Typical Cell Passaging Protocol Using Accutase

Accutase is formulated at a concentration that is ready to use, once defrosted. (Note: Never defrost a bottle of Accutase at 37°C.) A defrosted bottle of Accutase can be removed from the refrigerator and immediately applied to cells. It does not need to be and should not be pre-warmed to 37°C. Accutase contains proteolytic and collagenolytic enzymes to gently break down the cell adhesion structure on the outside of cells that attaches them to the bottom of the flask.

This entire procedure should be done in a laminar flow hood using proper aseptic technique.

1. Carefully aspirate all the media from the cell culture flask. (Rinsing with PBS is not necessary.)
2. Immediately add enough Accutase to the flask to cover the cells. (Typically, 2.5 to 5 ml for a T25 flask depending upon confluency and density of the cell culture.)
3. Set the flask aside at room temperature for 5 to 10 minutes up to a maximum of 1 hr. Check the flask frequently to see if the cells have rounded rather than merely shrunken or no longer appear “spidery” while remaining attached to the bottom of the flask.
4. Once the cells have turned into “balls”, smack the flask against the palm of your hand to dislodge any “stickers”.
5. Gently disperse the cells and take a sample of the cell suspension to determine the viable cell density.
6. Add an aliquot of the detached cells to fresh media in new flasks. Place the flasks into the 37°C incubator. No neutralization steps are required. The cells will reattach within a few minutes depending upon cell type.