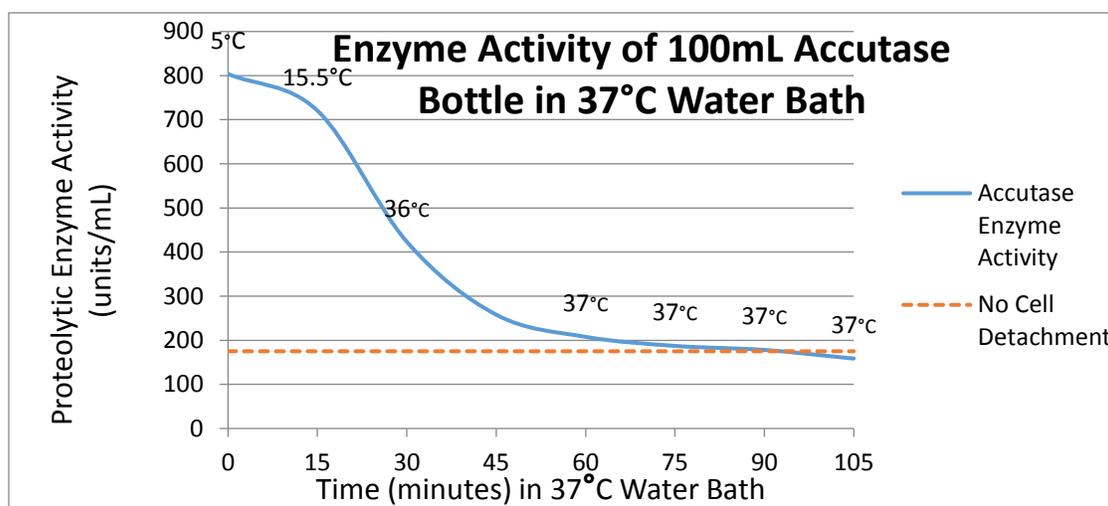




Heat Inactivation of Accutase®

Accutase, cell detachment solution, is a gentle yet effective product when used correctly. One key factor in using the product correctly is to defrost it properly. The enzymes in Accutase are temperature sensitive; When stored at 4°C, a bottle of Accutase can be kept for a couple of months, however, once raised to 37°C, it quickly loses its effectiveness. Defrosting Accutase in a 37°C bath for too long can inactivate Accutase. To illustrate this property of Accutase, the following experiment was conducted.

The time required for a bottle of Accutase to become inactivated when exposed to 37°C was determined by measuring the proteolytic enzyme activity of Accutase as it was brought to, and held at, 37°C. It was established that the Accutase required about 90 minutes to be deactivated. This can be observed in the graph below at the point in which the enzyme activity falls below 175 units per mL (proteolytic enzyme activity must be above 175 units/mL to properly detach cells). This time frame, however, includes the amount of time required for the sample to reach 37°C. When the acclimation time is removed, the Accutase requires about an hour to be inactivated. This is important to note, as the volume of Accutase used in cell passaging varies, thus causing the acclimation time frame to vary. The following graph contains the data collected in this experiment.



In this graph, the blue line represents the proteolytic enzyme activity of Accutase while measured over time. Time zero represents Accutase at 4°C. The temperature of the sample at the given time is noted above the blue line. The red dotted line represents the level of enzyme activity which will not detach cells.

Conclusion: It is very important that Accutase is defrosted correctly. Leaving a bottle in a 37°C water bath until it is visibly defrosted could degrade the performance of Accutase as it will be inactivated after 1 hour at 37°C. It is therefore recommend that Accutase be defrosted overnight in the refrigerator or in a bath of cold water. It should also be noted from this data that placing your cells in the incubator for longer than an hour will not increase the amount of cells detached.