iPSC-Derived Neural Progenitor Cells (NPC) With Media

Applied StemCell has developed an efficient integration-free method to differentiate high-quality neural progenitor cells (NPCs) from human iPSCs. The differentiated NPCs recapitulate the phenotype and functional parameters of primary and *in vivo* neural progenitor cells.

We provide NPCs differentiated from an integration-free, control human iPSC line (ASE-9211). These high-purity (≥95%) cells express high levels of the NPC biomarker Nestin.

To harness the full potential of our neural progenitor cells, we also provide optimized NPC Culture Media (ASE-9740MM) that supports robust maintenance and functionality of the NPCs in culture.

Kit Components

Catalog #	Product	Size
ASE-9740	**iPSC-Derived Neural Progenitor Cells (NPC) With Media**	1 Kit

Parental Tissue Information	
Parental Cell Line	Control Human iPSCs (ASE-9211, p8)
Tissue Source	Human Skin Fibroblast (HSF)
Sex	Male
Age	Neonatal
Race	African-American
Clinical Information	Normal

Intended Use

The ASE-9740 iPSC-differentiated NPCs can be used as control lines to compare phenotype and functionality of patient-derived, genome edited iPSC-derived neural progenitor cells for drug screening applications. The NPCs can also be used for neurotoxicity assays.

This product is for research use only and not intended for human or animal diagnostic or therapeutic uses.

Quality Assurance

Each lot of ASE-9740 NPCs cells has been tested for growth, viability, and purity (≥95%) following recovery from cryopreservation, and for the expression of NPC markers. Additionally, these iPSCs have been tested to confirm the absence of bacterial, fungal, and mycoplasma contamination.

All quality control test results are reported on a lot-specific Certificate of Analysis, available at appliedstemcell.com or upon request.

Storage Instructions and Stability

Store the components of the kit at the appropriate storage conditions as indicated in the media and materials table, immediately upon arrival.

Shelf-life of the product is contingent upon proper storage conditions.

Precautions

The safety and efficacy of this product in diagnostic or other clinical uses have not been established. This product is for research use only and not intended for human or animal diagnostic or therapeutic uses.

Please be aware that the following scenario can occur: Liquid nitrogen can leak into the vials while they are submerged in a liquid nitrogen freezer. Durning rapid thawing, the liquid nitrogen returns to the gas phase, resulting in a dangerous build-up of pressure within the vial. This can result in the vial exploding and expelling not only the vial contents but also the vial cap and plastic fragments of the vial. When retrieving the cell vial from a liquid nitrogen freezer, carefully twist open the cap to release any built-up nitrogen gas prior to thawing the cells.

Refer to the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.

Directions for Use

Provided Materials in Starter Kit

Catalog#	Component	Amount	Storage	Shelf Life
ASE-9740-C	NPCs	≥ 1.0×10^6 cells/vial	Liq. N_2	12 months
ASE-9740MM	NPC Culture Media	100 mL	-20°C	12 months

Required Materials (Not Provided)

The below reagents are recommended for use with the NPCs. If you use reagents other than those recommended, we suggest that you do a batch-test to validate integrity of the cells and culture protocol. Primary antibodies are listed in the table, and the corresponding secondary antibodies were purchased from ThermoFisher.

Product	Vendor	Catalog#
Matrigel®	Corning	354230
Nestin	R&D Systems	MAB1259
Tuj1	R&D Systems	MAB1195
GFAP	R&D Systems	AF2954

Protocol

1. Coating Cell Culture Vessels with Coating Matrix

    1.1. Coat the plates with 80 µg/mL Matrigel®.

    Note: Please follow manufacturer's instructions on coating plates using Matrigel®.

    1.2. Incubate at room temperature for at least 1 hour before use.

2. Preparation of NPC Culture Media

    2.1. Thaw the NPC Culture Media at room temperature before thawing the cryopreserved NPCs.
    2.2. The Culture Media should be aliquoted and stored at -20°C if it will not be used immediately.

    Note: The media can be stored at 4°C for up to 2 weeks or at -20°C for up to 12 months.

3. Thawing and Culturing Cryopreserved NPCs

    3.1. To thaw the cryopreserved NPCs, remove one vial from the storage unit.
    3.2. Immerse the vial in the water bath (up to 2/3 of the vial height) and thaw the cells rapidly until only a small piece of ice is still visible (approximately one minute).

    Note: Do not shake the vial during thawing.

    3.3. Bring the vial to the biological cabinet immediately and spray the outside of the vial thoroughly with 70% ethanol and wipe it with an autoclaved paper towel.
    3.4. Remove the cells from the vial using a p1000 micropipette (or serological pipette) and transfer it slowly, drop-wise while swirling into a 15 mL conical tube containing 5 mL of pre-warmed NPC culture medium. Wash the vial with 1 mL medium from the 15 mL conical tube and transfer it back to the tube.

    Note: Do not mix cells up and down and avoid generating bubbles.

    3.5. Centrifuge cells at 250 x g for 5 minutes at room temperature.
    3.6. Aspirate the medium very carefully using a vacuum (or pipette if preferred), leaving only a drop of liquid in the tube.

    Note: Take extra care not to remove or disturb the cell pellet during aspiration of medium.

    3.7. Using a p1000 micropipette, add 1 mL of the pre-warmed NPC culture medium into the tube and gently re-suspend cells by pipetting up and down 2-3 times.
    3.8. Remove a 10 µL aliquot of the cell suspension and mix it with 10 µL of Trypan blue solution.
    3.9. Count the cells.

3.10. Aspirate the coating matrix from the pre-warmed cell culture vessel.
3.11. Seed the NPCs at a density ranging from 100,000-150,000 live cells/cm2 in NPC Culture Media.
3.12. Distribute the cells evenly.
3.13. Place the cell culture vessels in the incubator (37°C/ 5% CO2/ humidity control) overnight.
3.14. We recommend you fully change the media daily and split the cells every 3-4 days.

Data

Characterization of ASE-9740 NPCs

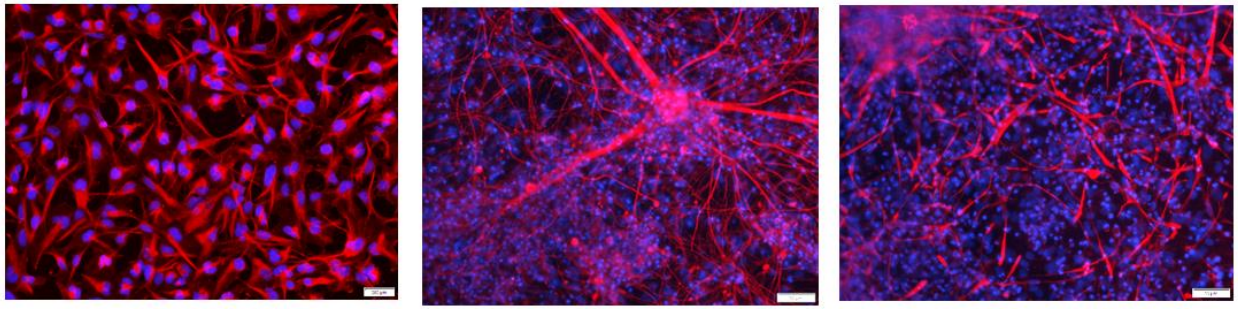

Figure 1. **Immunostaining of the ASE-9740 Neural Progenitor Cells derived from Applied StemCell's control iPSC line ASE-9211.** The neural progenitor cells (NPCs) (Left, Red: Nestin; Blue: DAPI) can be further differentiated into neurons (Middle, Red: Tuj1; Blue: DAPI) and astrocytes (Right, Red: GFAP; Blue: DAPI).

Related Products

Catalog #	Product	Size
ASE-9211	Human iPSC Line	1 x 10^6 cells per vial
ASE-9710-1	iPSC-Derived Retinal Pigment Epithelium (RPE) Cells With Media	1 Kit
ASE-9715	iPSC-Derived Human Photoreceptor Kit	1 Kit

Technical Support

CONTACT US

For more information or assistance, contact Customer Service at:

- Email: info@appliedstemcell.com
- Direct line: (408) 773-8007

WEBSITE RESOURCES

Visit appliedstemcell.com for technical resources and information including:

- Safety Data Sheets (SDS)
- Certificates of Analysis (when available)
- FAQs
- Product literature
- Complete contact information

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Effective Date: 15 September 2026