



Freeze-Thaw Accutase[®] Stability Report

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Innovative Cell Technologies ships Accutase/Accumax on Blue Ice bricks instead of dry ice. As a result, the Accutase/Accumax may arrive at your lab partially defrosted, is the product still good? Why does ICT do this? Why not dry ice?

- Accutase/Accumax does not need to remain frozen until used. It can be defrosted and frozen several times without affecting its performance. See data at the end of this document.
- Dry Ice is bad for the environment. It is frozen CO₂. It turns into global warming gas.
- Dry Ice is more expensive than blue ice, this increases the shipping charges we must charge you, the customer.
- Dry Ice, i.e. CO₂ gas can get inside the Accutase bottles during shipping, causing the Accutase to turn an ugly yellow color when defrosted. While still OK to use, you, as the customer are convinced the product is bad and want it replaced. Increases our shipping costs.

For these reasons, we ship Accutase/Accumax on Blue Ice. If we could guarantee that our products would arrive at the customer's site below room temperature, we would not even ship our products frozen, as they are stable for many months at refrigerator temperature (4-8C). If our products arrive at your lab with an "ice cube" in the bottle, the product is fine. If our products arrive at your lab completely defrosted and they are still cool to the touch, the product is fine. After removal from the shipping box, the products can be placed in the refrigerator for later use or refrozen. Your choice.

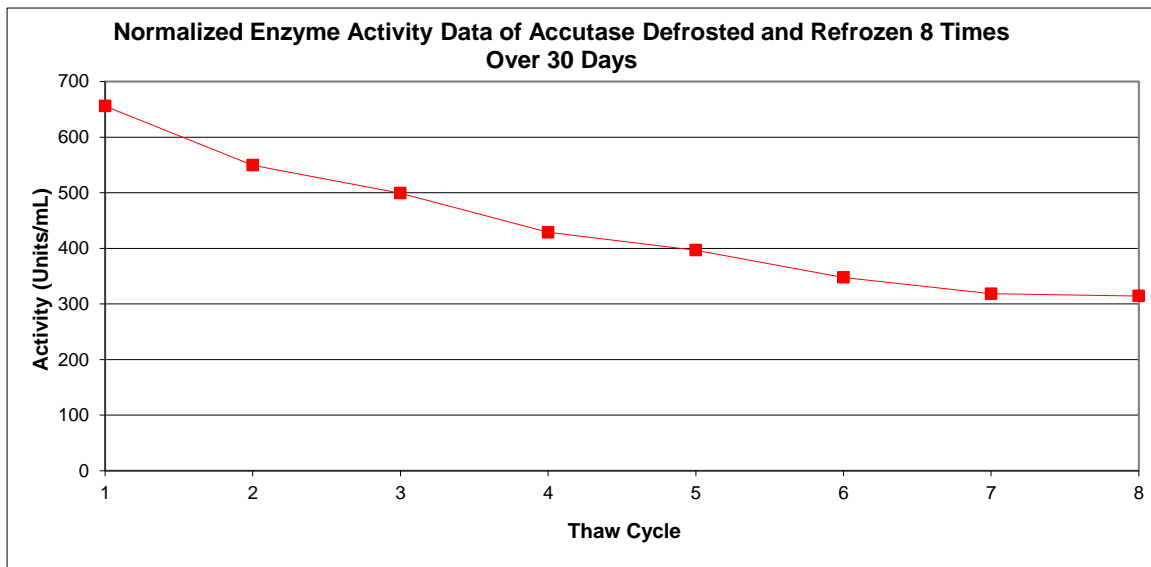
For those of you that find this hard to believe or want some hard data, this section is for you. First, we present the anecdotal data. ICT has been shipping Accutase/Accumax on Blue Ice for 15 years all over the world. That is close to 1 million bottles we have produced and shipped. If we had a problem with our shipping methods, wouldn't you think we would have changed them a long time ago? Otherwise, we would be out of business. Have you ever made home-made soup? And made too much and decided to freeze some down for another meal? Later, you pull a frozen container of the soup out of the freezer to defrost, but a few hours later, you get invited out to dinner. Did you throw out the partially defrosted soup or did you put it back in the freezer for another time?

ICT has developed 3 chromogenic enzyme activity assays that correlate with cell detachment efficiency. We test every lot of Accutase/Accumax we produce with these three assays as part of our QC release criteria. (The results of one of these assays can be



found on every one of our C of A's). We know from multiple in house studies that an average of the 3 chromogenic enzyme activities of 200 units is required to detach 90% of the cells in 5 minutes in a confluent tissue flask.

Here is an experiment where we took three different lots of Accutase, then defrosted them completely and refroze the product 8 times retesting their enzyme activity levels each time over 30 days.



This data shows there is degradation of the enzyme activity of the Accutase each time the Accutase is thawed and refrozen. However, the degradation is not enough to significantly affect the cell detachment performance of the product even after 8 cycles.

Now, back to the original question, “My Accutase/Accumax arrived partially thawed, is it still OK to use?” If the Accutase/Accumax can survive eight complete freeze/thaw cycles, can it survive one partial freeze/thaw cycle? Yes.

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