Dissociation of NSCs and Neurospheres with Accutase

Dissociation of adherent human or rat NSCs

1. Aspirate the medium from culture dish
2. Add 2 ml of Accutase to culture dish.
3. Incubate for 2 - 5 minutes at 37 °C until individual single cells start to round up.
4. Gently rinse to remove cells from the plate's surface.
5. Transfer cell suspension to 15 ml conical tube. Gently pipette up and down until cells are in a single cell suspension.
6. Add 8 ml of medium to rinse any remaining cells from the dish's surface and transfer to the conical tube (from Step 5).
7. Take a 20 µl sample of the cell suspension to determine viable cell density.
8. Centrifuge conical tube containing the cell suspension at 200 g for 4 minutes.
9. Aspirate supernatant, resuspend in fresh medium and plate on coated dish(s). Incubate at 36 - 38°C in a humidified atmosphere of 4 to 6% CO_2 in air.

Dissociation of human or rat neurosphere cultures

1. Remove neurosphere cell suspension from culture dish and transfer to a 15 ml conical tube.
2. Let neurospheres settle down in the tube (~2-5 minutes) before proceeding to Step 3. Alternatively, the cells can be centrifuged at 100 g for 1 minute.
3. Gently aspirate medium leaving the neurospheres at the bottom of tube with approximately 100 µl of media remaining.
4. Resuspend neurospheres in 5 ml D-PBS.
5. Let neurospheres settle down in the tube (~2-5 minutes) before proceeding to Step 6. Alternatively, the cells can be centrifuged at 100 g for 1 minute.
6. Gently aspirate D-PBS leaving the neurospheres at the bottom of tube with approximately 100 µl of D-PBS remaining.
7. Add 1 ml of Accutase to the neurospheres and incubate 10 minutes at room temperature.
8. Using the proper sized pipette tip (i.e.1000 µl), pipette up and down until all the neurospheres are in a single cell suspension.

9. Add 4 ml of fresh medium to the tube.
10. Centrifuge the cells at 200 g for 4 minutes.
11. Gently aspirate the supernatant.
12. Resuspend cells in fresh medium, transfer to a new culture dish and incubate at 36-38°C in a humidified atmosphere of 4 to 6% CO_2 in air.