NEUROSCIENCES INNOPROFILE™

Ref. P10101

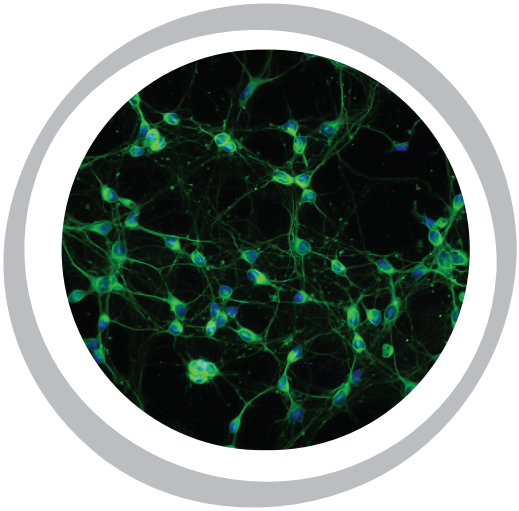
The tissue of the central nervous system is made up of two classes of cells that may be broadly categorized as neurons and glia. Neurons are anatomic, functional, and trophic units of the brain. Despite great variabilty in size and shape, all neurons share common morphologic features, which are those 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.

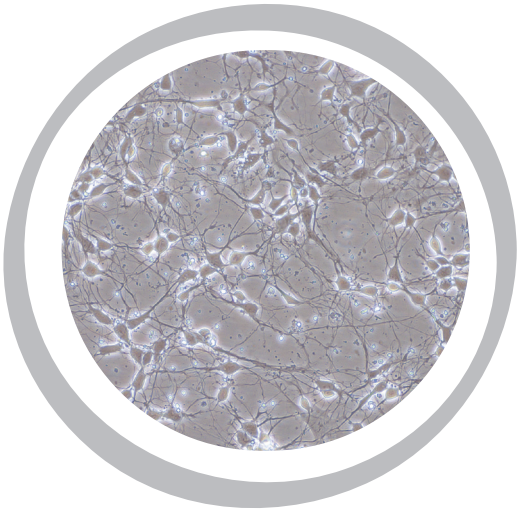
Rat hippocampal neurons from Innoprot are isolated E18 rat embryos, cryopreserved at secondary cultures and delivered frozen. Each vial contains > 1 x 10^6 cells in 0.5 ml volume. Hippocampal neurons are characterized by immunofluorescent method with antibodies to neurafilament, MAP2. Hippocampal neurons are guaranteed to further culture in the conditions provided by Innoprot.

Innoprot

HIPPOCAMPAL NEURONS

from Sprague/Dawley Rat Brains





Recommended Medium

BrainPhys™ Neuronal Medium (Cat. 05790-Stemcell Technologies)
Neurocult™ SM1 Neuronal Supplement (Cat. 05711-Stemcell T.)

Product Characterization

Immunofluorescent method for Neurafilament and MAP2.
Negative for mycoplasma, bacteria, yeast and fungi.

Product Use

These products are for research use only. Not approved for human or veterinary use, for application to humans or animals, or for use in vitro diagnostic or clinical procedures.
Important: Cryopreserved cells are very delicate. Thaw the vial in a 37°C waterbath and return them to culture as quickly as possible

NEUROSCIENCES INNOPROFILE™

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_2 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 order:

1. Coat culture vessel with poly-L-Lysine or poly-L-Ornitine.
Note: It is important that neurons are plated in poly-L-lysine or poly-L-Ornitine coated culture vessels that promote cell attachment and neurites outgrowth (coat flask or plate with poly-Ornitine at 0.003% W/V or 30 µg/ml concentration for one hour and wash the flask or plate with sterile water three times).

2. Medium preparation: 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 the medium to recover the entire volume.

3. Set up culture: Prepare one T-25 or M96 flask for each cryovial. Add the appropriate amount of neuronal medium supplemented with10% inactivated FBS to facilitate cell adhesion to the vessel (recommend for 4 ml/T-25 flask or 200 µl per well for M96) and allow the flask to equilibrate in 37°C, 5% CO_2 incubator for at least 30 min.

4. 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.

5. Pipet the correct volume of cell suspension and transfer into the equilibrated culture vessels or 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.

6. Return the culture plate to the incubator at 37°C, 5% CO_2. A higher seeding density than 10,000/cm^2 is recommended (10,000 - 20,000 cells/cm^2).

7. For best result, do not disturb the culture for at least 16 hours after the culture has been initiated. Change the thawing medium the next day to remove the residual DMSO, iFBS and unattached cells adding just the neuronal medium (ref. P60157), then change half medium every 2-3 days. Minimum handling is recommended.

8. Optionally the antimitotic compound FUDR can be added to the culture medium from the second day *in vitro*. This is recommended for long assays where it is necessary to avoid glial cell proliferation. A health culture will display normal neuron morphology, and nonvacuole cytoplasm with multiple processes.

Caution: Handling animal derived products is potentially bioharzadous. Although each cell strain testes negative for microbial, diagnostic tests are not necessarily 100% accurate, therefore, proper precautions mush 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 (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).

INNOVATIVE TECHNOLOGIES IN BIOLOGICAL SYSTEMS, S.L.
Parque Tecnológico Bizkaia, Edifício 502, 1ª Planta | 48160 | Derio | Bizkaia
Tel.: +34 944005355 | Fax: +34 946579925
innoprot@innoprot.com | www.innoprot.com

Innoprot