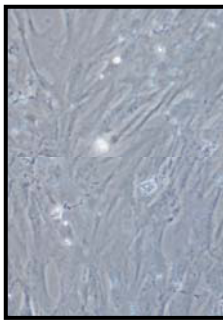


NEUROSCIENCES INNOPROFILE™

RAT HIPPOCAMPAL ASTROCYTES



Product Type:	Cryo-preserved Hippocampal Astrocytes
Catalog Number:	P10203
Source:	2 Days Sprague/Dawley Rat Brains
Number of Cells:	1 x 10 ⁶ Astrocytes / vial (1ml)
Storage:	Liquid Nitrogen

Rat Astrocytes (RA) from Innoprot are isolated from 2 days rat brain. RA are cryopreserved at passage one and delivered frozen. Each vial contains > 1 x 10⁶ cells in 1 ml volume. RA are characterized by immunofluorescent method with antibody to GFAP. RA are negative for mycoplasma, bacteria, yeast and fungi. RA are guaranteed to further expand for 15 population doublings in the conditions

Astrocytes make up the majority of the cells in the mammalian brain. They are the most variable in type, most intimately associated with all parts of neurons, and thus most functionally interesting in their relationships with neurons. They provide structural, trophic, and metabolic support to neurons and modulate synaptic activity. Astrocytes have been implicated in the pathological processes of many neurological diseases. Long-term recovery after brain injury, through neurite outgrowth, synaptic plasticity, or neuron regeneration, is influenced by astrocyte surface molecule expression and trophic factor release.

Recommended Medium

- IMDM+FBS (10%)

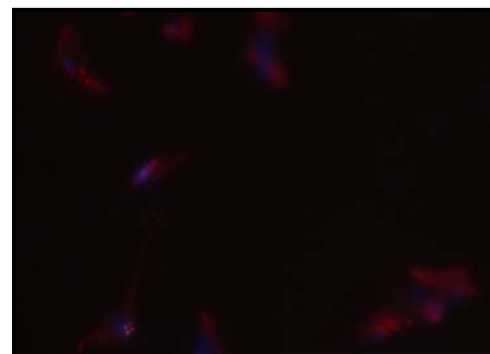


Figure 1. Immunofluorescent staining of passage one rat hippocampal astrocytes with anti GFAP (astrocytes marker) antibody and DAPI

Product Characterization

Immunofluorescent method

- GFAP

The cells test negative for HIV-1, HBV, HCV, 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

INSTRUCTIONS FOR CULTURING CELLS

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!

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% CO₂ incubator and allow equilibrating for 2 hours. After cells have equilibrated, remove shipping medium from the culture vessel and replace with fresh medium.
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 37°C, 5% CO₂ 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.

Set up culture after receiving the order:

1. Coat culture vessel with poly-L-Lysine.
Note: It is important that neurons are plated in laminin or poly-L-Lysine coated culture vessels that promote cell attachment and neurites outgrowth (coat flask or plate with poly-Lysine at 2 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.
5. Transfer the cells with the frozen medium to 15 ml tube and add 10 ml of culture medium. Centrifuge at 1000 g during 5 minutes. Discard the supernatant. Resuspend the pellet in the suitable volume of medium with 10% of bovine fetal serum and culture at 37°C, 5% CO₂ in the incubator. A higher seeding density (>10,000/cm²) is recommended.
7. 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 unattached cells, then every other day thereafter. A health culture will display normal astrocyte morphology and form a monolayer in 5 days.

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

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