR™ Plus in a 37°C water bath.
2. Wipe the outside of the vial of cells with 70% ethanol or isopropanol.
3. In a biosafety cabinet, twist the cap a quarter-turn to relieve internal pressure, then retighten.
4. Quickly thaw the cells in a 37°C water bath by gently shaking the vial. Remove the vial when a small frozen cell pellet remains. Do not vortex the cells.

   NOTE: ThawSTAR® CFT2 Automated Thawing System may be used instead of a water bath for optimal thawing in a sterile and controlled manner. For complete instructions, refer to the PIS (Document #10000010334), available at www.stemcell.com, or contact us to request a copy.
5. Wipe the outside of the vial with 70% ethanol or isopropanol.
6. In a biosafety cabinet, use a 2 mL serological pipette to transfer the contents of the cryovial to a 15 mL conical tube.

   NOTE: Using a 2 mL serological pipette instead of a 1 mL pipettor will minimize breakage of cell aggregates.
7. Add 5 - 7 mL of warm mTeSR™ Plus dropwise to the 15 mL tube, gently mixing as the medium is added.
8. Centrifuge the cells at 300 x g for 5 minutes at room temperature.
9. Aspirate the medium, leaving the cell pellet intact. Resuspend the cell pellet in 1 mL of mTeSR™ Plus by gently flicking the tube. Avoid pipetting up and down and take care to maintain the cells as aggregates.
10. When cells are ready to be plated, aspirate the Corning® Matrigel® solution from a coated 6-well plate and add 2 mL of mTeSR™ Plus to each well.
11. Aliquot the 1 mL cell suspension into a coated 6-well plate containing 2 mL of mTeSR™ Plus at six different densities: (1) 150 µL, (2) 100 µL, (3) 75 µL, (4) 50 µL, (5) 25 µL, and (6) 15 µL.

    NOTE: Gently flick the tube as many times as needed to ensure equal distribution of the cell aggregates between the wells.

    NOTE: The remaining 585 µL of cell suspension can be seeded into two wells of a separate 6-well plate to initiate backup cultures.
12. Place the plates in a 37°C and 5% CO_2 incubator. Move the plate in several quick, short, back-and-forth and side-to-side motions to distribute the cell aggregates. Do not disturb the plate for 24 hours.

    NOTE: Uneven distribution of aggregates may result in increased differentiation of hiPSCs.
13. Perform medium changes as desired using mTeSR™ Plus and visually assess cultures daily to monitor growth and morphology. See Figure 1 for expected growth characteristics during the first seven days after thawing. Medium can be changed daily, every other day, or up to two consecutive days of feeding can be skipped when using mTeSR™ Plus. To skip two consecutive days of feeding, add twice the volume of medium.
14. On Day 6 - 8, select the well with optimal hiPSC colony density for passaging (Figure 2). The culture should consist of healthy hiPSC colonies that are medium to large, compact, and have centers that are dense compared to their edges.
15. Use a microscope to visually identify regions of differentiation. Mark these using a felt tip or lens marker on the bottom of the plate. Remove regions of differentiation by scraping with a pipette tip.

    NOTE: Removing regions of differentiated cells is optional in subsequent passages.
16. Wash each well with 1 mL of D-PBS (Without Ca++ and Mg++). Aspirate to remove D-PBS from the wells.

17. Passage hiPSCs from the optimal well using ReLeSR™. Add 1 mL of ReLeSR™ to each well and aspirate to completely remove ReLeSR™ immediately or within 1 minute (if working with multiple wells), so that colonies are exposed to only the residual liquid. For SCTi003-A-2, incubate the culture at room temperature for 4 - 6 minutes and split at a ratio of 1 in 30 to 1 in 60 every 6 - 8 days.

    NOTE: When incubating cells with ReLeSR™, monitor cell detachment under the microscope. To prevent differentiated regions from lifting off the surface of the well, determine the optimal incubation time for this cell line in your own laboratory.

    NOTE: If the colonies are too dense or too sparse, adjust the split ratio accordingly at the next time of passaging. For complete instructions on passaging hiPSCs cultured in mTeSR™ Plus using ReLeSR™, refer to the Technical Manual: Maintenance of Human Pluripotent Stem Cells in mTeSR™ Plus (Document #10000007757, see section 5.1), available at www.stemcell.com, or contact us to request a copy.

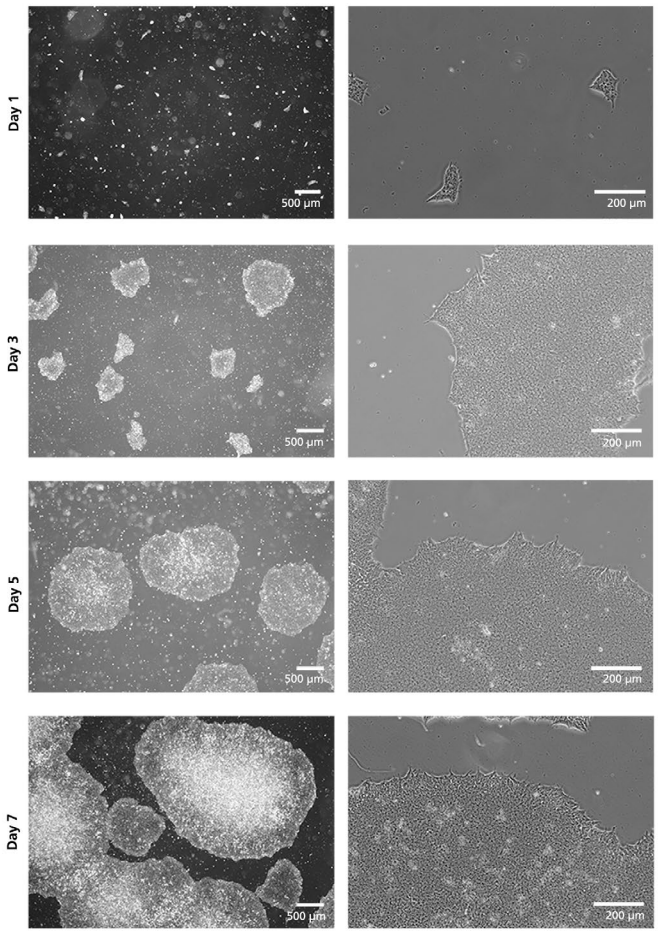

Figure 1. Recovery of SCTi003-A-2 hiPSCs on Days 1 - 7 After Thaw

SCTi003-A-2 hiPSCs were recovered in mTeSR™ Plus on Corning® Matrigel® hESC-Qualified Matrix and imaged at a magnification of 20X (left) and 100X (right) for seven days. For this concentration of cellular aggregates at thaw, Day 7 would be the optimal time for passaging.

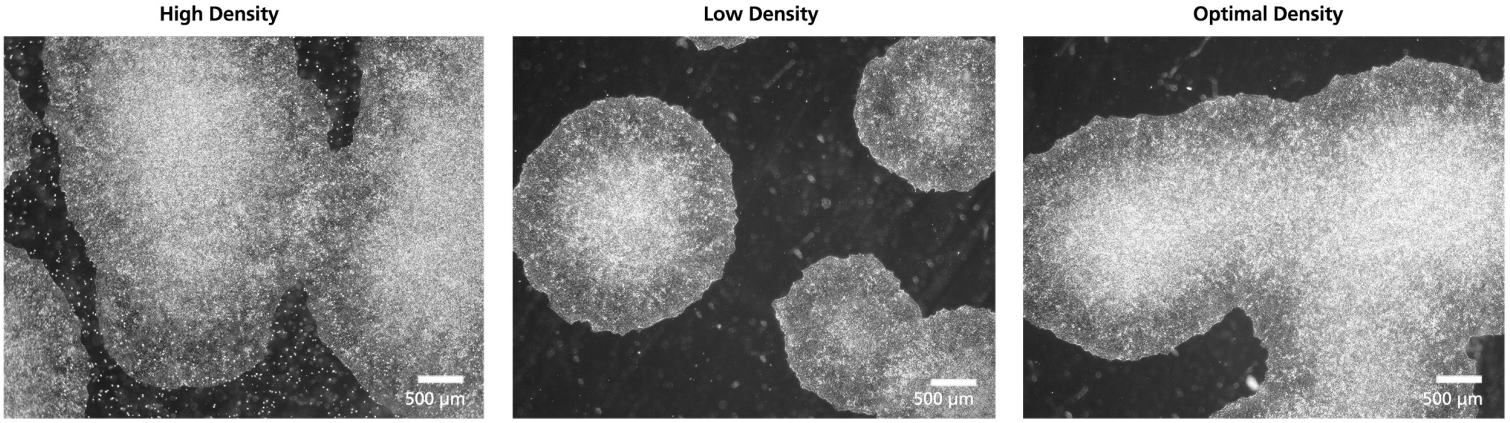

Figure 2. SCTi003-A-2 hiPSCs Demonstrate Varying Colony Densities When Seeded at a Range of Starting Concentrations

SCTi003-A-2 hiPSCs were thawed at a range of concentrations and expanded in mTeSR™ Plus for seven days. Final colony densities were imaged at a magnification of 20X. It is recommended that a culture is passaged once it has reached an optimal density consisting of medium-to-large, multilayered colonies that have begun to merge.

Notes and Tips

The ideal mean cell aggregate size obtained after step 17 of the Directions for Use is approximately 50 - 200 µm.

Accessory Products

PRODUCT NAME	CATALOG #
Anti-Human OCT4 (OCT3) Antibody, Clone 3A2A20	60093
Anti-Human TRA-1-60 Antibody, Clone TRA-1-60R	60064
CloneR™2	100-0691
Conical tubes, 15 mL	e.g. 38009
CryoStor® CS10	07930
D-PBS (Without Ca++ and Mg++)	37350
Falcon® 6-Well Flat-Bottom Plate, Tissue Culture-Treated	38016
hPSC Genetic Analysis Kit	07550
Human Pluripotent Stem Cell Trilineage Differentiation qPCR Array	07515
mTeSR™ Plus	100-0276
ReLeSR™	100-0483
Serological pipettes, 2 mL	e.g. 38002
STEMdiff™ Trilineage Differentiation Kit	05230
ThawSTAR® CFT2 Automated Thawing System	100-0650
Trypan Blue	07050

References

Sasaguri H et al. (2022) Recent Advances in the Modeling of Alzheimer's Disease. Front Neurosci 16:807473.

Thordardottir S & Graff C. (2018) Findings from the Swedish Study on Familial Alzheimer's Disease Including the APP Swedish Double Mutation. J Alzheimers Dis. 64(s1):S491–6.