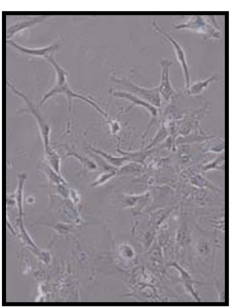
REF: P10101

NEUROSCIENCES INNOPROFILE™

RAT BRAIN HIPPOCAMPAL NEURONS



Product Type:	Cryo-preserved Brain Hippocampal Neurons
Catalog Number:	P10101
Source:	Day 18 embryonic Sprague/Dawley Rat Brains
Format:	> 1 x10^6 cells in Cryopreserved vials
Storage:	Liquid Nitrogen

Rat Neurons (RN) from Innoprot are isolated from 18 days gestation rat embrions. RN are cryopreserved at secondary cultures and delivered frozen. Each vial contains > 1 x 10^6 cells in 1 ml volume. RN are characterized by immunofluorescent method with antibodies to neurafilament, MAP2. RN are guaranteed to further culture in the conditions provided by Innoprot.

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.

Recommended Medium

- Neuronal Medium *(serum-free)* (Reference: P60157)

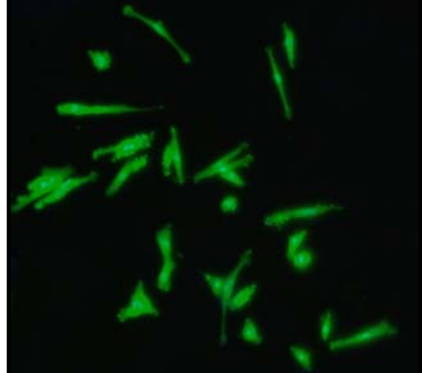


Fig.1 Inmunofluorescent staining of passage two rat hippocampal neurons with anti MAP-2 antibody in green (neuronal marker) and DAPI in blue. (20X)

Product Characterization

Immunofluorescent method

- Neurafilament
- 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.

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

INSTRUCTIONS FOR CULTURING CELLS

IMPORTANT: Cryopreserved cells are very delicate. Thaw the vial in a 37 ºC 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 37ºC, 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-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 3 µl/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 medium to the vessel (recommend for 4 ml/T-25 flask or 200 µl per well for M96) and allow the flask to equilibrate in 37ºC, 5% CO_2 incubator for at least 30 min.

4. Thawing of cells: Place the vial in a 37ºC 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. Transfer the cells with the freezing medium to 15 ml tube and add 10 ml of culture medium. Centrifuge at 230 g during 5 minutes. Discard the supernatant. Resuspend the pellet in the suitable volume of medium and culture at 37ºC, 5% CO_2 in the incubator. A higher seeding density (>10,000/cm^2) is recommended.

6. 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. A health culture will display normal neuron morphology, and nonvacuole cytoplasm with multiple processes..

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

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 [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).

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