Alphabioregen

Moving Research from 2D to 3D

GFP Expressing Human Brain Astrocytes

Catalog Number	HBMP201
Product Name	GFP Expressing Human Brain Astrocytes (GFP-HBA)
Storage	37°C $C0_2$ incubator or Liquid Nitrogen
Product Format	Proliferating culture or Frozen vial
Cells Number	>90% confluent in T25 flask />$5x10^5$ Frozen Vial

*Caution: The handling of human derived products has the potential to be biologically hazardous. All Cell strains tested negative for HIV, HBV, and HCV DNA in diagnostic tests. Proper precautions must be taken to avoid exposure. Always wear proper protective equipment (Gloves, safety glasses, etc.) when handling these materials. We recommend following the universal procedures for handling products of human origin as the minimum precaution against contamination.

GENERAL INFORMATION

GFP Expressing Human Brain Astrocytes were isolated from normal human brain Astrocytes and transfected with GFP-Lentiviral particles. Puromycin resistant, GFP-HBA is shipped in proliferating culture or frozen vial with a confluence of > 90% (cells are provide @ passage 3). Astrocyte-Growth medium (CAT# AGPM-03) containing 5% fetal bovine serum and growth supplement is recommended for culture. Cells have an average population doubling level >8 when cultured. When you receive the cells, leave the flask in 37°C C02 incubator for 1 hour. Then, replace the transport medium with fresh Astrocyte-Growth medium (CAT#AGPM-03). Let the cells grow for 24 hours before subculture.

CELL CHARACTERIZATION

Cytoplasmic GFAP	>98% positive by immunofluorescence
GFP Expressing Human Brain Astrocytes are negative for	HIV-1, HBV,HCV, and mycoplasma

PRODUCT USE AND SHIPPING STATUS

Product Use	GFP Expressing Human Brain Astrocytes cells are for research use only
Shipping Status	Proliferating cells in T25 Flask or Frozen vial

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SUBCULTURE PROTOCOL

***If Cells arrive frozen:**

When you receive the cells in a frozen vial, you can transfer the vial of cells into -80°C freezer for short period storage or a liquid nitrogen tank for long term storage.

1) Coating T25 flasks. Add 2 ml AlphaBioCoat (CAT# AC001) into a T25 flask and ensure entire interior surface is coated with the solution. After 30 minutes, dispose of AlphaBioCoat (CAT# AC001) by aspiration. Gently rinse and aspirate flask with Phosphate Buffer Solution (1XPBS-001). The flask is now ready for use (no need for overnight incubation when coated with CAT# AC001)

2) If you are using the coated flask the same day, add about 4 ml of Astrocyte-Growth media (CAT# AGPM-03) 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.

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

4) Transfer the vial into your Biosafety cabinet.

5) Using a 2 or 5ml pipet, pipet the cells out of the vial.

6) Transfer your cell suspension in to your coated plate that have the 4 ml media in it.

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

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

9) Change media after 48 hours.

If Cells arrive in a T25-flask:

*Coating T25 flasks. Add 2 ml AlphaBioCoat (CAT#AC001) into 2- T25 flasks and ensure entire interior surface is coated with the solution. After 30 minutes, dispose of AlphaBioCoat (CAT#AC001) by aspiration. Gently rinse and aspirate the flask with Phosphate Buffer Solution (CAT# IXPBS-001). The flask is now ready for use (no need for overnight incubation when coated with CAT#AC001). Add fresh media to flask, if color changes from pink to yellow, discard the media, and add fresh media to each flask.

1. Inspect to make sure Flask is at 90% confluence, if not remove transport media, and add 5ml of fresh media to the flask. Place flask in 37°C incubator until cells are at 90% confluence. Change media every 48 hours.

2. If flask is at 90% confluence, aspirate transport media from flask.

3. Rinse T25 flask containing cells with 5 ml 1XPBS-001.

4. Gently aspirate out the 1XPBS-001 after rinsing, and discard.

5. Add 2ml of to T25 flask **AlphabioDetach** Cell Detachment Solution (CAT# ADF001) containing cells (ensure entire interior surface is covered).

6. 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. Strike the flask against palm of hand to detach cells

7. Suspend the cells with 10ml of Astrocyte-Growth medium (CAT# AGPM-03) and transfer equally into 2 pre-coated T25 flasks (the cells are now at a subculture ratio of 1:2.

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

9. Proliferating cells culture: Astrocytes-Growth medium (CAT# AGPM-03) should be changed every 2 days. The cells normally become confluent within 7 days (when split at a 1:2 ratio)

10. Use Astrocyte-Basal media (CAT# AGPM-04) containing 0.5% FBS to induce quiescent cells (after 18-24 hours).