Neural Stem Cells
(iPSC from Blood Cells; Female)

Product Information

Catalog Number

ASE-9234F (Female)

Description

Neural Stem Cells (NSCs) are cryo-preserved and derived from a footprint-free, karyotype normal human iPSC line. NSCs were derived using EB formation method and isolation of neural rosettes. Obtained neuroepithelial cells represent a high purity NSC population, in which >95% of cells express key NSC markers, SOX1 and Nestin. NSC lines in combination with our optimized maintenance media allows customers to obtain cells characterized by high recovery rate, and can be expanded for up to 5 passages without loss of their differentiation capacity. The NSCs have a great capacity to differentiate into various types of neurons and glial cells.

Amount

≥1.0x10^6 viable cells/vial

Characterization of Neural Stem Cells

Neural Stem Cells (NSCs) can be assessed by their morphology and by immunostaining of SOX1 marker and Nestin. Percentage of NSCs can be determined by a count of SOX1 positive NSCs divided by the total number of cells (DAPI staining of nuclei).

Quality Control

NSC purity: ≥90% SOX1+/Nestin+
Recovery of frozen cells: 80% viability

Shipping

Dry ice

Storage and Stability

Store in liquid nitrogen freezer immediately upon receipt. This product is stable for at least 6 months from the date of receiving when stored as directed.

Safety Precaution

PLEASE READ BEFORE HANDLING ANY FROZEN VIALS. Please wear the appropriate Personal Protection Equipment (lab coat, thermal gloves, safety goggles and a face shield) when handling the cells. Handle the frozen vials with due caution. Please be aware that the following scenario can occur: Liquid nitrogen can leak into the vials when the vials are submerged in liquid nitrogen. Upon 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.

Restricted Use

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

Warranty

Performance of amsbio dopaminergic neurons has been extensively tested with other components. amsbio will not hold responsibility if media other than the recommended maturation media is used.

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Additional Reagents Required but not Provided

- NSC maintenance media, AMSBIO, Cat# ASE-9234SM
- BD Matrigel™, hESC-qualified Matrix features, BD Biosciences, Cat# 354277
- DMEM, life Technologies, Cat# 11995081
- Accutase, Life Technologies, Cat# 11995081
- DMSO, Sigma, Cat# D2650
- Primary antibodies:
  - Purified Mouse anti-human SOX1, BD Biosciences, Cat# 560749
  - Rabbit anti-Nestin, BioLegend, Cat# PRB-570C

Protocol

1. Handling Upon Receiving

Neural Stem Cells on dry ice at ambient temperature. Single or multiple vials withcryopreserved cells are packed in a transparent bag, which is buried in dry ice. Upon receiving the product,check the integrity of the packages and the presence of dry ice (contact AMSBIO, if the integrity of apackage has been compromised, e.g. no dry ice in the package).

Cells can be plated immediately after arrival or transferred into liquid nitrogen. Do not remove the vials from dry ice during transportation to storage units. Immediately transfer components (especially the cryopreserved neural stem cells) to storage units, avoiding prolonged exposure to room temperature.

2. Preparation of Culture Vessels and Media

The handling procedures described below have been extensively tested for all of NSC lines using specified substrate coating and optimized maintenance media. The usershould follow these procedures closely. The user assumes all responsibility for the failure of the experiment should there be any deviation from these procedures.

2.1 Coating Cell Culture Vessels with Matrigel

Cell culture vessels should be coated one day before or on the day of plating the cells. Please read producer's manual for handling BD Matrigel hESC-qualified Matrix.

Important producer's notes:

It is extremely important that BD Matrigel hESC- qualified Matrix and all culture ware or media coming in contact with Corning Matrigel hESC-qualified Matrix should be pre-chilled/ice-cold since BD Matrigel hESC-qualified Matrix will start to gel above 10°C.

The dilution is calculated for each lot based on the protein concentration. Prepare aliquots according to the dilution factor provided on the Certificate of Analysis (use 1.5-2.0 mL tubes). The volume of the aliquots is typically between 270-350 µL.

2.1.1. Pre-chill pipettes tips and dishes at 4°C.
2.1.2. Thaw an aliquot (typically between 270-350 µL) of Corning Matrigel hESC-qualified Matrix at 4°C (approximately 45-60 minutes).
2.1.3. Transfer the aliquot on ice into biological safety cabinet.
2.1.4. Prepare a 25 mL aliquot of cold DMEM/F12 in 50 mL conical tube and keep on ice.
2.1.5. Using p1000 micropipette and cold tips, transfer 1000 µL of the cold DMEM/F12 from the above tube into the tube with Matrigel and mix up several times. Transfer the Matrigel solution to the 50 mL conical tube containing cold DMEM/F12 and mix several times with a serological pipette (keep on ice).

2.1.6. Immediately, coat pre-chilled culture dishes with Matrigel/DMEM solution (volume 120-150 µL/cm^2).
2.1.7. Distribute coating matrix evenly and incubate the vessels at room temperature (15-25°C) for at least 1 hour before use.
2.1.8. Coated dishes can be used immediately or can be stored at 4°C for up to 7 days (aseptic conditions).
2.1.9. Pre-warm vessels at 37°C before use.
2.1.10. Aspirate Matrigel just before seeding the NSCs. Do not let the surface dry.

Table 1. Recommended volumes of coating reagents for various vessels.

Vessel	Approx. Surface Area	Matrigel
96 well plate	0.33 cm^2/well	50 µL/well
4 or 24 well plate	2 cm^2/well	250 µL/well
35 mm dish	10 cm^2	1.5 mL
60 mm dish	20 cm^2	2.5 mL

2.2 Preparation of complete NSC Maintenance Medium

Use sterile techniques when preparing reagents and materials. Thaw frozen supplements at room temperature (15-25°C). It is advised to use thawed components within 7 days to formulate complete medium, however if desired, thawed components can be allocated and re-frozen once. Mix all components of NSC Maintenance Media as described in Table 2. Complete medium shall be stored at 2-8°C and used within 7-10 days. Pre-warm aliquots of complete medium at 37°C before use. Change medium every alternate day.

Table 2. Formulation of Complete NSC Maintenance Media (e.g. 250 mL size)

Component	Storage	Volume Provided	Formulation Per 50 mL	Optional one time re-freezing
NSC Maintenance Basal Medium	2-8°C	1x250 mL	50 mL	yes
NSC Maintenance Supplement A	-20°C	1x5 mL	1 mL	yes
NSC Maintenance Supplement B	-20°C	1x50 µL	10 µL	yes

3. Thawing and Culturing Cryopreserved NSCs

All steps described here must be performed in accordance with aseptic cell culture standards. All media and vessels used for cell culture must be pre-warmed to 37°C prior to use. For the entire process of NSC maintenance, one type of complete medium is required: NSC Maintenance Medium + Supplement A + Supplement B.

3.1 Shortly before thawing cells, place pre-warmed complete NSC Maintenance Medium, DMEM and coated cell culture vessels in a biosafety cabinet.
3.2 Transfer a 9 mL aliquot of pre-warmed DMEM into a 15 mL conical tube. This aliquot will be used for recovery of the NSCs from frozen stock.
3.3 Prepare a 5 mL aliquot of the complete NSC Maintenance medium (volume for 60 mm culture dish). For your convenience, we recommend seeding the cells from one vial onto a 60 mm dish after thawing. However, other sizes of vessels of customer's choice can be used. Please seed the cells at density of 8 x 10^5 cells/ cm^2 to 10 x 10^5 cells/ cm^2, for good recovery.

3.4 To thaw cryopreserved NSCs, remove the vial from LN2 storage unit and place immediately onto dry ice (the vial must be buried in the dry ice).
3.5 Bring the dry ice container with the vial to the location of the 37°C water bath.
3.6 Immerse the vial in the water bath (up to ⅔ of the vial) and thaw cells until only a small piece of ice is still visible (approximately 1 minute).

Note: *Do not shake the vial during thawing.*

3.7 Immediately bring the vial to the biological cabinet, spraying it thoroughly with 70% ethanol and wiping it with an autoclaved paper towel.
3.8 Remove cells from the vial using a p1000 micropipette (or serological pipette) and transfer drop-wise while swirling into the 15 mL conical tube containing 9 mL of pre-warmed DMEM (Step 2). Wash the vial with 1 mL of DMEM 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.*

3.9 Centrifuge cells at 400 x g for 5 minutes at room temperature.
3.10 Aspirate the DMEM very carefully using a vacuum (or pipette if preferred), leaving only a drop of liquid in the tube. Take extra care not to remove or disturb the cell pellet during aspiration of DMEM.
3.11 Using a p1000 micropipette, add 1 mL of NSC Maintenance medium (Step 3) into the tube and gently re-suspend cells by pipetting up and down 4-6 times.
3.12 Remove a 10 µL aliquot of cell suspension and mix it with 10 µL of Trypan blue solution if needing to perform a cell count. Count the cells.
3.13 Aspirate Matrigel solution from pre-warmed 60 mm cell culture dish and immediately transfer 4 mL of NSC Maintenance Medium into that dish.
3.14 Transfer 1 mL of thawed NSCs (Step 11) into the dish.
3.15 Distribute cells evenly and place the dish in the cell culture incubator (37°C/ 5% CO_2/ humidity control).
3.16 Monitor cell survival the following day.
3.17 Change medium every other day until the NSCs are ready to be passaged.

4. Passaging NSCs

4.1 Prepare Matrigel coated cell culture dishes as described earlier. If dishes were stored at 4°C, pre-warm them at 37°C.
4.2 Pre-warm accutase solution, DMEM and complete NSC Maintenance Medium at 37°C.
4.3 To harvest NSCs, remove media from a confluent dish and add accutase immediately to the dish (approximately 100 µL/cm^2). For 60 mm dish use 2.5 mL.
4.4 Incubate at 37°C for 3-5 minutes until cells detach. Use a P-1000 pipette to wash off cells from the dish and transfer them to a 15 mL conical tube. Rinse the dish with an equal volume of pre-warmed DMEM (e.g. 2.5 mL for 60 mm dish) and transfer to the same tube.
4.5 Centrifuge cells at 400 x g for 5 minutes.
4.6 Aspirate supernatant and re-suspend pellet thoroughly in 2-4 mL of complete NSC medium.
4.7 Optional: Perform a cell count if necessary. Passage can be performed without cell count.
4.8 Aspirate Matrigel solution from earlier prepared dish(es) and add NSC Maintenance Medium to each dish to cover the bottom. For customer's convenience, we recommend seeding cells from one 60 mm dish onto one 100 mm dish. Cells can also be plated at a ratio of 1:4 on the same size dishes as the parental dish.
4.9 Transfer the cells to each plate at a density of 8-10 x 10^5 cells/cm^2.
4.10 Distribute cells evenly and place dishes in the cell culture incubator (37°C/ 5% CO_2/ humidity control).
4.11 Change medium every other day until NSCs become confluent.

5. Cryopreservation of NSCs

5.1 Pre-warm accutase solution and DMEM at 37°C.
5.2 To harvest NSCs, remove media from NSC plate and add accutase to the plate (100 µL/cm^2). For 60 mm dish use 2.5 mL.

5.3 Incubate at 37°C for 3-5 minutes until cells detach. Use a P-1000 pipette to wash the cells off of the dish with the accutase and transfer cells to a 15 mL conical tube. Rinse the dish with an equal volume of pre-warmed DMEM (e.g. 2.5 mL for 60 mm dish) and transfer to the same tube to collect remaining cells.
5.4 Centrifuge cells at 400 x g for 5 minutes.
5.5 Quickly aspirate supernatant and re-suspend pellet in NSC Maintenance medium. Count cells using cell counter or compatible method.
5.6 Dilute cells in NSC maintenance medium to a concentration of 4-5 x 10^6 /mL cells.
5.7 Add an equal volume of cold NSC Maintenance Medium containing 20% DMSO and gently mix 3-4 times using serologic pipette.
5.8 Aliquot cell suspension into cryo-vials, (1 mL per vial).
5.9 Immediately freeze at -80°C using isopropanol Mr. Freeze container (or compatible unit).
5.10 The following day, transfer vials into liquid nitrogen.

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