Human Neural Stem Cell (hNSC)

Name:	Human Neural Stem Cell (hNSC)
Cat. #:	P801
Application:	Human Neural stem cells (hNSCs) are neural progenitor cells that can differentiate into neuron and glia cells. NSCs have great potential for the study of neurogenesis and neurodegenerative diseases, and therapeutic transplantation studies. This product is for research use only.
Product Size:	One frozen vial of ~1x10^6 cells
Product Description:	Human Neural Stem Cells are derived from human induced pluripotent stem cells (hiPSCs) generated by genome footprint-free episomal reprogramming method. When culturing in bFGF and EGF containing medium, these cells are able to consistently proliferate without differentiating to neural lineages. When culturing in proper differentiation condition, these hNSCs can differentiate into multiple neural and glial lineages.
Shipping / Storage:	Ship in **dry ice,** store at gas phase of liquid nitrogen immediately upon receipt.
Shelf Life:	6 months
Remark:	hNSCs 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.

Protocol **(**example of culturing cells in 6-well plate**)**

Preparation of neural stem cell culture medium

1. Thaw N-2 supplement (100X) (Cat. #: 17502-048, Life Technologies) and B-27 serum free supplement (50X) (Cat. #: 17504-044) **overnight at 4°C**.

2. Make neural stem cell culture medium **(NSC medium)**:

N-2 supplement	5 mL
B-27 supplement	10 mL
bFGF (Cat. #: bFGF-50, 101Bio)	20ng / mL
EGF (Cat. #: EGF-50, 101Bio)	20ng / mL
DMEM/F12 medium (Cat. #: 15-090-CV, Cellgro)	500 mL

Mix well and filter through a 0.2 µm, low-protein binding filter.

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 (Cat. #: P3655, Sigma) on ice using sterile tissue culture-grade water (20µg/mL). Aliquot the diluted poly-l-ornithin and keep the aliquots in -80°C until use.
2. Aliquot laminin (Cat. #: L2020, Sigma) on ice and keep the aliquots in -80°C until use.
3. Coat 6-well plate with poly-l-ornithine (20 µg/mL) 1 mL / well, and incubate at 37°C for 2 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 (Cat. #: 354277, BD) 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.

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 of mL conical tube. Add 5 mL of warm **NSC medium** to the tube, gently mixing as the medium is added.
3. Centrifuge cells at 200**x g** for 5 minutes at room temperature.
4. While centrifuging, remove the matrigel or laminin solution from a coated tissue culture 6-well plate. Add 1 mL of warm **NSC medium** 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 **NSC medium**, following by transferring the cells to the **matrigel** or **poly-l-ornithine and laminin coated** 6-well plate.

6. Shake the plate in side to side, forward to back motions to evenly distribute the cells within the wells. 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. Pass the cells when the density reaches 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** (Cat. #: SCR005, Millipore). Incubate at 37°C for 2-3 minutes.
4. Neutralize the accutase with 1 ml of **NSC medium**. 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 **NSC medium** to collect any remaining cells, and add it to the 15 mL tube.
6. Centrifuge the 15 mL conical tube containing the cells at 200x **g** for 5 minutes at room temperature.
7. Aspirate the supernatant and resuspend the pellet in **NSC medium** by gently pipetting up and down.
8. Split the hNSCs at 1:6 ratio and plate into a new plate coated with matrigel or poly-l-ornithine and laminin (Remove the coating solution before plating the cells) in **NSC medium.**
9. Shake the plate in side to side, forward to back motions to evenly distribute the cells within the wells. 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 as following and keep in 4°C till use.

   Add 9 mL of **NSC medium** with 1 mL of DMSO in a 15 mL conical tube. Prepare neural stem cell freezing medium immediately before use. Do not store.
2. Perform steps 1 to 6 of "**Passaging the hNSCs"** as described above.
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 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.

-- The end –