SNCA3x_0KO_C1

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Description

SNCA3x_0KO_C1 is a CRISPR-engineered induced pluripotent stem cell (iPSC) with SNCA frameshift mutations from a donor with an SNCA triplication. This cell line is part of a panel of cells that can be used to study alpha-synuclein gene expression dosage from the endogenous locus and has applications in Parkinson's disease research.

Organism: *Homo sapiens*, human
Tissue: Skin
Age: 42 years
Gender: Male
Growth properties: Adherent
Disease: Parkinsons disease
Cells per vial: ≥ 1.0 x 10^6
Volume: 1.0 mL

Storage Conditions

Product format: Frozen
Storage conditions: Vapor phase of liquid nitrogen

Intended Use

This product is intended for laboratory research use only. It is not intended for any animal or human therapeutic use, any human or animal consumption, or any diagnostic use.

BSL 2

ATCC determines the biosafety level of a material based on our risk assessment as guided by the current edition of *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, U.S. Department of Health and Human Services. It is your responsibility to understand the hazards associated with the material per your organization's policies and procedures as well as any other applicable regulations as enforced by your local or national agencies.

ATCC highly recommends that appropriate personal protective equipment is always used when handling vials. For cultures that require storage in liquid nitrogen, it is important to note that some vials may leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vial exploding or blowing off its cap with dangerous force creating flying debris. Unless necessary, ATCC recommends that these cultures be stored in the vapor phase of liquid nitrogen rather than submersed in liquid nitrogen.

Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at www.atcc.org.

Growth Conditions

Temperature: 37°C
Atmosphere: 95% Air, 5% CO_2

Handling Procedures

Complete medium:

The basal medium for this cell line is StemFlex™ Basal Medium. To make the complete medium add the following to 450 mL basal medium:

- 50 mL StemFlex™ Supplement 10X
- 1 µM of Thiazovivin: 1 mM Thiazovivin/DMSO solution - prepared using 1 mg Thiazovivin/ 3.1212 ml DMSO

Note: Thiazovivin/DMSO solution is used only at Start-Up and Day 0 of all subcultures and removed next day - substituted with StemFlex™ Basal Medium.

Handling Procedure:

To ensure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at -70°C. Storage at -70°C will result in loss of viability.

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).
2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. All of the operations from this point on should be carried out under strict aseptic conditions.
3. Transfer the vial contents to a centrifuge tube containing 9.0 mL complete culture medium. and spin at approximately 125 x *g* for 5 to 7 minutes.
4. Resuspend cell pellet with the recommended complete medium (see the specific batch information for the culture recommended dilution ratio). It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the complete growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6). pH (7.0 to 7.6).
5. Incubate the culture at 37°C in a suitable incubator. A 5% CO_2 in air atmosphere is recommended if using the medium described on this product sheet.

Subculturing procedure:

Volumes used in this protocol are for 75 cm^2 flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes. Corning® T-75 flasks (catalog #430641) are recommended for subculturing this product.

Notes: CBM coated culture vessels used. Do NOT use CellSTACKs Note on dissociation medium

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with PBS.
3. Add 2.0 to 3.0 mL of 0.5mM EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting.
5. **Centrifuge cells to remove dissociation agent and resuspend in complete media .**
6. Add appropriate aliquots of the cell suspension to new CBM coated culture vessels.
   Cultures can be established between 2 x 10^4 and 1 x 10^5 viable cells/cm^2. Do not exceed 7 x 10^4 cells/cm^2.
7. Incubate cultures at 37°C.

Interval: Maintain cultures at a cell concentration between 1.5 X 10^4 and 6 X 10^4 cell/cm^2.

Subcultivation Ratio: A subcultivation ratio of 1:3 to 1:8 is recommended

Medium Renewal: 2 to 3 times per week

Notes: Prepare in advance CBM coated cell culture flasks prior to subculturing. Media changes must be performed every other day. Subculture at ≤ 70% confluence. For all startups dilute 1 mL of cell vial with 4 mL of complete medium and perform cell counts prior to centrifugation.

Reagents for cryopreservation: BAMBANKER™ Serum Free Cell Freezing Medium (Fisher Scientific catalog # NC9582225)

Material Citation

If use of this material results in a scientific publication, please cite the material in the

following manner: SNCA3x_0KO_C1 (ATCC SNCA3x_0KO_C1)

References

References and other information relating to this material are available at www.atcc.org.

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