PRODUCT INFORMATION

Human Neural Hindbrain Stem Cell Line Kit

Catalog Number: Y40060

Product: Human Neural Hindbrain ~1.5 x 10^6 cells per vial
RHB-A® 500 mL bottle

Characteristics:
- Homogeneous, adherent human neural hindbrain stem cells
- Stable, serum-free maintenance and expansion
- Multilineage neural differentiation

Applications: Applications of human neural hindbrain stem cells include:
- Adherent monolayer expansion
- Investigation and evaluation of biological and functional differentiation properties
- Adherent monolayer neural differentiation[1]
- Compound library screening[2]

Description: This Human Neural Stem Cell (hNSC) line is derived from donated fetal brain tissue. Tissue was procured from voluntary, consenting, unpaid donors in compliance with government requirements and regional ethical committee approval. The donor had no known health issues, genetic disorders, or genetic predispositions to disease, and the cell line has not been genetically modified.

Storage: Upon receipt, store the cell vial in the vapor phase of liquid nitrogen and the bottle of medium at –20°C until ready to use.
The cells are stable for 1 year from date of receipt and the medium is stable until the expiration date (see label) under proper storage conditions.

Quality Control: SC Proven products undergo rigorous quality-control procedures before release. Each lot of hNSCs is tested for growth and viability following thawing. In addition, each lot is tested for the expression of Nestin, SOX2, 3CB2, and Vimentin, and the absence of, or minimal expression of, MAP2.

Recommended Use:

Human NSCs are grown in RHB-A supplemented with 20 ng/mL of recombinant human epidermal growth factor (EGF)* and 20 ng/mL recombinant human fibroblast growth factor (FGF-2/bFGF)* in laminin-coated tissue culture flasks at 37°C, 5% carbon dioxide (CO_2) in humidified conditions.

* Recombinant human EGF and bFGF are not supplied. Takara Bio Inc. recommends using Animal-Free Recombinant Human EGF (Peprotech, Cat. #AF-100-15) and Animal-Free Recombinant Human FGF-basic (Peprotech, Cat. #AF-100-18B).

Culture Flasks:

Pre-coat standard cell-culture-treated tissue culture flasks with 10 μg/mL of laminin (Appendix 1) for 3 hr at 37℃ before thawing or passaging the cells.

Recommendations for Passaging:

Cells will reach near confluency (80 to 90%) and will be ready to be split 1:2 (see NOTE below) after five to seven days of monolayer culture. hNSCs should be seeded at 3 - 4 x 10^4 cells/cm^2.

NOTE: For enzyme-based passaging, Takara Bio Inc. recommends using StemPro Accutase Cell Dissociation Reagent (Thermo Fisher Scientific, Cat. #A1110501)

Recommendations for Thawing hNSCs:

1. Prewarm 10 mL of RHB-A medium without growth factors and 10 mL of RHB-A medium containing recombinant human EGF (20 ng/mL) and FGF-2/bFGF (20 ng/mL).
2. When ready, quickly thaw the vial of frozen cells in a 37℃ water bath.
3. Once thawed, immediately transfer the cells to the tube containing the warm media without growth factors. Centrifuge at 200*g* for 5 min, and then carefully discard the supernatant.
4. Resuspend the cells in RHB-A medium containing recombinant human EGF (20 ng/mL) and bFGF (20 ng/mL).
5. Remove the laminin from the pre-coated culture flask(s) and add the cells at a seeding density of 3 - 4 x 10^4 cells/cm^2.
6. Incubate at 37℃ and 5% CO_2 in humidified conditions.
7. Observe the cells daily and change the media on alternate days.

Recommendations for Expansion and Maintenance of hNSCs:

Passage the cells every five to seven days depending on growth rate and requirements. hNSCs are routinely passaged with Accutase using standard cell culture techniques:

1. Remove existing media.
2. Rinse the cells gently with 1X PBS (without Calcium and Magnesium) and aspirate.
3. Add Accutase (1 mL for a T25 flask, 3 mL for a T75 flask, and 5 mL for a T175 flask) and incubate at 37℃ for approximately 5 min until the cells detach from the plastic; gently tap the flask to release cells if necessary.
4. Add media and transfer to a centrifuge tube.
5. Centrifuge the cell suspension at 200*g* for 5 min.
6. Aspirate the supernatant and gently resuspend the cell pellet in growth medium using a 5 mL pipette.
7. Count the cells, and determine cell viability if desired.
8. Resuspend the cells in the required volume of RHB-A medium containing recombinant human EGF (20 ng/mL) and FGF-2/bFGF (20 ng/mL).
9. Remove the laminin from the pre-coated culture flask(s) and add the cells at a seeding density of 3 - 4 x 10^4 cells/cm^2.
10. Incubate at 37℃ and 5% CO_2 in humidified conditions.
11. Observe the cells daily and change the media on alternate days.

Recommendations for Differentiating hNSCs:

The following optimized protocol for differentiation of hNSCs is based on Hook, L., *et al.* (2011)[1] and Conti, L., *et al.* (2005)[3], though investigators may wish to individually develop and/or tailor their conditions. hNSCs will reach near confluency (80 to 90%) and will be ready for differentiation after five to six days in monolayer culture.

1. (Day 0): Pre-coat a culture plate with poly-L-ornithine (see NOTE below) for 20 min and wash twice with PBS, followed by coating with 10 µg/mL of laminin (Appendix 1) for 3 hr at 37°C before plating the cells.

   NOTE: Takara Bio Inc. recommends using Poly-L-ornithine solution (Sigma-Aldrich, Cat. #P4957)

2. Prepare a single-cell suspension (see NOTE below), remove the laminin from the pre-coated culture plate, and seed the cells at a density of 2.5 x 10^4 cells/cm^2 in Differentiation Medium 1 (Appendix 2). Incubate at 37°C and 5% CO_2 in humidified conditions.

   NOTE: For enzyme-based, single-cell dissociation, Takara Bio Inc. recommends using StemPro Accutase Cell Dissociation Reagent (Thermo Fisher Scientific, Cat. #A1110501)

3. (Days 1 to 6): Observe the cells daily and change the media on alternate days.

4. (Days 7 to 13): Replace media completely with Differentiation Medium 2 (Appendix 2). Incubate at 37°C and 5% CO_2 in humidified conditions. Observe the cells daily and change the media on alternate days.

5. (Day 14): Replace media completely with Differentiation Medium 3 (Appendix 2). Incubate at 37°C and 5% CO_2 in humidified conditions. Observe the cells daily and change approximately 80% of the media volume on alternate days until the desired differentiation endpoint is reached.

Recommendations for Freezing hNSCs:

1. Prepare cell freezing mixture: 10% DMSO (Sigma-Aldrich, Cat. #D2650), 10% human serum albumin, and 80% supplemented growth media.
2. Prepare a cell suspension from a culture that is in log-phase growth, as above, and centrifuge at 200*g* for 5 min to pellet the cells.
3. Remove supernatant and discard. Resuspend the cells in the freezing mixture and transfer to cryovials.
4. Immediately transfer vials to a room temperature freezing pot ('Mr Frosty'; Thermo Fisher Scientific, CRY-120-010T) containing isopropyl alcohol to maintain a 1°C/min cooling rate, and place at –80°C. Transfer to the liquid nitrogen vapour phase after 24 - 72 hr.

Appendices:

【 Appendix 1 】

Takara Bio Inc. recommends using Natural Mouse Laminin (Thermo Fisher Scientific, Cat. #23017-015), or Laminin from Engelbreth-Holm-Swarm murine sarcoma basement membrane (Sigma-Aldrich, Cat. #L2020).

The recommended volumes of 10 µg/mL laminin to use for coating culture flasks are shown below. NOTE: Laminin should be diluted with cold PBS (4°C).

Flask Size	Volume of 10 µg/mL Laminin
T25	2 mL
T75	7 mL
T175	14 mL

【 Appendix 2 】

Differentiation Medium 1.
RHB-Basal™ medium (Cat. #Y40000) supplemented with 0.5% NDiff® N2-AF (Cat. #Y40110), 0.5% B-27 Supplement (50X), serum free (Thermo Fisher Scientific, Cat. #17504044) and 10 ng/mL Animal-Free Recombinant Human FGF-basic (Peprotech, Cat. #AF-100-18B).

Differentiation Medium 2.
1:1 v/v of RHB-Basal and Neurobasal Medium, minus phenol red (Thermo Fisher Scientific, Cat. #12348017) mediums supplemented with 0.25% NDiff N2-AF, 0.5% B-27 Supplement (50X), serum free, 10 ng/mL bFGF and 0.5% GlutaMAX (Thermo Fisher Scientific, Cat. #35050-038).

Differentiation Medium 3.
Neurobasal medium supplemented with 1% B-27 Supplement (50X), serum free, 20 ng/mL Recombinant Human/Murine/Rat BDNF (Peprotech, Cat. #450-02) and 1% GlutaMAX.

References:

1. Hook L, Vives J, Fulton N, Leveridge M, Lingard S, Bootman MD, Falk A, Pollard SM, Allsopp TE, Dalma-Weiszhausz D, Tsukamoto A, Uchida N, and Gorba T. Non-immortalized human neural stem (NS) cells as a scalable platform for cellular assays. *Neurochem Int*. (2011) **59**(3): 432-444.
2. McLaren D, Gorba T, Marguerie de Rotrou A, Pillai G, Chappell C, Stacey A, Lingard S, Falk A, Smith A, Koch P, Brüstle O, Vickers R, Tinsley J, Flanders D, Bello P, and Craig S. Automated large-scale culture and medium-throughput chemical screen for modulators of proliferation and viability of human induced pluripotent stem cell-derived neuroepithelial-like stem cells. *J Biomol Screen*. (2013) **18**(3): 258-268.
3. Conti L, Pollard SM, Gorba T, Reitano E, Toselli M, Biella G, Sun Y, Sanzone S, Ying QL, Cattaneo E, and Smith A. Niche-independent symmetrical self-renewal of a mammalian tissue stem cell. *PLoS Biol*. (2005) **3**(9): e283.