invitrogen™

Passaging Rat Fetal Neural Stem Cells (Adherent Culture)

You may maintain Rat Fetal Neural Stem Cells (NSCs) (Cat. nos. N7744-100, N7744-200) as an adherent culture on CELLStart™, fibronectin, or poly-L-ornithine coated, tissue-culture treated flasks, plates or dishes. Subculture adherent cells when they are 75–90% confluent, before colonies start contacting each other.

Materials Needed

- Culture vessels containing Rat Fetal NSCs at 75–90% confluency
- CELLStart™ (Cat. no. 10142-01), fibronectin (Cat. no. 33016-015), or poly-L-ornithine coated, tissue-culture treated flasks, plates, or Petri dishes
- Complete StemPro® NSC SFM (Cat. no. A10509-01), pre-warmed to 37°C
- 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)

Passaging Adherent Rat Fetal NSCs

1. Aspirate the complete StemPro® NSC SFM from the cells.
2. Rinse the surface of the cell layer 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 1 mL of pre-warmed StemPro® Accutase®. Cells will be lifted off from the culture dish right after the application of StemPro® Accutase® (within approximately 30 seconds).
5. Once you observe cell detachment, gently pipette up and down break clumps into a single cell suspension. Stop the cell dissociation reaction by adding 4 mL of complete StemPro® NSC SFM. Disperse the medium by pipetting over the cell layer surface several times
6. Transfer the cells to a 15-mL or a 50-mL conical tube and centrifuge at 300 × *g* for 4 minutes at room temperature. Aspirate and discard the medium.
7. Resuspend the cell pellet in a minimal volume of pre-warmed complete StemPro® NSC SFM and remove a sample for counting.
8. Determine the total number of cells and percent viability using your method of choice. If necessary, add complete StemPro® NSC SFM to the cells to achieve the desired cell concentration and recount the cells.
9. Add complete StemPro® NSC SFM to each tube containing cells so that the final viable cell concentration is 5×10^4 cells per cm^2.

   Note: If you are culturing Rat Fetal NSCs in growth medium other than complete StemPro® NSC SFM, make sure to supplement the medium every day with bFGF to 10 ng/mL to maintain your cells undifferentiated.
10. Add the appropriate volume of cells to each culture vessel and incubate at 37°C, 5% CO_2 and 90% humidity.
11. When cells reach 80–90% confluency (3–4 days after seeding), completely remove the medium, and replace with an equal volume of complete StemPro® NSC SFM.

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