



**Mouse Neurons-raphe
(MN-r)**
Catalog Number: M1510

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 MN-r from ScienCell Research Laboratories are isolated from E14 mouse brain raphe. MN-r are cryopreserved at primary cultures and delivered frozen. Each vial contains $>1 \times 10^6$ cells in 1 ml volume. MN-r are characterized by immunofluorescent method with antibodies to neurofilament, MAP2, and beta-tubulin III. MN-r are negative for mycoplasma, bacteria, yeast and fungi. MN-r 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 culturing of MN-r *in vitro*.

Product Use

MN-r 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!

Set up culture after receiving the ordering:

1. Prepare a poly-L-lysine coated flask (2 $\mu\text{g}/\text{cm}^2$, T-45 flask is recommended). Add 5 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 the flask in incubator overnight (minimum one hour at 37°C incubator).
2. 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.
3. Rinse the poly-L-lysine coated flask with sterile water twice and add 15 ml of complete medium to the flask. Leave the flask in the hood and go to thaw the cells.
4. 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, rinse the vial with 70% ethanol and transfer it to a sterile field. Remove the cap, being careful not to touch the interior threads with fingers. Using 1 ml eppendorf pipette gently resuspend the contents of the vial.
5. Dispense the contents of the vial into the equilibrated, poly-L-lysine coated culture vessels. A seeding density of 20,000 cells/cm² 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. It is also important that neurons are plated in poly-L-lysine coated culture vessels that promote cell attachment.
6. Replace the cap or cover, and gently rock the vessel to distribute the cells evenly. Loosen cap if necessary to permit gas exchange.
7. Return the culture vessels to the incubator.
8. For best result, do not disturb the culture for at least 16 hours after the culture has been initiated. Change the growth medium the next day to remove the residual DMSO and unattached cells, then every other day thereafter.

Maintenance of Culture:

1. Change the medium to fresh supplemented medium the next morning after establishing a culture from cryopreserved cells.

2. Change the medium every two to three days thereafter.

It is not recommended that MN-r be subcultured beyond their initial plating.

Caution: Handling animal derived products is potentially biohazardous. 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).