Smart Note

What are enzyme assays?

Enzymes are a special kind of protein found in cells of living organisms. They're made up of long chains of amino acids held together by peptide bonds. No two types of enzymes have the same amino acid structure, and each enzyme has its own unique shape. Automation of enzyme assays is becoming increasingly important and instrumentation is being developed to satisfy this need.

Enzyme assays are laboratory methods for measuring enzymatic activity. They are vital for the study of enzyme kinetics and enzyme inhibition. Usually the assay is carried out by determining the enzyme activity with, and without activation by an added coenzyme. The activity can be monitored by measuring changes in concentration of substrates or products during the reaction.

Enzyme activity is the rate of enzyme reaction—generally expressed as units of substrate converted (or product formed) per time unit. Enzyme kinetics is the study of the chemical reactions that are catalyzed by enzymes.

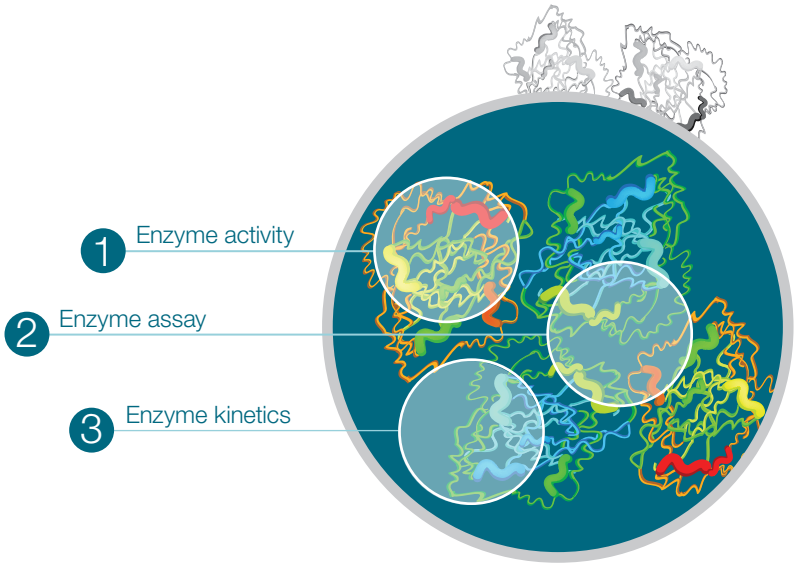

Figure 1. Three facets of enzyme analysis.

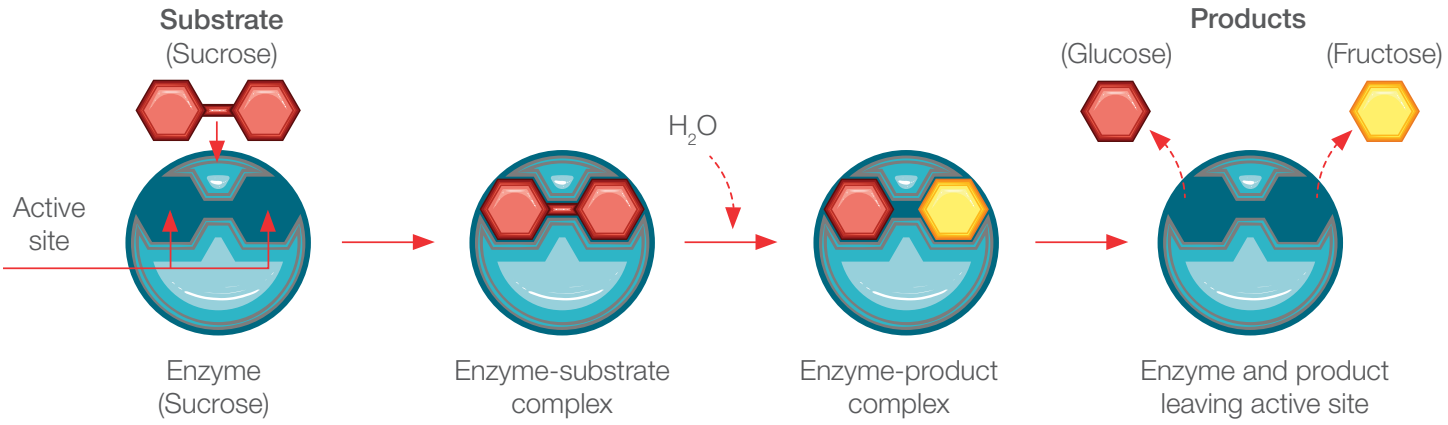

Figure 2. Specific enzyme action.

What are the enzyme assay methods?

Enzyme assay is performed to determine the amount of enzyme in the sample and it is also used for identifying a special enzyme. The enzymatic assay can be direct or indirect, or coupled. In the case of direct assay, substrate is added to the sample and the end product formed is determined. The substrate or assay reagent is directly modified by the enzyme, but the signal is generated by interaction with another reagent or another reaction. The other reagents or reactions may be the second, third, or even the forth reaction from the initial enzyme reaction.

Enzyme assays—what are the method choices?

Most enzyme assays are based on spectroscopic techniques, with the two dominant types being absorption and fluorescence. The spectrophotometric assay is a classic enzyme test, which remains as the most widely used assay for the lowest cost. During a spectrophotometric assay, the operator follows the course of an enzyme reaction by measuring the changes in the intensity of the light absorbed or scattered by the reaction solution. All the steps involved are manual and lead to inconsistent results. This method is suitable when analyzing a few samples or enzyme-type routine operations.

Enzyme assays based on photometry, fluorometry, 96-, 384-, or even 1536-well format microplate offers a high throughput alternative to the traditional spectrophotometers. The microplate format is convenient for high throughput analysis using a 200 µL assay volume and are commonly used in life science applications. However, the microplate method suffers from temperature stabilization, absorption correction, and edge effect.

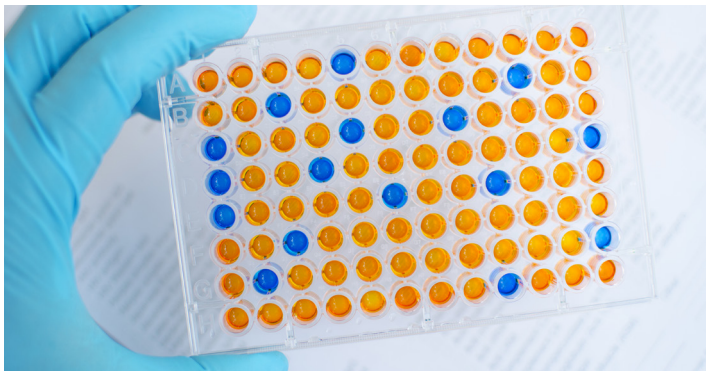

Figure 3. Enzyme assay by high throughput microplate.

The absorbance is measured vertically on microplates through the well, so several factors affect the liquid pathlength, and thus the absorbance. Therefore, in photometric microplate measurements pathlength correction is required for calculating the enzyme assay.

The primary cause for the “edge effect” phenomenon is evaporation and is commonly associated with 96-well microplates. “Edge effect” is an issue attributed to the increased evaporation rate of circumferential wells compared to the centrally located wells. Often a great deal of work goes into assay development. They have limited incubation temperature, temperature stability, and precision, which limits the application range.

For some enzyme assays it is necessary to quench or stop the reaction at a specific time to prevent further production of the product. For example, samples may be taken at 5-minute intervals for a predetermined period of time with the product being measured by high performance liquid chromatography (HPLC). Each chromatographic analysis may take 30 minutes to complete.

Enzyme assays by the Thermo Scientific Gallery Enzyme Master enzyme analyzer—a better way to automate enzyme assay analysis

The Thermo Scientific™ Gallery™ Enzyme Master enzyme analyzer automates the critical steps involved in reliable enzyme analysis—sequential addition of buffers, substrates, and reagents—including incubation time and precise incubation temperature suitable for a specific enzyme type. It can efficiently manage various enzymes and measuring conditions—all in a single instrument that offers consistent and reliable performance.

In comparison to the spectrophotometer or direct read microplate systems, the Gallery Enzyme Master systems offer a wide incubation temperature range from 25 °C to 60 °C expanding the application possibilities. All the substrate additions and measurements are done in disposable low volume cuvette allowing the system to perform real time kinetic measurement. Superior temperature control and lack of edge effects assures confidence in results. Thanks to dedicated software, enzyme workflows are incredibly simple with practically no change over time from one method to another. With flexible method parameters for each enzyme type-measuring wavelength, blank measurement, buffer addition, reagents additions, substrate addition, enzyme specific incubation temperature, enzyme specific incubation time, and data collection duration-the enzyme assay method development and transfer are effortless and reliable from R&D to QA/QC labs.

The Gallery Enzyme Master system intelligently interprets all the samples and automatically groups the samples that require the same temperature. It completes samples at the lowest temperature first then heats the incubation chamber to next setting and completes those, repeating the process until all samples are done.

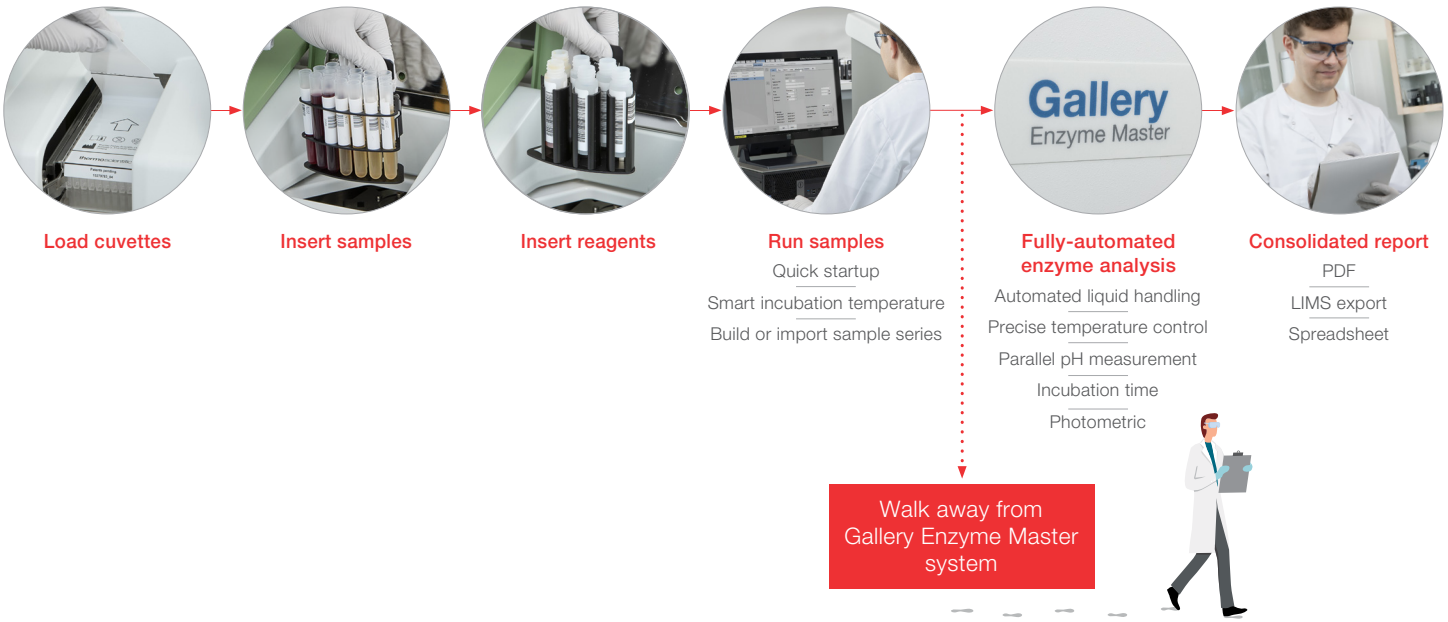

Figure 4. Gallery Enzyme Master system workflow.

thermo scientific

The Gallery Enzyme Master systems can efficiently manage various enzymes and measuring conditions—all in a single instrument that offers consistent and reliable performance.

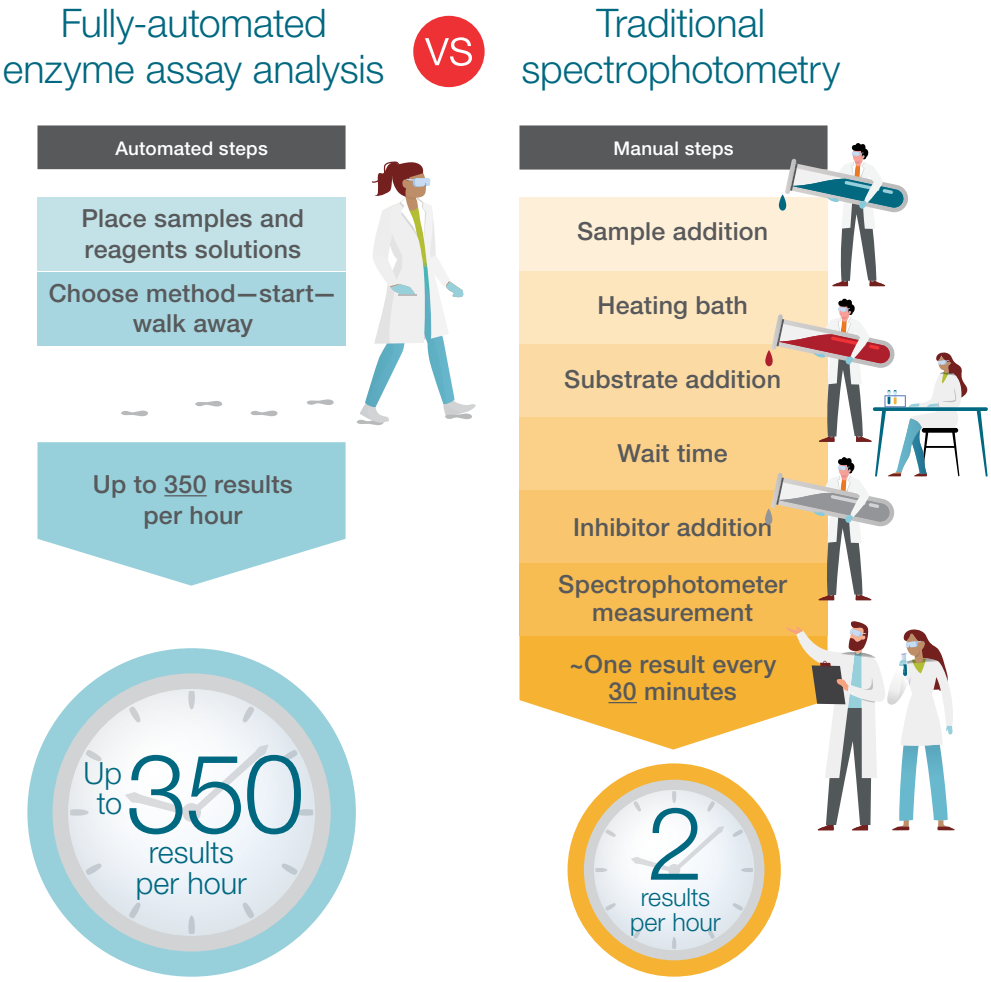

Figure 5. Fully-automated enzyme analyzer versus spectrophotometer.

Find out more at **thermofisher.com/enzymeanalysis**

ThermoFisher SCIENTIFIC