INSTITUTO FERREYRA

Laboratorio Bollo
Instituto Ferreyra

Primary cultures of Human and Mouse astrocytes

Human and mouse astrocytes are cultured according to the procedures described in [1,2,3,4.]

Protocol for Astrocyte Isolation from Human Brain Tissue

Human astrocytes are obtained from brain biopsies of anonymous patient waste tissues, and purified from non-infiltrated tumor brain regions at least 2 cm away from the contrast-enhancing tumor core or from the entry cortex of epilepsy surgeries. The protocols have received approval from the Institutional Ethics Committee of Hospital Privado Universitario de Córdoba, Argentina. Human brain tissue should be collected and transported in transport solution (see formulation below) and kept on ice. Place 1X HANKS solution in the refrigerator ahead of time to ensure it is well chilled before use. Tissue must be processed immediately.

1) In the hood, place the tissue in a sterile dish and cover it with 1X HANKS (HBSS) solution. Keep the dish on a cold pack to maintain low temperature.
2) Under a dissection microscope, use forceps and scissors to remove blood vessels and meninges (retaining the clearer part of the tissue).
3) Transfer the cleaned tissue to a sterile culture dish and chop the tissue into small pieces using scissors.
4) Transfer the minced tissue to a new sterile culture dish and cover with 0.25% trypsin-EDTA (ThermoFisher # 25200056). Incubate at 37°C, 5% CO_2 for 10-15 minutes. *Note: monitor cell dissociation under the microscope every 4–5 minutes.*
5) Transfer the tissue to a 15 ml Falcon tube. Add an equal volume of Astrocyte Medium (see receipt below) to neutralize trypsin. Centrifuge at ~300 × g for 5 minutes.
6) Remove the supernatant and resuspend the pellet in 1 ml of astrocyte medium. Dissociate the tissue mechanically by pipetting up and down using a micropipette with a 1000 µL tip (~10 times).
7) Pass the homogenate through a 40 µm cell strainer (JET BIOFIL #CSS013040) into a 50 ml Falcon tube.
8) If the original tissue had a volume of approximately 0.6 cm^3, plate the content of the 50 ml Falcon tube into a T75 flask containing 8–10 ml of astrocyte medium (pre-warmed to 37 °C). *Note: if the tissue had a smaller volume, plate the cells in one or two T25 flasks.* Place the flask in the incubator at 37°C, 5% CO_2 overnight.
9) The next day, collect the medium and replace it with 10 ml of fresh pre-warmed to 37 °C Astrocyte Medium.
10) **Culture Maintenance**: allow cultures to grow for 7–21 days. Optimal confluency and purity are usually reached by day 8. Change the medium every 3–4 days.
11) Cells can also be cryopreserved. To increase cell numbers, the initial T75 flask (passage 0) can be trypsinized and split into two flasks (passage 1). Once cultures reach 85–95% confluency, cells can be frozen. Based on our experience, these human astrocytes can be passaged up to passage 5 without losing their phenotype.

INSTITUTO FERREYRA

Laboratorio Bollo
Instituto Ferreyra

Protocol for Astrocyte Isolation from Mouse Brain Tissue

1) Remove the brain from a mouse pup (preferably P0–P7), and dissect the brain region of interest. If cortical astrocytes are to be used, follow the protocol described in Schildge et al. (2013)[4], and use four mouse pup cortices per T75 tissue culture flask.
2) Immediately place the dissected tissue in a 50 ml Falcon tube containing chilled PBS with 1X Penicillin/Streptomycin to rinse.
3) In the hood, transfer the brain to a sterile dish. Using a sterile single-edge razor blade, finely mince the brain into small pieces. Gently remove excess PBS. Add 0.25% trypsin-EDTA (ThermoFisher # 25200056) to the culture dish. Incubate at 37 °C for 10-15 minutes. *Note: monitor cell dissociation under the microscope every 4–5 minutes.*
4) Transfer tissue into a 15 ml Falcon tube and add an equal volume of astrocyte medium to neutralize the trypsin. Centrifuge at ~300 × g for 5 minutes. Carefully decant the supernatant, and resuspend the pellet in 1 ml of astrocyte medium.
5) Titrate the tissue up and down using a micropipette with a 1000 µL tip (~10 times) until no visible clumps remain.
6) Filter ~1 ml of the dissociated cell suspension through a 40 µm cell strainer (JET BIOFIL #CSS013040) into a clean 50 ml Falcon tube.
7) Plate the cell suspension into a T75 flask containing 8–10 ml of astrocyte medium. Incubate overnight at 37 °C with 5% CO_2.
8) The next day, collect the medium and replace it with 10 ml of fresh pre-warmed to 37 °C Astrocyte Medium.
9) **Culture Maintenance**: allow cultures to grow for 7–21 days. Optimal confluency and purity are usually reached by day 8. Change the medium every 3–4 days. Astrocytes are usually used for experiments at day 21 to 28 to ensure a mature phenotype of isolated astrocytes. At this point the expected yield is about 1.5-2 x10^6 cells per T75 tissue culture flask.
10) **To obtain enriched astrocyte culture**: Around day 7, when astrocytes reach confluency and microglia appear loosely attached on top, shake the flask at 180 rpm for 30 min to remove microglia. Discard the supernatant and add 20 ml of fresh astrocyte medium. Continue shaking at 240 rpm for 6 hr to eliminate oligodendrocyte precursor cells. Rinse the astrocyte layer twice with PBS, then add 5 ml of 0.25% trypsin-EDTA (ThermoFisher # 25200056) and incubate at 37 °C. Monitor detachment every 5 minutes, tapping the flask gently if needed. Once detached, add 5 ml of astrocyte medium, centrifuge at 180 × g for 5 min, discard the supernatant, and resuspend the pellet in 40 ml of medium. Plate the cells into two T75 flasks and incubate.

Astrocyte medium:

- 89% DMEM F12 (ThermoFisher #11330032)
- 10% Fetal Bovine Serum (FBS)
- 1% Penicillin-Streptomycin (ThermoFisher #15140122)

Transport solution (for human tissue):

Final concentration: ---------- For 100 ml of transport solution:
88 mM NaCl ---------- (8.8 ml of 1 M NaCl)
1 mM KCl ---------- (100 µl of 1 M KCl)
0.8 mM $CaCl_2$ ---------- (80 µl of 1 M $CaCl_2$)
0.82 mM $MgSO_4$ ---------- (82 µl of 1 M $MgSO_4$)
2.4 mM $NaHCO_3$ ---------- (240 µl of 1 M $NaHCO_3$)
10 mM HEPES, pH 7.5 -------- (2 ml of 0.5 M HEPES)
---------- (88.71 ml of MilliQ H_2O)

Supplement with Primocin (InvivoGen #ant-pm-05) → final dilution 1:500.
Filter through a 0.1 µm filter before use.
Note: Add Primocin to the transport solution and also to the culture medium until the first passage is performed.

References

1. Lin, D.T., Wu, J., Holstein, D., Upadhyay, G., Rourk, W., Muller, E., and Lechleiter, J.D. (2007). Ca2+ signaling, mitochondria and sensitivity to oxidative stress in aging astrocytes. Neurobiol Aging 28, 99-111. 10.1016/j.neurobiolaging.2005.11.004.
2. Chen, Y., Holstein, D.M., Aime, S., Bollo, M., and Lechleiter, J.D. (2016). Calcineurin beta protects brain after injury by activating the unfolded protein response. Neurobiol Dis 94, 139-156. 10.1016/j.nbd.2016.06.011.
3. Feliziani, C., Fernandez, M., Quassollo, G., Holstein, D., Bairo, S.M., Paton, J.C., Paton, A.W., de Batista, J., Lechleiter, J.D., and Bollo, M. (2022). Ca(2+) signalling system initiated by endoplasmic reticulum stress stimulates PERK activation. Cell calcium 106, 102622. 10.1016/j.ceca.2022.102622.
4. Schildge, S., Bohrer, C., Beck, K., and Schachtrup, C. (2013). Isolation and culture of mouse cortical astrocytes. J Vis Exp. 10.3791/50079.

Contact info:

PhD. Mariana Bollo - mbollo@immf.uncor.edu

Biol. Julia Dimundo - jdimundo@immf.uncor.edu