Neural Progenitor Cells Derived from ATCC-BYS012
Normal; Human (ATCC® ACS-5004™)

Please read this FIRST

Storage Temp. Liquid Nitrogen Vapor Phase (~130°C or colder)

Biosafety Level 2

Intended Use

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

Astrocyte, oligodendrocyte, and neuron differentiation; drug screening

Complete Growth 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 GROWTH Medium

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner:

Neural Progenitor Cells Derived from ATCC-BYS012 Normal; Human (ATCC® ACS-5004™)

SAFETY PRECAUTION

ATCC highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. It is important to note that some vials leak when submerged 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 vessel exploding or blowing off its cap with dangerous force creating flying debris.

Unpacking & 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.

Handling Procedure for Frozen Cells

Note: Coat plates with CellMatrix (ATCC® ACS-3035) and culture the NPCs with NPC Growth Medium (ATCC® ACS-3003). 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 -80°C. Storage at -80°C will result in loss of viability. Preparation of CellMatrix Basement Membrane Gel (ATCC® ACS-3035™) coated plates:

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

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Or contact your local distributor
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- 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

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Handling Procedure for Flask Cultures

1. Prepare CellMatrix-coated 12-well or 6-well plates as described above.
2. Change the media at 100% media change rate every other day (1.5 mL media/well of a 12-well plate) and monitor NPC growth daily.
3. Passage NPCs with diluted Accutase when cells are about 95% confluence. Note: Don’t split NPCs when they are <85% confluence
4. Prepare diluted Accutase by mixing Accutase (StemCell Technologies Cat #07920) with equal volume of 1xDPBS
5. Aspirate the media and add 1 mL diluted Accutase per well of 12-well plate.
6. Incubate the plate at 37°C incubator until majority of cells are detached. Note: It may take 3-5 min to dissociate NPCs.
7. Add 1 mL DMEM:F12 medium per well when majority of cells are detached.
8. Transfer cells into a 15 mL conical tube.
9. Perform cell count by a Vi-Cell Cell Analyzer or hemocytometer.
10. Centrifuge cells at 270 x g for 5 minutes at room temperature.
11. Aspirate the supernatant and discard. Gently tap the bottom of the tube to loosen the cell pellet.
12. Add 5 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.
13. Aspirate NPC solution and add 2 mL the complete NPC growth media per well of a 12-well plate.
14. Reseed NPCs at a seeding density of 40,000 viable cells/cm² (e.g. Seed 0.15x10⁶ cells/well of a 12-well plate).
15. Incubate the plate at 37°C with 5% CO₂ overnight.
16. Change the media at 100% media change rate next day and change the media every other day thereafter.
17. Monitor NPC growth daily and subculture NPCs with diluted Accutase when cells are about 95% confluence described above.

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/cm² on CellMatrix-coated dishes/plates.

References

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

Biosafety Level: 2

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the current publication of the Biosafety in Microbiological and Biomedical Laboratories from the U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes for Health.

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