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Expanding Rat Primary Cortical Astrocytes

You may expand Rat Primary Cortical Astrocytes (Cat. no. N7745-100) as an adherent culture on uncoated, tissue-culture treated flasks, plates or dishes. Harvest your cells when 100% confluent.

Note: We recommend that you use Rat Primary Cortical Astrocytes right after recovery. After thawing Rat Primary Cortical Astrocytes, expand the cells once to have a 1.5 to 2-fold increase in their number, and harvest them to use in your experiments.

Materials Needed

- Culture vessels containing Rat Primary Cortical Astrocytes (100% confluent)
- Uncoated, tissue-culture treated culture vessel
- Astrocyte growth medium*, pre-warmed to 37°C
  *85% D-MEM (high glucose) (Cat. no. 11995-065) and 15% Fetal Bovine Serum (Cat. no. 16000-036)
- Disposable, sterile 15-mL or 50-mL conical tubes
- 37°C incubator with humidified atmosphere of 5% CO_2
- Dulbecco's Phosphate Buffered Saline (D-PBS) without Ca^{2+}, Mg^{2+}, or phenol red (Cat. no. 14190-144)
- StemPro® Accutase® (Cat. no. A11105-01), pre-warmed to 37°C
- Hemacytometer, cell counter and Trypan Blue (Cat. no. 15250-061), LIVE/DEAD® Cell Vitality Assay Kit (Cat. no. L34951), or the Countess™ Automated Cell Counter (Cat. no. C10227)

Protocol

Note: Rat Primary Cortical Astrocytes readily stick to the plastic used in cell culture dishes and centrifuge tubes. Prior to use, rinse all material that will come in contact with the cells with medium to prevent cells from sticking to the plastic.

1. Remove the spent growth medium from the culture dish containing the cells, and store in a sterile tube to use as a washing solution.
2. Rinse the surface of the cell layer once with D-PBS without Ca^{2+} and Mg^{2+} (approximately 2 mL D-PBS per 10 cm^2 culture surface area) by adding the D-PBS to the side of the vessel opposite the attached cell layer, and rocking back and forth several times.
3. Aspirate the D-PBS and discard.
4. To detach the cells, add 3 mL of pre-warmed StemPro® Accutase® Cell Dissociation Reagent per T75 flask; adjust volume accordingly for culture dishes of other sizes.
5. Incubate for up to 20 minutes at 37°C. Rock the cells every 5 minutes, and check for cell detachment and dissociation toward single cell under the microscope.
6. Once you observe cell detachment, gently pipette up and down to break clumps into a single cell suspension. Stop the cell dissociation reaction by an adding equal volume of the spent medium from step 1. Disperse the medium by pipetting over the cell layer surface several times.
7. Transfer the cells to a new 15-mL or 50-mL pre-rinsed conical tube, and centrifuge at 250 × *g* for 5 minutes at room temperature. Aspirate and discard the supernatant.
8. Gently resuspend the cell pellet in pre-warmed astrocyte growth medium and remove a sample for counting.
9. Determine the total number of cells and percent viability using your method of choice. If necessary, add astrocyte growth medium to the cells to achieve the desired cell concentration and recount the cells.
10. Plate cells in an uncoated tissue-culture treated vessel at a seeding density of 2×10^4 cells per cm^2.
11. Incubate cells at 37°C, 5% CO_2, and 90% humidity, and change growth medium every 4–5 days.

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