

## **BASIC RULES FOR HANDLING AN ENZYME**

### **Up-dated October 2011**

For the novice--basic hints to guide you through your first enzymatic reaction.

For the expert--a refresher course and an aid for training your students.

- I. For best stability, enzymes should be stored in their original commercial form (lyophilized, ammonium sulfate suspension, etc.) at the appropriate temperature as specified on the label.
- II. Enzymes should be diluted for use with an ice-cold buffer or distilled water, as appropriate for the enzyme.
- III. Diluted enzyme solutions are generally unstable. The amount of enzyme required for the experiment should be diluted within 1-2 hours of use. Enzymes should not be diluted for long-term storage.
- IV. Do not shake crystalline suspensions, since oxygen tends to denature the enzyme. The material should be resuspended with gentle swirling or rolling on the laboratory bench.
- V. Vials containing lyophilized enzymes (as well as cofactors, such as NADH and NADPH) should be warmed to room temperature before opening. This prevents condensation of moisture onto the powder, which can cause loss of activity or degradation. If the reagent is hygroscopic, one such mishandling may well ruin the entire vial.
- VI. Enzyme solutions or suspensions normally stored at 4<sup>o</sup> or -20<sup>o</sup> C should be kept in an ice bath when used at the laboratory bench.
- VII. Avoid repeated freeze-thawing of diluted enzymes and lyophilizates in solution. Store in small aliquots. The stability of individual enzymes may vary greatly and often should be determined empirically under your exact conditions.
- VIII. Detergents and preservatives should be used with caution, since they may affect enzyme activity.
- IX. Enzymes, especially those that have been diluted, should be checked for activity periodically to ensure that any slight loss in activity is taken into account when designing an experimental protocol. Our products are guaranteed through the control date printed on the vial, only when stored in the original form and at the correct temperature.
- X. Enzymes should be handled carefully to avoid contamination of any kind. Use a fresh pipette for each aliquot that is removed from the parent vial. Never return unused material to the parent vial. Wearing gloves protects both you and the enzyme.

XI. Adjust the pH of the enzyme buffer at the temperature at which it will be used. Many common buffers are exceptionally sensitive to temperature. The pH of a solution containing the buffer Tris decreases 0.3 pH units for every 10 °C rise in temperature. More details on this phenomenon can be found in the Lab Hints section of BM Biochemical Vol. 1, No. 3, August 1984.

XII. Detailed information is available on many enzymes. The most complete references are:

Methods in Enzymology, published by Academic Press, Editors-in-chief: Sidney P. Colowick and Nathan O. Kaplan. There are more than 110 volumes in this series, covering an extensive range of topics.

The Enzymes, 3rd edition, edited by Paul D. Boyer, an excellent, broad series with less emphasis on methodology than Methods in Enzymology.

Methods of Enzymatic Analysis, 2nd and 3rd editions, Editor-in-chief: Hans U. Bergmeyer, published by Verlag Chemie. In-depth discussion of techniques of analysis which use enzymes or which assay enzymes.