Human Brain Microglia Cells – Immortalized

#Cat: NB-26-00180-500 Size:500,000cells

Catalog #: NB-26-00180-500

Cell #: >5x10^5 cells

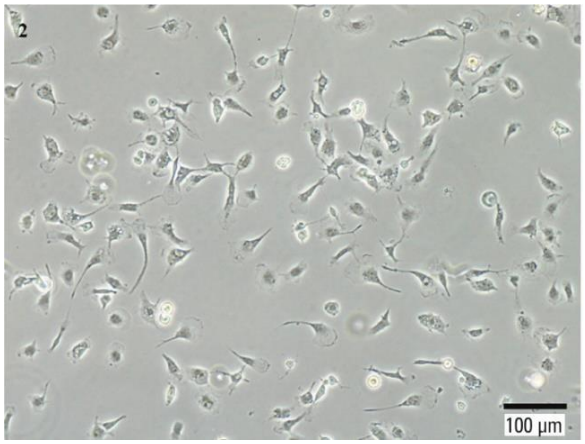
Storage: Liquid Nitrogen until ready for culture.

While Culturing keep in 37°C CO_2 incubator

Product Format: Frozen Vial

General Information

Human brain microglia cells were isolated from healthy human brain tissue. Human Brain Microglia Cells Immortalized were selected from HBMgs infected with lentiviruses expressing hTert with puromycin. HBMgs can be cultured in the long term (tested >20 passages). It is recommended to use Microglia Cell culture growth Medium for the culturing of HBMgs.



Product is for Research use only.

Frozen Vials are shipped in a Dry Ice Package.

Charaterization of The Cells

1. Cytoplasmic F4/80 Positive
2. CD68 Positive
3. HBMgs are negative for HIV-1, HBV, HCV, and mycoplasma.

Handling of Arriving Cells

1. Check all containers for leakage or breakage.
2. Remove the frozen cells from the dry ice packaging and immediately place the cells at a temperature below -130°C, preferably in liquid nitrogen vapor, until ready for use.
3. To ensure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at -70°C. Storage at -70°C will result in loss of viability.

Medium

We recommend customer use our Alpha-glia Expansion Medium (NB-26-01694) to culture these cells.

Protocol For Thawing The Cells

To ensure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at -70°C. Storage at -70°C will result in loss of viability.

1. Prepare an AlphaBioCoat (cat. NB-26-01690) coated flask (T-25 flask is recommended). Add 5 ml of sterile Alphabiocoat solution to a T-25 flask. Leave the flask in an incubator for a (minimum of one hour at 37C incubator).
2. Rinse the AlphaBiocoat coated flask with sterile 1xPBS twice and add 7 ml of complete medium to the flask. Leave the flask in the hood and go to thaw the cells.
3. Warm medium before thawing the cells.
4. Place the vial of cells in a 37C water bath, hold and rotate the vial gently until the contents are completely thawed. Remove the vial from the water bath immediately, wipe it dry, rinse the vial with 70% ethanol and transfer it to a sterile field. Remove the cap, being careful not to touch the interior threads with fingers. Using 1 ml Eppendorf pipette gently resuspend the contents of the vial.
5. Dispense the contents of the vial into the equilibrated, AlphaBiocoat coated culture vessels. A seeding density of more than 10,000 cells/cm2 is recommended.

6. Replace the cap or cover, and gently rock the vessel to distribute the cells evenly. Loosen cap if necessary to permit gas exchange.
7. Return the culture vessels to the incubator (For best result, do not disturb the culture for at least 16 hours after the culture has been initiated.
8. Change the growth medium the next day to remove the residual DMSO by centrifuging the unattached cells down, resuspending the cells with 5ml fresh medium, and adding the cells back to the flask. Then change the medium every other day thereafter).
9. Change the medium every two to three days thereafter. centrifuge unattached cells down, resuspend the cells with 5ml fresh medium and add the cells back to the flask. These cells are low-attached cells – it will take weeks before they can fully be attached to the plate. Floating cells are not dead cells.

Subculturing The Cell:

1. Prepare an AlphaBioCoat (cat. NB-26-01690) coated flask (T-25 flask is recommended). Add 5 ml of sterile Alphabiocoat solution to a T-25 flask. Leave the flask in an incubator for a (minimum of one hour at 37C incubator).
2. Rinse the AlphaBiocoat coated flask with sterile 1xPBS twice and add 7 ml of complete medium to the flask. Leave the flask in the hood and go to thaw the cells.
3. Warm medium
4. * Check your cells under a microscope, most of the cells should be floating. Dispense the cells into a centrifuge tube.
5. ** If you have attached cells, remove them by gently washing them with 1x PBS. Discard the 1X PBS and add 1 ml of cell detachment solution. Place the flask with attached cells into the incubator for a few minutes. Once detached place cells into the same centrifuge tube with floating cells.
6. Centrifuge for 10 minutes at 1000 RPM. Once the centrifuge stops, remove the old media, and add new media to the centrifuge tube.
7. Resuspend the cells, and plate them 1:2
8. Return the culture vessels to the incubator (For best result, do not disturb the culture for at least 16 hours after the culture has been initiated.
9. Change the medium every two to three days thereafter. centrifuge unattached cells down, resuspend the cells with 5ml fresh medium, and add the cells back to the flask. These cells are low-attached cells – it will take weeks before they can fully be attached to the plate. **Floating cells are not dead cells.**