



## Mouse Neurons-hippocampal (MN-h)

Catalog Number: M1540-57

### 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, which are those of the key elements of a highly complex communication network. The neurons are the dynamically polarized cells that serve as the major signaling unit of the nervous system. The brain is known to contain billions of neurons, each being able to contact at least thousands of other neurons [2].

MN-h from ScienCell Research Laboratories are isolated from the E-18 C57BL/6 mouse brain hippocampus. MN-h were cryopreserved at primary culture and delivered frozen. Each vial contains  $>1 \times 10^6$  cells in 1 ml volume. MN-h are characterized by immunofluorescent method with antibodies to neurofilament, MAP2, and beta-tubulin III. MN-h are negative for mycoplasma, bacteria, yeast and fungi. MN-h are guaranteed to further culture in the conditions provided by ScienCell Research Laboratories.

### Recommended Medium

It is recommended to use neuronal medium (NM, Cat. No. 1521) for the culture of mouse neurons *in vitro*.

### Product Use

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

### Storage

Transfer cells directly and immediately from dry ice to liquid nitrogen upon receiving and keep the cells in liquid nitrogen until cell culture is needed for experiments.

### Shipping

Dry ice.

### Reference

- [1] Parent, A. (1996) Neurons in Carpenter's Human Neuroanatomy. 9th ed., pp131-198, Williams & Wilkins, Quebec, Canada.
- [2] Alberts, B., Bray, D., Lewis, J., Raff, M., Roberts, M., Watson, J. D. (1989) Molecular biology of the cell. 2nd ed., New York: Garland.

# Instruction for culturing cells

---

Caution: Cryopreserved cells are very delicate. Thaw the vial in a 37°C waterbath and return them to culture as quickly as possible with minimal handling!

## Unpacking

1. For cryopreserved cells: If there is dry ice in the package and you are not going to culture cells right way, place cryovial(s) immediately into liquid nitrogen. If there is no dry ice left in the package, thaw and culture the cells immediately.
2. For proliferating cells: Spray the culture vessel (flask, plate or slide) with 70% ethanol for disinfection. Transfer the cells into 37°C, 5% CO<sub>2</sub> incubator and allow equilibrating for 2 hours. After cells have equilibrated, remove shipping medium from the culture vessel and replace with fresh medium.

## Set up culture after receiving the ordering

3. Prepare a poly-L-lysine coated flask (2 µg/cm<sup>2</sup>, T-45 flask is recommended). Add 10 ml of sterile water to a T-45 flask and then add 9 µl of poly-L-lysine stock solution (10 mg/ml, ScienCell cat. no. 0413). Leave flask in incubator overnight (minimum one hour at 37°C incubator).

*Note: It is important that neurons are plated in laminin or poly-L-lysine coated culture vessels that promote cell attachment and neurites outgrowth.*

4. Prepare complete medium: decontaminate the external surfaces of medium and medium supplements with 70% ethanol and transfer them to sterile field. Aseptically open each supplement tube and add them to the basal medium with a pipette. Rinse each tube with medium to recover the entire volume.
5. Rinse the poly-L-lysine coated flask with sterile water twice and add 20 ml of complete medium to the flask. Leave the flask in the hood and go to thaw the cells.
6. Set up culture: Prepare one T-45 flask for each cryovial. Add the appropriate amount of medium to the vessel (recommend for 10 ml/T-45 flask) and allow the flask to equilibrate in 37°C, 5% CO<sub>2</sub> incubator for at least 30 min.
7. Thawing of cells: Place the vial in a 37°C waterbath, hold and rotate the vial gently until the contents are completely thawed. Remove the vial from the waterbath immediately, wipe it dry, and transfer it to a sterile field. Rinse the vial with 70% ethanol, and then wipe to remove excess. Remove the cap, being careful not to touch the interior threads with fingers.
8. Using 1 ml eppendorf pipette gently resuspend the cells in the vial and transfer them to equilibrated culture vessels (a T-45 flask). A high seeding density (>10,000/cm<sup>2</sup>) is recommended.

*Note: Dilution and centrifugation of cells after thawing are not recommended since these actions are more harmful to the cells than the effect of DMSO residue in the culture.*

9. Replace the cap or cover, and gently rock the vessel to distribute the cells evenly. Loosen caps if necessary to permit gas exchange. Return the culture vessels to the incubator.
10. Change the medium 4 - 6 hours after plating to remove the residual DMSO and unattached cells, then every other day thereafter. A health culture will display normal neuron morphology (unipolar or dipolar processes with small and round phase-bright cell body in early days of culture).

*Caution: Handling human derived products is potentially biohazardous. Although each cell strain tests negative for HIV, HBV and HCV DNA, proper precautions must be taken to avoid inadvertent exposure. Always wear gloves and safety glasses when working 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, W. E., and Polt, S. S. (1988) Guidelines to avoid personal contamination by infective agents in research laboratories that use human tissues. *J Tissue Culture Methods*. 11(4).