Dissociation of Neural Stem Cells (NSCs) and Neurospheres with Accutase® Cell Detachment Solution

Protocol

CORNING

Accutase® is an effective solution for routinely detaching cells from standard tissue culture-treated vessels, as well as advanced surface treatments or coatings. Accutase is useful for cell detachment and for preparing single-cell suspensions from clumped cell populations for sub-culturing cells, analytical studies, and for accurate cell counting. Accutase is free of mammalian or bacterial-derived products, which reduces the risk of contamination.

Accutase is formulated at a concentration that is ready to use, once thawed. **(Note: Never thaw Accutase at 37°C.)** A thawed bottle of Accutase can be removed from the refrigerator and immediately applied to cells. **It should not be pre-warmed to 37°C.** Accutase contains proteolytic activity that gently breaks down cellular adhesion molecules and enables cell detachment from the bottom of the flask.

Protocol for dissociation of adherent human or rat NSCs

1. Aspirate the medium from a culture dish.
2. Add 2 mL of Accutase (Corning Cat. No. 25-058-CI) to the culture dish.
3. Incubate for 2 to 5 minutes at 37°C until the cells start to round-up and detach.
4. Gently rinse to remove cells from the plate's surface.
5. Transfer cell suspension to a 15 mL conical tube. Gently pipet up and down until cells are in a single-cell suspension.
6. Add 8 mL of medium to rinse any remaining cells from the surface of the dish 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 x g for 4 minutes.
9. Aspirate supernatant, resuspend in fresh medium to desired cell density, and plate on coated dish(es). Incubate at 36 to 38°C in a humidified atmosphere of 4% to 6% CO_2.

Protocol for 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 to 5 minutes) before proceeding to Step 3. Alternatively, the cells can be centrifuged at 100 x g for 1 minute.
3. Gently aspirate medium leaving the neurospheres at the bottom of the tube with approximately 100 µL of media remaining.
4. Resuspend neurospheres in 5 mL Dulbecco's Phosphate-Buffered Saline (DPBS).
5. Let neurospheres settle down in the tube (~2 to 5 minutes) before proceeding to Step 6. Alternatively, the cells can be centrifuged at 100 x g for 1 minute.
6. Gently aspirate DPBS leaving the neurospheres at the bottom of tube with approximately 100 µL of DPBS remaining.
7. Add 1 mL of Accutase to the neurospheres and incubate for 10 minutes at room temperature.
8. Using the proper sized pipet tip (i.e., 1000 µl), pipet 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 x g for 4 minutes.
11. Gently aspirate the supernatant.
12. Resuspend cells in fresh medium to desired cell density, transfer to a new culture dish, and incubate at 36°C to 38°C in a humidified atmosphere of 4% to 6% CO_2.

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At Corning, cells are in our culture. In our continuous efforts to improve efficiencies and develop new tools and technologies for life science researchers, we have scientists working in Corning R&D labs across the globe, doing what you do every day. From seeding starter cultures to expanding cells for assays, our technical experts understand your challenges and your increased need for more reliable cells and cellular material.

It is this expertise, plus a 160-year history of Corning innovation and manufacturing excellence, that puts us in a unique position to offer a beginning-to-end portfolio of high-quality, reliable cell culture consumables.

For additional product or technical information, please visit www.corning.com/lifesciences/media or call 1.800.235.5476.

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