Product Specification Sheet

Product Name	**Human Neural Stem Cells**
Description	Neural stem cells (NSCs) are neural progenitor cells that are capable to differentiate to different kinds of cells in the nervous system when under defined conditions. Taking the advantage of their consistent propagation, NSCs have great potential for the study of neurogenesis and neurodegenerative diseases, and furthermore, for uses in clinical transplantation applications. ALSTEM's human Neural Stem Cell Line is derived from human induced pluripotent stem cells, which are generated from adult skin fibroblasts using ALSTEM's unique footprint-free episomal iPSC reprogramming method. The cells are able to proliferate regularly without differentiating to neural lineage cells when culturing in bFGF and EGF containing medium.
Catalog Number	hNSC11
Size	1 x 10^6 cells/vial
Shipping	Dry Ice
Storage and Stability	Store in gas phase of liquid nitrogen immediately upon receipt. This product is stable for 6 months when stored as directed.
Quality Control	Human NSCs were grown in N2/B27 medium containing bFGF and EGF. Each lot of hNSCs is tested for growth and viability after recovery from cryopreservation. In addition, each lot is tested for expression of Nestin to ensure its undifferentiating characteristics.
Safety Precaution	**ALSTEM highly recommends that protective gloves, a lab coat, and a full-face mask always be worn when handling frozen vials.** It is important to note that some liquid nitrogen can leak into the vials when submersed in liquid nitrogen. Upon thawing, the liquid nitrogen returns to the gas phase, resulting in excessive pressure within the vial that can cause the vial to explode or expel the cap with dangerous force.
Restricted Use	For Research Use Only. Not for use in diagnostic or therapeutic procedures.

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Protocol (hNSC11)

Procedure

CULTURE CONDITION FOR HUMAN NEURAL STEM CELLS

Preparation of neural stem cell culture medium

1. Thaw N-2 supplement (100X, Life Technologies, Cat. # 17502-048) and B-27 serum free supplement (50X, Life Technologies, Cat. # 17504-044) overnight at 4° C.
2. Add the 5 ml of thawed N-2 supplement, 10 ml of thawed B-27 supplement, 20 ng/ml bFGF (Stem-RD, Cat. # bFGF-50) and 20 ng/ml EGF (StemRD, Cat. # EGF-50) to 500 ml of DMEM/F12 medium (Cellgro, Cat. # 15-090-CV) aseptically, and mix well. Filter through a 0.2 µm, low-protein binding filter, if desired.
3. Aliquot into appropriate amount according to usage and store the aliquots at 4° C.

Coating plates with poly-l-ornithine and laminin

1. Dilute poly-l-ornithine (Sigma, Cat. # P3655) on ice using sterile tissue culture-grade water. Aliquot the diluted poly-l-ornithine and keep the aliquots in -80° C until use.
2. Aliquot laminin (Sigma, Cat. # L2020) on ice and keep the aliquots in -80° C until use.
3. Add 1 ml of poly-l-ornithine (20 µg/mL) to a well of a 6-well plate, and incubate at 37° C for two hours.
4. Aspirate poly-l-ornithine and rinse the well twice with 1 ml of DPBS.
5. Add 1 ml of laminin solution (5 µg/mL) in the well and incubate at 37° C for 2 hours.
6. Aspirate the laminin before plating cells or store plate at 4° C until needed.

Coating plates with Matrigel (alternative plate coating method)

Matrigel (BD, Cat. # 354277) should be aliquoted and stored at -80° C for long-term use.

1. Thaw the matrigel on ice until liquid. Dilute matrigel 1:50 with pre-chilled KO DMEM/F12.
2. Immediately use the diluted matrigel solution to coat tissue culture-treated plates. For a 6-well plate, use 0.8 ml of diluted matrigel solution per well, and swirl the plate to spread the matrigel solution evenly across the surface.
3. Keep the coated plate at 37° C for 1 hour or overnight at 4° C. If plate has been stored at 4° C, allow the plate to incubate at 37° C for at least 30 minutes before removing the matrigel solution.

Preparation of neural stem cell freezing medium

1. Add 9 ml of neural stem cell culture medium with 1 ml of DMSO in a 15 ml conical tube. Keep it at 4° C until use. Prepare neural stem cell freezing medium immediately before use. Do not store.

Thawing cryopreserved hNSC

1. Quickly thaw the NSCs in a 37° C water bath by gently shaking the cryovial continuously until half thawed. Remove the cryovial from the water bath and spray with 70% ethanol to sterilize.
2. Transfer the contents of the cryovial to a 15 ml conical tube. Add 5 ml of warm N2/B27 medium containing bFGF and EGF to the tube, gently mixing as the medium is added.

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3. Centrifuge cells at 200 x *g* for 5 min at room temperature.
4. While centrifuging, remove the matrigel solution from a coated tissue culture 6-well plate. Add 1 ml of warm N2/B27 medium containing bFGF and EGF to one well of 6-well plate.
5. After centrifugation, aspirate the medium from 15 ml tube. Gently resuspend the cell pellet in 1 ml N2/B27 medium containing bFGF and EGF, following by transferring the cells to the matrigel coated 6-well plate.
6. Move the plate in quick side-to-side, forward-to-back motions to evenly distribute the cells within the wells, and then place the plate into the 37° C incubator. Culture the cells at 37° C with 5% CO_2 and 95% humidity.
7. Change medium in every two days.

Passaging the hNSCs (6-well plate format)

1. Passage cells when the cells reach 80-90% confluence.
2. Aspirate medium from the hNSC culture and rinse with DPBS (2 ml/well).
3. Add 0.5 ml per well of accutase (Millipore, Cat. # SCR005). Keep the plate at 37° C for 2-3 minutes.
4. Neutralize the accutase with 1 ml of N2/B27 medium containing bFGF and EGF. Gently rinse each well 2-3 times to lift up most of the hNSCs.
5. Transfer the detached cells to a 15 ml conical tube and rinse the well with an additional 2 ml of N2/B27 containing bFGF and EGF to collect any remaining cells. Add the rinse to the 15 ml tube.
6. Centrifuge the 15 ml conical tube containing the cells at 200 x *g* for 5 min at room temperature.
7. Aspirate the supernatant. Resuspend the pellet in N2/B27 containing bFGF and EGF by gently pipetting up and down.
8. Plate the hNSCs using 1:6 ratios with 2 ml N2/B27 medium containing bFGF and EGF in a new plate coated with poly-l-ornithine and laminin or matrigel (remove the coating solution before plating the cells).
9. Move the plate in quick side-to-side, forward-to-back motions to evenly distribute the cells within the wells, and then place the plate into the 37° C incubator. Culture the cells at 37° C with 5% CO_2 and 95% humidity.
10. Change medium once per two days.

Cryopreserving hNSCs

1. Prepare the hNSCs freezing medium and keep the freezing medium in 4° C until use.
2. Perform steps 1-6 from "Passaging the hNSCs."
3. Gently aspirate the supernatant and loosen the cell pellet by tapping the bottom of the tube.
4. Gently resuspend the pellet in freezing medium.
5. Transfer 1 ml of cells with freezing medium into each labeled cryogenic vial.
6. Place vials into an isopropanol freezing container and place the container at -80° C overnight.
7. Transfer the vial to a liquid nitrogen tank the next day.

WARNING

Do not use cryogenic vials for storage in the liquid phase of liquid nitrogen. Such use may cause entrapment of liquid nitrogen inside the vial and lead to pressure buildup resulting in possible explosion or biohazard release. Use appropriate safety procedures which are outlined by the ATCC when handling and disposing of vials. ALSTEM highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials.

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