Neural Progenitor Cells Derived f
MAP2p-Nanoluc-Halotag

ACS-5007™

Description

- **Organism** *Homo sapiens*, human
- **Cell Type** neural progenitor cell
- **Tissue** Umbilical cord; CD34+ cord blood
- **Age** neonate
- **Gender** Male
- **Morphology** short spindle shape
- **Growth properties** Adherent
- **Disease** Normal
- **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.

Astrocyte, oligodendrocyte, and neuron differentiation; drug screening; Quantitative measurement of ealy neuron differentiation.

BSL 1

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: 8.0 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 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

- **Handling Procedure**

Coat plates with Cell Basement Membrane Gel (ATCC® ACS-3035) and culture the NPCs with NPC Growth Medium (ATCC® ACS-3003).

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 Gel (ATCC® ACS-3035™) coated plates:

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

- **Cryopreservation**

  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 CellMatrix-coated dishes/plates.

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 XCL-1 MAP2p-Nanoluc-Halotag (ATCC ACS-5007)

References

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

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