NEUROSCIENCES INNOPROFILE™

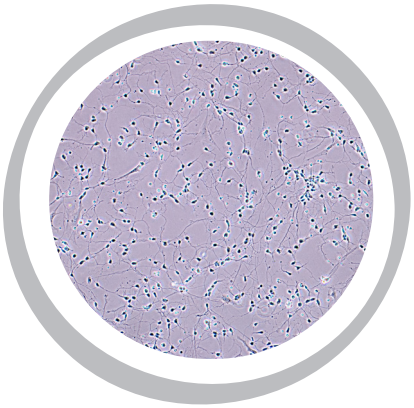
Ref. P10109

SPINAL MOTOR NEURONS

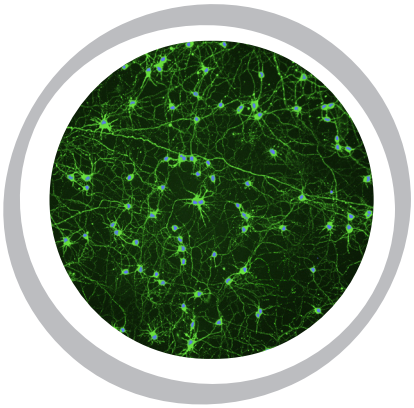
from Sprague/Dawley Rat

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.

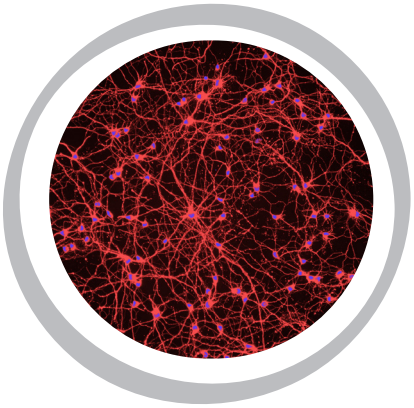
Rat Spinal Motor Neurons from Innoprot are isolated from E15 rat embryos, cryopreserved at secondary cultures and delivered frozen. Each vial contains > 5 x 10^5 cells in 0.5 ml volume. Spinal motor neurons are characterized by immunofluorescent method with antibodies to β-III-Tubulin, p75NTR and ChAT. Spinal motor neurons are guaranteed to further culture in the conditions provided by Innoprot.



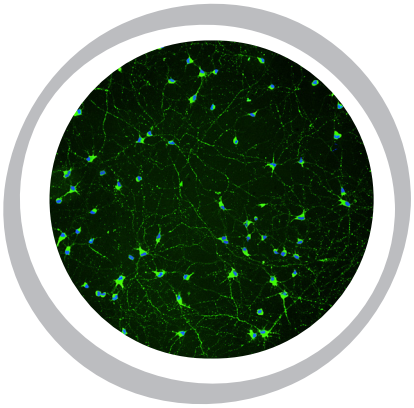
Phase-contrast



p75NTR



β-III-Tubulin



ChAT

Recommended Medium

BrainPhys™ Neuronal Medium + 2% NeuroCult™ SM1 (Stemcell #05792)+ 1% Anti-Anti (Gibco #15240062), supplemented with 50 ng/ml BDNF (Alomone #B-250) and 50 ng/ml GDNF (Alomone #G-240).

Product Characterization

Immunofluorescent method for β-III-Tubulin (Millipore #AB15708C3), p75NTR (Alomone #ANT-007) and ChAT (Invitrogen # MA5-47259). 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 with minimal handling.

Innoprot

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Unpacking

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.

Set up culture after receiving the order:

1. Coat culture vessel with 0.1 mg/ml poly-D-lysine and incubate at 37°C for one hour. Wash the plate with sterile water three times. Next, coat the plate with cold Geltrex Matrix (Gibco #A1413201) diluted 1:10 in culture medium or Matrigel Growth Factor Reduced Basement Membrane Matrix (Corning #356231) diluted 1:10 in culture medium. Immediately remove the excess and incubate again at 37°C for one hour. Note: It is important that neurons are plated in coated culture vessels that promote cell attachment and neurites outgrowth.

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 40-50 M96 wells for each cryovial. Prepare the appropriate amount of neuronal medium supplemented with 10% heat-inactivated FBS to facilitate cell adhesion to the plate (recommended final volume: 200 µl per well) and allow medium to equilibrate in 37°C, 5% CO_2 incubator for at least 30 min. Note: FBS is added only at the time of plating.

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, rinse it with 70% ethanol, wipe it dry, and transfer it to a sterile field. Remove the cap, being careful not to touch the interior threads with fingers.

5. Pipet the cell suspension, transfer it into the equilibrated culture medium, mix gently and distribute the appropriate volume to each well. A higher seeding density is recommended (>10,000 - 20,000 cells/cm^2). Return the culture plate to the incubator at 37°C, 5% CO_2.

6. Once the cells are attached to the plate, very carefully change the growth medium to remove the residual DMSO, unattached cells and FBS. The first change of medium can be done between the first 4 and 16 hours after starting the culture. To avoid glial cell proliferation, add the antimitotic compound FUDR at 10 µM to the culture medium from day *in vitro* 2 to day *in vitro* 6. Change medium every other day thereafter. A healthy culture will display normal neuron morphology with multiple processes and nonvacuole cytoplasm.

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).

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