Rev. 1

# Mouse Neurons-raphe (MN-r)

Catalog #M1510-57

## 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, 2]. Neurons are dynamically polarized cells responsible for electrochemically transmitting information throughout the nervous system [2]. Scientists have characterized hundreds of neuron types based on location, morphology and gene expression [1-3]. The raphe nuclei are clusters of mostly serotonergic neurons located in the brain stem. Raphe neurons send projections to the forebrain, brain stem and spinal cord to regulate mood, memory and circadian rhythms. Primary mouse neurons-raphe (MN-r) are a useful *in vitro* model for studying conditions such as Alzheimer's disease, depression, and narcolepsy.

MN-r from ScienCell Research Laboratories are isolated from embryonic day 14 C57BL/6 mouse brain raphe. MN-r are cryopreserved at P0 and delivered frozen. Each vial contains >1 x $10^6$ cells in 1 ml volume. MN-r are characterized by immunofluorescence with antibodies specific to β-tubulin III. MN-r are negative for mycoplasma, bacteria, yeast, and fungi. MN-r are guaranteed to further culture under the conditions provided by ScienCell Research Laboratories; however, *MN-r 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 MN-r i*n 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

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.

# Instructions for culturing primary cells

**Caution: Cryopreserved primary cells are very delicate. Thaw the vial in a 37ºC 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 MN-r. It is recommended that MN-r are used for experiments as quickly as possible after thawing the cells.* ***MN-r cannot be subcultured or passaged, as the cells do not proliferate.***

**Initiating the culture:**

**Note:** ScienCell primary cells must be cultured in a 37ºC, 5% $CO_2$ 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/cm$^2$ 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 37ºC 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 medium (NM, Cat. #1521). Thaw NGS and P/S solution at 37ºC. Gently tilt the tubes several times to ensure the contents are completely mixed before adding to the medium. Decontaminate the external surfaces of medium bottle and medium supplement tubes with 70% ethanol and transfer them to a sterile field. Add NGS and P/S solution to the medium and mix well.

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

3. Rinse the poly-$_L$-lysine-coated vessel twice with sterile water and then add the volume of complete medium recommended in Table 1 or Table 2. Leave the plate(s) in the sterile field and proceed to thaw the cryopreserved cells.

4. Place the frozen vial in a 37ºC 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.

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

5. Carefully remove the cap without touching the interior threads and gently resuspend the cell suspension. A seeding density of 10,000-20,000 cells/cm$^2$ is recommended depending on your experiments. We recommend following Table 1 for seeding MN-r onto 6-well, 12-well, or 24-well plates. For seeding MN-r on 60 mm plates, use Table 2.

**Table 1**
**Recommended cell suspension volume per vial using a 6-well, 12-well, or 24 well format**

| Well format | Surface area/well (approx. values) | Volume of media/well | Volume of cell suspension from vial/well | # of wells/vial |
|---|---|---|---|---|
| 6-well | 9.6 $cm^2$ | 3.0 ml | 150 µl | 6 wells |
| 12-well | 3.9 $cm^2$ | 2.0 ml | 60 µl | 15 wells |
| 24-well | 1.9 $cm^2$ | 1.0 ml | 30 µl | 30 wells |

**Table 2**
**Recommended cell suspension volume per vial using 60 mm plates**

| Plate Format | Surface area/plate (approx. values) | Volume of cell suspension from vial/plate | # of plates/vial | Volume of media (ml)/plate |
|---|---|---|---|---|
| 60 mm | 21 $cm^2$ | 300 µl | 3 | 3.0 ml |

6. Pipet the correct volume of cell suspension into each well of an equilibrated, poly-L-lysine-coated culture plate containing complete medium. Replace the lid of the culture plate and gently rock the plate to distribute the cells evenly.
7. Return the culture plate to the incubator.
8. For best results, do not disturb the culture for at least 16 hours after the culture has been initiated. Change the culture medium the next morning after establishing a culture from cryopreserved cells to remove residual DMSO and unattached cells.
9. Once the neurons attach, the cells can be used for experiments. Cells may take a few days to grow neurites in culture.

**Note:** We do not recommend cryopreservation of primary cells by the end user. Refreezing cells may damage them and affect cell performance. ScienCell does not guarantee primary cells cryopreserved by the end user.

*Caution: Handling animal-derived products is potentially biohazardous. 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.