

Passaging Rat Fetal Neural Stem Cells (Neurospheres)

You may maintain Rat Fetal Neural Stem Cells (NSCs) (Cat. nos. N7744-100, N7744-200) as neurospheres in a suspension culture. Passage NSCs in suspension when neurospheres are larger than 3.5 mm in diameter.

Materials Needed

- Culture vessels containing Rat Fetal NSCs at 75–90% confluency
- Uncoated, 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% CO₂
- Dulbecco's Phosphate Buffered Saline (D-PBS) without Ca²⁺, Mg²⁺, 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 Rat Fetal NSCs in Suspension (Neurospheres)

1. Transfer medium with neurospheres into a 15-mL or 50-mL sterile conical tube.
2. Leave the tube at room temperature to let the neurospheres settle at the bottom of the tube by gravity. Alternatively, you may centrifuge the neurospheres at 200 × g for 2 minutes.
3. Aspirate the medium carefully to leave the neurospheres in a minimal volume of medium
4. Wash the neurospheres with 10 mL of D-PBS without Ca²⁺ and Mg²⁺, and leave a minimal volume of D-PBS.
5. Add 1 mL of pre-warmed StemPro® Accutase® to the neurospheres, and incubate for 10 minutes at room temperature.
6. After incubation, gently pipette the cells up and down to get a single cell suspension.
7. Stop the StemPro® Accutase® treatment by adding 4 mL of complete StemPro® NSC SFM.
8. Centrifuge cells at 300 × g for 4 minutes at room temperature. Aspirate and discard the medium.
9. Resuspend the cell pellet in a minimal volume of pre-warmed complete StemPro® NSC SFM and remove a sample for counting.
10. 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.
11. Seed the cells in fresh complete StemPro® NSC SFM at 2 × 10⁵ viable cells per cm² in a suspension dish or a non-coated flask or Petri dish.
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.
12. Incubate the cells at 37°C, 5% CO₂, and 90% humidity.

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