invitrogen™

Thawing and Establishing Rat Primary Cortical Astrocytes

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

- Rat Primary Cortical Astrocytes (Cat. no. N7745-100), stored in liquid nitrogen
- 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 conical tubes, pre-rinsed with growth medium
- 37°C water bath
- 37°C incubator with humidified atmosphere of 5% CO_2
- Flame-polished and autoclaved glass Pasteur pipettes, or plastic Pasteur pipettes pre-rinsed with growth medium
- 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 cells from liquid nitrogen storage, and **immediately** transfer the cells to a 37°C water bath to prevent crystal formation.
2. Quickly thaw the vial of cells by gently swirling it in the 37°C water bath and removing it when the last bit of ice has melted, typically < 2 minutes. Do not submerge the vial completely. **Do not** thaw the cells for longer than 2 minutes. **Do not** introduce bubbles into the cell suspension as it decreases cell viability.
3. When thawed, transfer the tube containing the cells into the laminar flow hood, and wash the outside of the tube with 70% isopropanol.
4. **Very gently** transfer the cells into a **pre-rinsed** 15-mL centrifuge tube using a Flame-polished and autoclaved glass Pasteur pipette, or a **pre-rinsed** plastic Pasteur pipette.
5. Rinse the vial with 1 mL of astrocyte growth medium, and **dropwise** add to the cells in the 15-mL centrifuge tube (one drop/second). Mix by gentle swirling after each drop.
6. Dropwise add 8 mL of astrocyte growth medium to the cell solution and mix gently.
7. Centrifuge the cells at 250 × *g* for 5 minutes.
8. Aspirate the supernatant and resuspend cells in 2 mL of astrocyte growth medium.
9. Determine the viable cell count using your method of choice.
10. Plate the cells at a seeding density of 2×10^4 cells per cm^2 on an uncoated, tissue-culture treated culture dish. If necessary, gently add growth medium to the cells to achieve the desired cell concentration and recount the cells.
11. Incubate at 37°C, 5% CO_2, and 90% humidity. Replace the medium with an equal volume of fresh, pre-warmed astrocyte growth medium every 4–5 days.
12. Passage cells when the culture is 100% confluent.

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