NEUROMICS

GFP Expressing Human Brain Astrocytes (GFP-HBA)

Catalog #: HMP201

Cell #: > $5X10^5$ cells in frozen vial

Storage: 37°C CO_2 incubator or Liquid Nitrogen

Product Format: Frozen

General Information

GFP Expressing Human Brain Microvascular Astroctyes were isolated from normal human brain cortical tissues and transfected with GFP-Lentiviral particles. Puromycin resistant, GFP-HBAs are shipped at passage 1 on dry ice in a vial. Astrocyte-Growth medium (cat#PGB003) containing 5% fetal bovine serum and growth supplement is recommended for culture. Cells have an average additional population doubling levels >15 when cultured.

Characterization of the cells

- Cytoplasmic GFAP: <98% positive by immunofluorescence

HBMECs are negative for HIV-1, HBV, HCV, and mycoplasma.

Recommended Products

- Astrocyte-Growth Media – PGB003
  - Astrocyte-Growth Media is freshly prepared by mixing two components: 450 ml of Astrocyte Basal Media and 50 ml of Astrocyte-Growth supplement.
- Smooth Coat Solution – SC300
  - Biocompatible complex of extracellular matrix binding solution

OR

- AlphaBioCoat Solution – AC001
  - Premium Smooth Coat Solution. Biocompatible complex of extracellular matrix binding solution with growth factors. Ideal for culturing cells from frozen.
- Cell Detachment Solution – ADF001
  - Contains protease and collagenase activities in an isotonic, phosphate buffer solution with EDTA to detach primary cells and cell lines
- 1X Phosphate Buffer Solution - PBS300

Shipping

Shipped on dry ice frozen in a vial.

Handling of Arriving Cells

Store in liquid nitrogen to keep the cells frozen or thaw cells according to the protocol for culture.

Note: Handling human derived products is potentially biohazardous. Although each cell strain tests negative for HIV, HBV and HCV DNA, 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.

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SUBCULTURE PROTOCOL

Note: If you have any questions or need clarification regarding the protocol for culturing these cells, please reach out to Dr. Jensen Auguste at (978) 608-1766 with your questions before beginning.

1. Coating T25 flasks:
    a. Add 2 ml AlphaBioCoat Solution (AC001) into a T25 flask and ensure entire interior surface is coated with solution. After 30 minutes, dispose of AlphaBioCoat Solution by aspiration. Gently rinse and aspirate flask with phosphate buffer solution (PBS300) twice. The flask is now ready for use (no need for overnight incubation when coated with AC001)
    b. If you are using the coated flask the same day, add about 4 ml of Astrocyte-Growth media to the coated flask. *If the media changes color from pink to yellow, aspirate and discard the media. Add 4ml of fresh media to the coated flask.

2. Thaw the cells in a 37°C water bath. Once you see a small amount of ice left in the vail, spray the vail with 70% Ethanol and wipe it down.

3. Transfer the vail into your Biosafety cabinet.

4. Using a 2 or 5ml pipet, pipet the cells out of the vial. Do not centrifuge the cells.

5. Transfer your cell suspension in to your coated flask (which contains the 4 ml media).

6. You should have a total working volume of 5ml of cell suspension in the flask; close the cap. Make sure cells are evenly distributer in the flask by moving the flask left and right five times. Move it up and down for and additional five times.

7. Place flask in a 37°C incubator with 5% C02. If flask is not vented, please loosen cap.

8. Change media after 48 hours.

9. Place flask in 37°C incubator until cells are at 90% confluence. Change media every 2 days.

10. When flask is at 90% confluence, aspirate media from flask.

11. Rinse T25 flask containing cells with 5 ml 1XPBS (cat#PBS300).

12. Gently aspirate out the PBS after rinsing, and discard.

13. Add 2ml of RT trypsin/ EDTA or Cell Detachement Solution (ADF001) to T25 flask containing cells (ensure entire interior surface is cover).

14. Place T25 flask containing cells into 37°C incubator for 1 or 2 minutes (cells will normally come off of the surface within 1 or 2 minutes). Monitor cell detachment. Palm of the hand can be used to strike the flask to help cells detach.

15. Suspend the cells with 10ml of Astrocyte-Growth medium (PGB003) and transfer equally into 2 pre-coated T25 flasks (the cells are now at a subculture ratio of 1:2).

16. There is no need to spin cells during subculture.

17. Proliferating cell culture: Astrocyte-Growth medium should be changed every 2 days. The cells normally become confluent within 7 days (when split at a 1:2 ratio)

18. Use Astrocyte Basal media (PGB004) containing 0.5% FBS to induce quiescent cells (after 18-24 hours).

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