Neural Progenitor Cells Derived from ATCC-DYS0530 Parkinson's Disease

ACS-5001™

Description

Organism: *Homo sapiens*, human
Cell Type: neural progenitor cell
Tissue: Skin; Dermis
Age: 63 years
Gender: Male
Morphology: short spindle shape
Growth properties: Adherent
Disease: Parkinsons disease
Cells per vial: 1.0 x 10^6
Volume: 1.0 mL

Storage Conditions

Product format: Frozen

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.

Astrocyte, oligodendrocyte, and neuron differentiation; drug screening

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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.

Cells contain Sendai virus (SeV) DNA sequences

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

Seeding density: Post-thaw: 4.0 x 10^4 viable cells/cm^2 on Cell Basement Membrane Gel-coated dishes/
Subculture: 4.0 x 10^4 viable cells/cm^2 on Cell Basement Membrane Gel-coated

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dishes/plates

Handling Procedures

Unpacking and storage instructions:

1. Check all containers for leakage or breakage.
2. Remove the frozen cells from the dry ice packaging and immediately place the cells at a temperature below -130°C, preferably in liquid nitrogen vapor, until ready for use.

Complete medium: Complete growth media for Neural Progenitor Cells (NPCs) includes DMEM: F12 (ATCC 30-2006) supplemented with the Growth Kit for Neural Progenitor Cell Expansion (ATCC ACS-3003). To make complete NPC medium add the following components of the kit to 464 mL DMEM: F12:

- 5 mL L-Alanyl-L-Glutamine
- 5 mL Non-Essential Amino Acids
- 10 mL NPC Growth Kit Component A
- 5 mL NPC Growth Kit Component B
- 1 mL NPC Growth Kit Component C
- 10 mL NPC Growth Kit Component D

Complete dopaminergic differentiation medium for NPCs is DMEM: F12 (ATCC 30-2006); supplemented with the Neural Progenitor Cell Dopaminergic Differentiation Kit (ATCC ACS-3004). To make complete NPC dopaminergic differentiation medium add the following components of this kit to 237 mL DMEM: F12:

- 2.5 mL L-Alanyl-L-Glutamine
- 2.5 mL Non-Essential Amino Acids + 0.5 mL Ascorbic Acid
- 5 mL Dopaminergic Differentiation Kit Component A
- 2.5 mL Dopaminergic Differentiation Kit Component B
- 0.5 mL Dopaminergic Differentiation Kit Component C

Also add CHIR-99021 (from Stemgent, catalog number 3003) to a final concentration of 5 µM (625 µl to 250 ml of media)

Handling Procedure:

Coat plates with Cell Basement Membrane Gel (ATCC ACS-3035) and culture the NPCs with NPC Growth Medium (ATCC ACS-3003) to provide a surface for the attachment of NPCs.

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Coating Procedure:

1. Thaw Cell Basement Membrane Gel on ice and swirl gently to mix. Important: Cell Basement Membrane Gel will solidify in 15 to 30 minutes above 15°C. Keep Cell Basement Membrane Gel, vials and pipette tips on ice at all times to prevent Cell Basement Membrane Gel from solidifying. If air bubbles form, they may be eliminated by centrifuging Cell Basement Membrane Gel at 300 x g for 10 minutes at 2°C to 8°C.
2. Determine the appropriate volume per aliquot based on concentration and usage. For seeding, plate cells at 40,000 viable cells/cm2 (1.50 x 106 cells/ well of 12-well plate).
3. Dilute Cell Basement Membrane in DMEM:F12 to a working concentration of 150 µg/mL. Add 0.5 ml diluted Cell Basement Membrane gel per well of 12 well plate.
4. Cell culture dishes coated with Cell Basement Membrane Basement Membrane Gel should be incubated at 37°C for one hour. Aspirate coating solution and immediately plate the cells. It is critical that the coating does not dry out.

To insure 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 –80°C. Storage at –80°C will result in loss of viability. Preparation of Cell Basement Membrane Basement Membrane Gel (ATCC ACS-3035) coated plates:

1. Thaw Cell Basement Membrane Basement Membrane Gel on ice or at 4°C
2. Prepare a 150 µg/ mL working concentration of Cell Basement Membrane in cold DMEM: F-12 medium
3. Add enough Cell Basement Membrane solution to cover the surface of the plate (e.g. 1 mL diluted Cell Basement Membrane/well of a 12-well plate)
4. Incubate for 1 hour at 37°C prior to use

Initiation of Cultures

1. Prepare complete NPC growth medium (ATCC ACS-3003) following the instructions in the package and pre-warm that medium as well as DMEM:F12 in a 37°C water bath for 15-30 min. If using a small volume of medium (50 mL or less), warm only the volume needed in a sterile conical tube. Avoid warming complete medium multiple times.
2. Obtain a 12-well plate with Cell Basement Membrane Basement Membrane. Aspirate the Cell Basement Membrane medium and directly add 1.5 mL of the complete NPC Growth Medium per well. Place the plate in the incubator for 15

minutes to allow the medium to reach its normal pH (7.0-7.6). Four to five wells of a 6-well plate may be needed for each vial of cells thawed.

3. Transfer 9 mL of pre-warmed DMEM:F12 into a 15 mL conical tube for recovery of the NPCs from the frozen stock.
4. Remove cryovial of frozen cells from liquid nitrogen storage.
5. Thaw the cells by gently swirling in a 37°C water bath. To reduce the possibility of contamination, keep the cap out of the water. Thawing should be rapid (approximately 1 to 2 minutes). Remove the cryovial from water bath when only a few ice crystals are remaining.
6. Sterilize the cryovial with 70% ethanol. All of the operations from this point on should be carried out under strict aseptic conditions.
7. Remove cells from the vial using a P1000 micropipette and transfer cells drop-wise into the 15 mL conical tube containing 9 mL DMEM:F12.
8. Centrifuge cells at 270 x g for 5 minutes at room temperature.
9. Aspirate the supernatant and discard. Gently tap the bottom of the tube to loosen the cell pellet.
10. Add 4 mL of the complete NPC Growth Medium to the tube. Gently resuspend the pellet by pipetting up and down 3 or 4 times to make a single-cell suspension.
11. Perform cell count by a Vi-Cell Analyzer or hemocytometer. Note: Don't perform cell count by a Vi-Cell Analyzer without removal of serum-free freezing medium.
12. Seed NPCs at a seeding density of 80,000 viable cells/cm^2 (e.g. 0.30 $x10^6$/well of a 12-well plate) onto a Cell Basement Membrane-coated plate described above.
13. Incubate the plate at 37°C with 5% CO_2 overnight.
14. Change medium at 100% media change rate (1 mL media/well) next day and every other day thereafter.
15. Monitor cell growth and passage the cells when they reach ~95% confluency

Note: Don't passage NPCs when the cells are <85% confluency.

Subculturing procedure:

Post thaw day 1, perform a 100% medium change and remove all cells that did not attach. Perform a 100% medium change every other day thereafter. Passage the cells cells with diluted Accutase (50% Accutase and 50% DPBS) when they reach ~95% confluence and reseed the NPCs at 40,000 viable cells/cm^2 on Cell Basement Membrane-coated dishes/plates.

Dopaminergic Differentiation: Seed NPCs at 10,000 cells/cm^2 in a 12-well plate pre-coated with Cell Basement Membrane. Culture overnight in complete NPC expansion medium. The next day, aspirate the medium and add 1.5 ml of pre-warmed complete Dopaminergic Differentiation Medium containing CHIR. Change the Dopaminergic media every other day (*e.g.*, Monday, Wednesday, and Friday) for 3 weeks as the following:

1. For each media change in the process, gently remove approximately 85% of the medium using a 5 ml serological pipette from each well and discard.
2. During the first week of the differentiation, slowly add 1.5 ml of fresh Dopaminergic Differentiation Medium to each well along the wall of the well on Monday and Wednesday; add 2 ml of of fresh Dopaminergic Differentiation Medium on Friday.
3. During the second and third week of the differentiation, slowly add 2 ml of fresh Dopaminergic Differentiation Medium to each well along the wall of the well on Mondays and Wednesdays; add 2.5 ml of of fresh Dopaminergic Differentiation Medium on Fridays.
4. Monitor NPC differentiation.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: Neural Progenitor Cells Derived from ATCC-DYS0530 Parkinson's Disease (ATCC ACS-5001)

References

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

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