

Considerations in Experimental Design

For enzyme characterization and purification, good numerical data suggest that the process was successful. How do we measure success?

Since an enzyme is a biological catalyst, we predict that a reaction will occur more rapidly in the presence of the enzyme. Thus, to **control** for enzyme activity, we incubate substrate without enzyme. Any product that forms in the absence of enzyme is interpreted to be from spontaneous chemical NOT biochemical reaction.

To ensure that a reaction has indeed occurred, we should expect that the rate of the reaction will increase with increasing substrate or with increasing enzyme in the presence of excess substrate. **Dose-dependence** of reaction rate is another prime indicator that a controlled reaction has occurred.

As well, we prepare at least **duplicates** for every reaction. Two identical mixtures should show the same behavior. In reality, we often do not see identical behavior even though we have added the same amount of each component. Statistically, it is rare to get identical results. Technical skill in pipetting, care in mixing the cells to get maintain a uniform suspension, degree of drying of each disc --- all contribute to technical and scientific variation. This is why we draw scatter plots, calculate average times, calculate standard deviations.

Repeating the experiment also helps. **Reproducibility** of results can occur within the same class period for the catalase assay, because the reaction takes only a few minutes. When different teams get similar results, this can be also treated as a contribution to overall confidence in the enzyme assay.

A scientist studying the same enzyme every day does not repeat the procedure exactly in machine fashion, but does include **external and internal standards**, no-enzyme and no-substrate **blanks**, etc. New shipments of substrate might also be tested to identify the material. Retractions embarrassing to the authors have had to be made on professional research reports when the scientists discovered that the presumed material was actually something else!

If enzyme activity can be measured more than one way, e.g., by floating disc and by a spectral shift in light absorbance by peroxide as it decomposes, the researcher again has increased confidence in the biochemical phenomenon.

By going back and repeating certain aspects of the study while accumulating new evidence, a researcher actually develops knowledge in a spiral fashion. It may look like running in circles from one point of view (enzyme assays every day) but the deepening knowledge generates intellectual and personal satisfaction and sometimes new discoveries that can even help researchers in other fields.