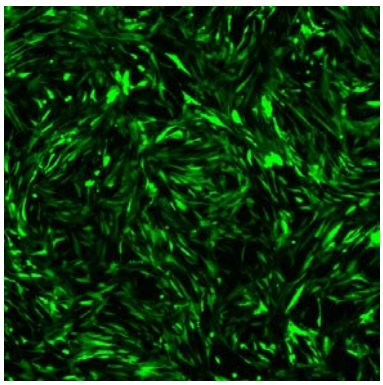
Innoprot
Speeding up drug discovery

REF: P10251-IM-tGFP

NEUROSCIENCES INNOPROFILE™

GREEN FLUORESCENT IMMORTALIZED HUMAN ASTROCYTES



Product Type:	Immortalized Human Astrocytes
Catalog Number:	P10251-IM-tGFP
Immortalization:	SV40 Large T Antigen
Number of cells:	> 1 x10^6 cells in Cryopreserved vials
Storage:	Liquid Nitrogen

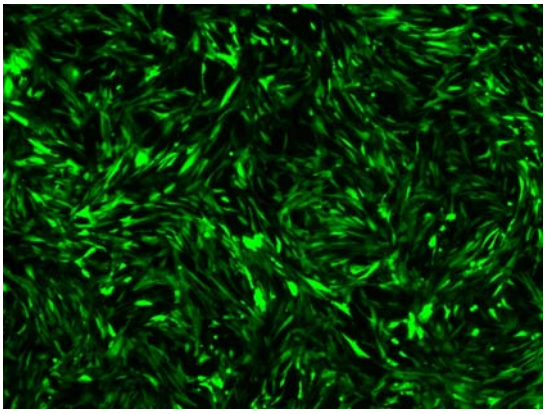
P10251-IM-tGFP has been obtained immortalizing Human Cortical Astrocytes with Lenti-SV40 Lentivirus and stably transfecting the green fluorescent protein tGFP. Green fluorescent Immortalized cells were controlled passaging side by side with the primary cells. Primary cells go into senescence after the 5th passage while the SV40-tranduced cells go beyond 30 passages.

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

- Astrocyte Medium

  (Reference: P60101)



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

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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!

Set up culture after receiving the order:

1. Prepare a poly-L-lysine coated flask (2 µg/cm^2, T-75 flask is recommended). Add 10 ml of sterile water to a T-75 flask and then add 150 µl of poly-L-lysine stock solution (1 mg/ml). Leave the flask in incubator overnight (minimum one hour at 37ºC incubator).

2. Prepare complete medium. Decontaminate the external surfaces of medium bottle and medium supplement tubes with 70% ethanol and transfer them to a sterile field. Aseptically transfer supplement to the basal medium with a pipette. Rinse the supplement tube with medium to recover the entire volume.

3. Rinse the poly-L-lysine-coated vessel twice with sterile water and then add 15 ml of complete medium. Leave the vessel 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.

5. Carefully remove the cap without touching the interior threads. Gently resuspend and dispense the contents of the vial into the equilibrated, poly-L-lysine-coated culture vessel. A seeding density of 5,000 cells/cm^2 is recommended.

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. It is also important that cells are plated in poly-L-lysine-coated culture vessels to promote cell attachment.

6. Replace the cap or lid of the culture vessel and gently rock the vessel to distribute the cells evenly. Loosen cap, if necessary, to allow gas exchange.

7. Return the culture vessel to the incubator.

8. For best results, do not disturb the culture for at least 16 hours after the culture has been initiated. Refresh culture medium the next day to remove residual DMSO and unattached cells, then every other day thereafter.

Maintenance of Culture:

1. Refresh supplemented culture medium the next morning after establishing a culture from cryopreserved cells.

2. Change the medium every three days thereafter, until the culture is approximately 70% confluent.

3. Once the culture reaches 70% confluency, change medium every other day until the culture is approximately 90% confluent.

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Subculture:

1. Subculture when the culture reaches 90% confluency or above.

2. Prepare poly-L-lysine-coated culture vessels (2 µg/cm^2) one day before subculture.

3. Warm complete medium, trypsin/EDTA solution (T/E Solution), T/E neutralization solution (TNS), and DPBS (Ca^{++}- and Mg^{++} -free) to room temperature. We do not recommend warming reagents and medium in a 37ºC water bath prior to use.

Note: DPBS, trypsin/EDTA solution & trypsin neutralization solution are included in the "Primary Cells Detach Kit provided by Innoprot (Cat. Nº P60305).

4. Rinse the cells with DPBS.

5. Add 8 ml of DPBS and then 2 ml of T/E solution into flask (in the case of a T-75 flask). Gently rock the flask to ensure complete coverage of cells by T/E solution. Incubate the flask in a 37oC incubator for 1 to 2 minutes or until cells completely round up. Use a microscope to monitor the change in cell morphology.

6. During incubation, prepare a 50 ml conical centrifuge tube with 5 ml of fetal bovine serum (FBS, Cat. #0500).

7. Transfer T/E solution from the flask to the 50 ml centrifuge tube (a small percent of cells may detach) and continue to incubate the flask at 37oC for another 1 to 2 minutes (no solution in the flask at this moment).

8. At the end of incubation, gently tap the side of the flask to dislodge cells from the surface. Check under a microscope to make sure that all cells detach.

9. Add 5 ml of TNS solution to the flask and transfer detached cells to the 50 ml centrifuge tube. Rinse the flask with another 5 ml of TNS to collect the residual cells.

10. Examine the flask under a microscope for a successful cell harvest by looking at the number of cells being left behind; there should be less than 5%.

11. Centrifuge the 50 ml centrifuge tube at 1000 rpm for 5 minutes. Resuspend cells in culture medium.

12. Count and plate cells in a new poly-L-lysine-coated culture vessel with the recommended cell density

Caution: *Handling human derived products is potentially bioharzadous. Although each cell strain testes negative for HIV, HBV and HCV DNA, 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).

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