SOP: **Propagation of Human Astrocytes-cerebellar (HAc, ScienCell Research Laboratories)**
Date modified: **11/25/2010**
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Ordering Information

Human Astrocytes-cerebellar (HAc) may be ordered as frozen ampoules. Each vial contains >1 x 10^6 cells in a 1ml volume.

To order frozen ampoules + media:

Name:	HAc – Human Astrocytes-cerebellar
Item #:	1810 (HAc - Cryopreserved ampoule)
	1801 (AM, Astrocyte Medium)

Notes:

The number of media kits purchased depends on the target number of cells to be generated. A rule of thumb is 10 media kits for every initial cryopreserved ampoule. It is strongly recommended to purchase all of the media that will be required for a complete expansion series, since media supply may be erratic. All culture flasks must be coated with poly-L-lysine (2ug/cm^2).

Materials List

1. AM Astrocyte Medium (ScienCell, Cat# 1801)
2. Poly-L-Lysine (10mg/ml, ScienCell, Cat# 0413)
3. Phosphate Buffered Saline (1X PBS) (Cellgro, Cat# 21-040-CM)
4. Accutase Enzyme Cell Detachment Medium (EBioscience, Cat# 00-4555)
5. T75, T225 tissue culture flasks
6. Corning conical centrifuge tubes (15mL and 50mL)
7. Graduated pipets (1, 5, 10, 25, 50mL)
8. Pen-Strep solution (if required; ScienCell typically supplies antibiotics)
9. Eppendorf Centrifuge 5810R
10. Hemocytometer
11. Micropipet w/ P20 tips
12. Microscope

Procedure

A. Initiation of culture from cryopreserved cells

1) Immediately place frozen cells in liquid nitrogen storage until ready to culture.
2) When ready to start cell culture, rapidly thaw cells by holding vial and gently rotating in 37°C water bath.
3) As soon as ice crystals disappear, swab outside surface of the ampoule with 70% ethanol, then dispense contents of vial into a T75 flask at **5,000 cells/cm^2 density**.
4) Let cells recover for 16 hours in a 37°C, 5% CO_2 humidified incubator.
5) The next morning, the diluted DMSO-containing shipping/cryopreservation medium is aspirated from the cell layer and replaced with fresh medium.

B. Sub-culture

1) Propagate cells until density reaches 80% confluence.
2) Aspirate medium.
3) Wash cells with warm 1X PBS.
4) Add 15mL of Accutase and return to incubator for 10-15 minutes, or until cells detach.
5) Immediately remove cells, rinse flask with warm 1X PBS to collect residual cells, and pellet at 500 x g for 5 minutes (4^oC).
6) Gently re-suspend cell pellet in warm medium.
7) Count cells with hemocytometer.
8) Add warmed medium to flasks.
9) Seed flasks at **5,000 cells/cm^2 density**.
10) Record each subculture event as a passage.

C. Maintenance

1) Change media the day after seeding and every OTHER day thereafter.
2) Increase media volume as confluency increases (volumes assume the use of
3) T225 flasks):
    a. 25% = 1mL/5 cm^2
    b. 25-45% = 1.5mL/5 cm^2
    c. 45%+ = 2mL/5 cm^2.
4) Per the above an exemplary schedule might be:
    a. day 1, plate into T225: use 50mL of media.
    b. day 2, change media, use 50mL of media.
    c. day 4, change media, use 100mL of media (if confluency is >50%).
    d. day 6, change media, use 100mL of media (or harvest if ready).
    e. day 7 or 8 (harvest when cells reach 6 x 10^6 cells/flask.

D. Harvest

1) Pass cells 3-4 times until the desired cell number is achieved (primary cells will senesce after 4-5 passages).
2) Remove cells from flasks according to protocol described above under “Sub-culture.”
3) Examine viability using Trypan blue staining (SOP TP-7).