



**Human Neurons-brain stem
(HN-bs)
Catalog #1560**

Cell Specification

The tissue of the central nervous system is made up of two classes of cells that may be broadly categorized as neurons and glia. Neurons are anatomic, functional, and trophic units of the brain [1]. Despite great variability in size and shape, all neurons share common morphological features, the key elements of a highly complex communication network. Neurons are dynamically polarized cells that serve as the major signaling unit of the nervous system. The brain is known to contain about 1×10^{11} neurons, each being able to contact at least 10,000 other neurons [2]. Brain stem neurons provide main motor and sensory innervation and integrative functions by regulating heart rate, breathing, sleep cycle, awareness, consciousness, and more [3]. Neurons isolated from different brain regions serve as excellent models for studies of region-specific pathologies and degeneration, neurotoxicology and brain development [4].

HN-bs from ScienCell Research Laboratories are isolated from human brain stem. HN-bs are cryopreserved at P0 and delivered frozen. Each vial contains $>1 \times 10^6$ cells in 1 ml volume. HN-bs are characterized by immunofluorescence with antibodies specific to β -tubulin III. HN-bs are negative for HIV-1, HBV, HCV, mycoplasma, bacteria, yeast, and fungi. HN-bs are guaranteed to further culture under the conditions provided by ScienCell Research Laboratories; however, *HN-bs are not recommended for expanding or long-term cultures since the cells do not proliferate in culture.*

Recommended Medium

It is recommended to use neuronal medium (NM, Cat. #1521) for culturing HN-bs *in vitro*.

Product Use

HN-bs are for research use only. They are not approved for human or animal use, or for application in *in vitro* diagnostic procedures.

Storage

Upon receiving, directly and immediately transfer the cells from dry ice to liquid nitrogen and keep the cells in liquid nitrogen until they are needed for experiments.

Shipping

Dry ice.

References

- [1] Parent A. (1996) "Neurons." In *Carpenter's Human Neuroanatomy* (9th ed., pp131-198). Quebec: Williams & Wilkins.
- [2] Alberts B, Bray D, Lewis J, Raff M, Roberts M, Watson JD. (1989) *Molecular Biology of the Cell* (2nd ed.). New York: Garland.
- [3] Smith JC, Abdala AP, Koizumi H, Rybak IA, Paton JF. (2007) "Spatial and functional architecture of the mammalian brain stem respiratory network: a hierarchy of three oscillatory mechanisms." *J Neurophysiol.* 98: 3370-87.
- [4] Mitchell J, Paul P, Chen HJ, Morris A, Payling M, Falchi M, Habgood J, Panoutsou S, Winkler S, Tisato V, Hajitou A, Smith B, Vance C, Shaw C, Mazarakis ND, de Belleruche J. (2010) "Familial amyotrophic lateral sclerosis is associated with a mutation in D-amino acid oxidase." *Proc Natl Acad Sci U S A.* 107: 7556-61.

Instructions for culturing cells

Caution: Cryopreserved primary cells are very delicate. Thaw the vial in a 37°C water bath and return the cells to culture as quickly as possible with minimal handling! Do not centrifuge the cells after thawing as this can damage the cells.

Note: Experiments should be well organized before thawing HN-bs. HN-bs cannot be subcultured or passaged, as the cells do not proliferate.

Initiating the culture:

Note: ScienCell primary cells must be cultured in a 37°, 5% CO₂ incubator. Cells are only warranted if ScienCell media and reagents are used and the recommended protocols are followed.

1. Prepare a poly-L-lysine-coated culture plate (2 µg/cm² is recommended). For example, add 2 ml of sterile water to one well of a 6-well plate and then add 20µl of poly-L-lysine stock solution (1 mg/ml, Cat. #0403). Leave the plate in a 37°C incubator overnight (or for a minimum of one hour).

Note: It is important that these cells are plated in poly-L-lysine-coated culture vessels to promote cell attachment.

2. Prepare complete Neuronal Medium (NM, Cat #1521). Decontaminate the external surfaces of medium bottle and medium supplement tubes with 70% ethanol and transfer them to a sterile field. Aseptically transfer supplement to the basal medium with a pipette. Rinse the supplement tube with medium to recover the entire volume.

Note: We recommend pre-warming the complete medium to room temperature (Not 37°C), prior to use.

3. Prepare a 50 ml conical tube with complete medium. We recommend adding the appropriate volume of complete medium to the conical tube, as shown in Table 1.

Table 1
Recommended medium volume for cell suspension using a 6-well, 12-well, or 24-well format

| Well format | Neuronal medium volume added to the conical tube |
|-------------|--|
| 6-well | 18 ml |
| 12-well | 24 ml |
| 24-well | 24 ml |

4. Leave the tube in the sterile field and proceed to thaw the cryopreserved cells.
5. Place the frozen vial in a 37°C water bath. Hold and rotate the vial gently until the contents completely thaw. Promptly remove the vial from the water bath, wipe it down with 70% ethanol, and transfer it to the sterile field. Carefully remove the cap without touching the interior threads.

- Gently dispense the contents of the vial into the conical tube containing the complete medium.

Note: One vial of cells is sufficient to thaw and plate onto an entire multiwell plate (6-well, 12-well, or 24-well plate). Centrifugation of cells after thawing is not recommended since these actions are more harmful to the cells than the effect of residual DMSO in the culture.

- Gently invert** the tube **1-2 times** to obtain a homogenous cell suspension in the medium. Resuspending primary neurons by pipetting up and down is NOT recommended.
- Gently aliquot the recommended cell suspension volumes onto 6-well, 12-well, or 24-well plates, as shown in Table 2.

Table 2
Recommended cell suspension volume per vial using a 6-well, 12-well, or 24-well format

| Well format | Volume of media/well | # of wells/vial |
|-------------|----------------------|-----------------|
| 6-well | 3.0 ml | 6 wells |
| 12-well | 2.0 ml | 12 wells |
| 24-well | 1.0 ml | 24 wells |

- Replace the lid of the culture plate and gently rock the plate to distribute the cells evenly.
- Return the culture vessel to the incubator.
- For best results, do not disturb the culture for at least 16 hours after the culture has been initiated. Refresh culture medium the next day to remove residual DMSO and unattached cells, then every other day thereafter.
- Cells may take a few days to grow neurites in culture.

It is not recommended that neurons be subcultured beyond their initial plating as these cells do not proliferate.

Caution: Handling human-derived products is potentially biohazardous. Although each cell strain tests negative for HIV, HBV, and HCV DNA, diagnostic tests are not necessarily 100% accurate, therefore, proper precautions must be taken to avoid inadvertent exposure. Always wear gloves and safety glasses when working with these materials. Never mouth pipette. We recommend following the universal procedures for handling products of human origin as the minimum precaution against contamination [1].

[1] Grizzle WE, Polt S. (1988) "Guidelines to avoid personal contamination by infective agents in research laboratories that use human tissues." *J Tissue Cult Methods*. 11: 191-9.