

# Smart Note



## What are the top 5 reasons to consider fully-automated enzyme assays?

### Why is accurate enzyme assay analysis important?

Enzymes play a pivotal role in a wide variety of industries. They not only speed manufacturing, they can improve quality, reduce waste, and optimize product yield—ensuring more cost-efficiency and higher profitability. Most enzyme assays are based on spectroscopic techniques, with the two dominant types being absorption and fluorescence.

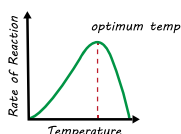
### What are the important factors that affect enzyme assay analysis?

Measuring enzyme activity is a precise job and can be influenced by many variables. Results accuracy is highly dependent on temperature stability. Just one degree temperature change can lead to a 4–8% variation in enzyme activity. For consistent and reproducible results, an enzyme assay should be carried out in well-defined conditions that can be duplicated in other laboratories. Variables such as pH and buffer type, ionic strength, and temperature must

#### Temperature

##### BIG influence

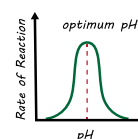
More heat = More kinetic energy



But if **too high** enzyme is denatured

#### pH

Enzymes have optimum pH



If **higher/lower** H<sup>+</sup> in acid / OH<sup>-</sup> in alkaline can **interfere** enzyme structure

#### Enzyme concentration

steady increase if substrate available

if substrate is **limited**

Increase enzyme concentration = increase rate of reaction

Rate of Reaction

Enzyme concentration

\*\*Until substrates amount are limited\*\*

#### Substrate concentration



Increase substrate concentration = increase rate of reaction

Rate of Reaction

Substrate concentration

\*\*Until active site of enzyme are used\*\*

Figure 1. Factors affecting enzyme activity.

be strictly controlled. pH is a critical parameter in method development and routine enzyme assay measurement. pH affects the enzyme activity, charge, and shape of the substrate, so that the substrate cannot bind to the active site or cannot be catalyzed to form a product. All enzymes have an ideal pH value, which is called optimal pH. Under the optimum pH conditions, each enzyme showed the maximum activity. Determination of the optimum pH in a coupled enzyme assay poses significant challenges because altering the pH of the reaction mixture can affect the performance of both enzymes. Fixing the other variable will allow to correlate the change in measuring parameter and absorbance directly to the enzyme assay or enzyme activity. Reliable enzyme assay development is critical and the automated enzyme analyzers simplify the overall method development and results reliability.

### 1 Method development and transferability

Developing a reliable analytical method for enzyme assay or enzyme activity involves many different steps. Overall method development is tedious and time consuming. Method development starts with identifying the key method variables through design of experiment (DoE), which requires many sets of samples to be tested for enzyme activities. Controlling several variables manually leads to inconsistent results and makes the overall method development tedious and unreliable.

The Thermo Scientific™ Gallery™ Enzyme Master Enzyme Analyzers offer a wide incubation temperature range from 25 to 60 °C expanding the application possibilities and automates the enzyme assay workflow. All the substrate additions and measurements are done in disposable low volume cuvettes allowing the system to perform real time

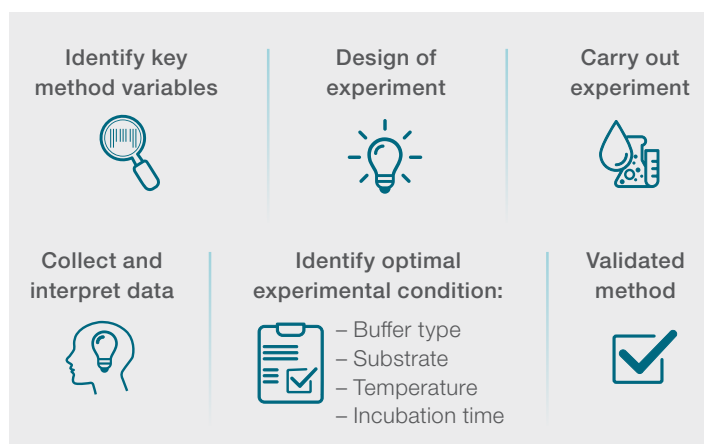


Figure 2. Overall method development is tedious and time consuming.

kinetic measurement. Superior temperature control and lack of edge effects assures confidence in results. Wide wavelength coverage from 340 to 880 nm with optional additional filters enhances the enzyme assay applications.

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-make the enzyme assay method development and transfer effortless and reliable from research and development to QA/QC labs.

### 2 Confidence in results

Numerous method variables and a consistent measurement condition is critical in achieving reproducible results. The addition of a sample, buffer, and substrate in a specific sequence and time interval are critical for enzyme assays. The Gallery Enzyme Master enzyme analyzer automates the critical steps involved in reliable enzyme analysis, including incubation time, incubation temperature, and precision liquid handling. It can efficiently manage many various enzymes and measuring conditions—all in a single instrument that offers consistent and reliable performance. Built-in barcode readers for samples and reagents eliminates the manual error and precise temperature controls to the  $\pm 0.3$  °C eliminates sample overheating. Superior temperature control and lack of edge effects assures confidence in results without compromising the throughput. The Thermo Scientific™ Gallery™ Plus Enzyme Master Enzyme Analyzer with the electrochemistry module (ECM) allows the parallel determination of sample pH, and parallel determination of optimal pH which make overall enzyme analysis easier, faster, and reliable.

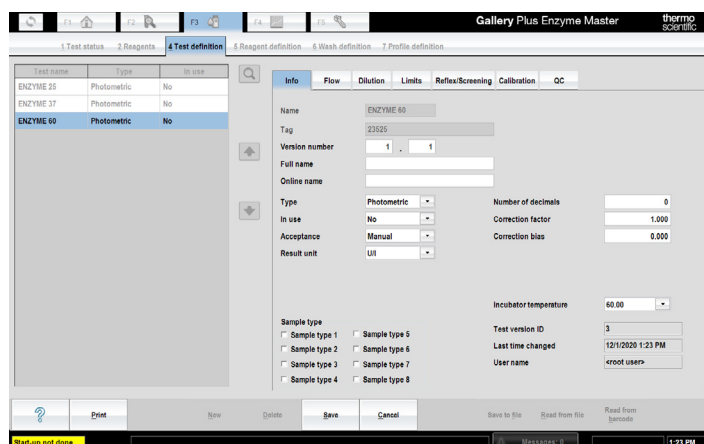


Figure 3. Method-specific incubation temperature.

### 3 Higher productivity

With the Gallery Enzyme Master system, samples are simultaneously prepared and waiting in the queue—delivering results one after another. With automated enzyme analysis, the first sample still requires longer time, 20–30 minutes, depending on the incubation time. All the subsequent samples are much faster due to system multitasking. While one sample is incubating at its set temperature, the second sample is prepared and waiting in the queue. After sample one is done, sample two is done one minute later, sample three one minute after that, and so on. The cumulative effect is exponentially more results in less time, with less hands-on work. The Quick Start function warms up the system to a specific incubation

temperature and substantially reduces the wait time. An optional ECM can perform parallel and simultaneous pH and conductivity measurements of up to 67 samples per hour.

### 4 Simplified workflow

The Gallery Enzyme Master system is a fully-integrated walkaway solution. The testing workflow is easy to learn and can be left unattended, which improves throughput, system uptime, and staff productivity. All necessary enzyme analysis steps are automated, providing true walkaway time for the operator. Thanks to dedicated software, enzyme workflows are incredibly simple with practically no change over time from one method to another.

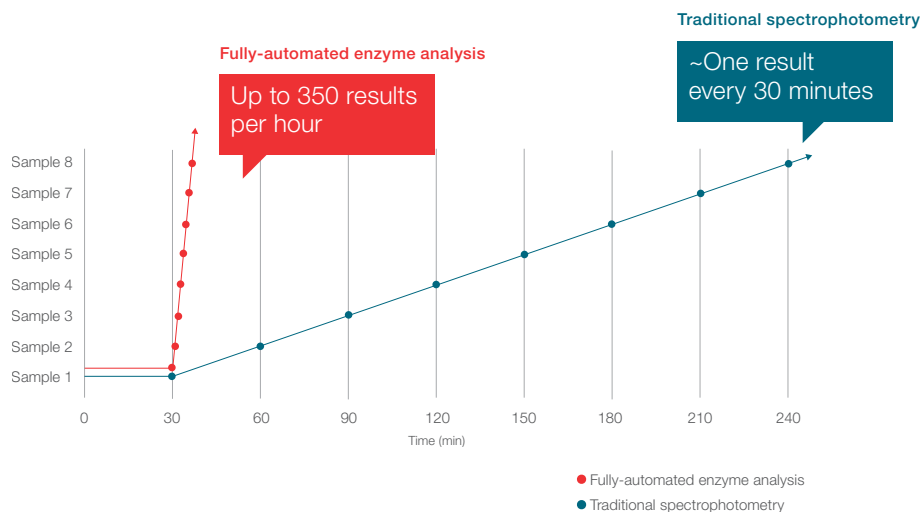


Figure 4. How automation improves productivity.

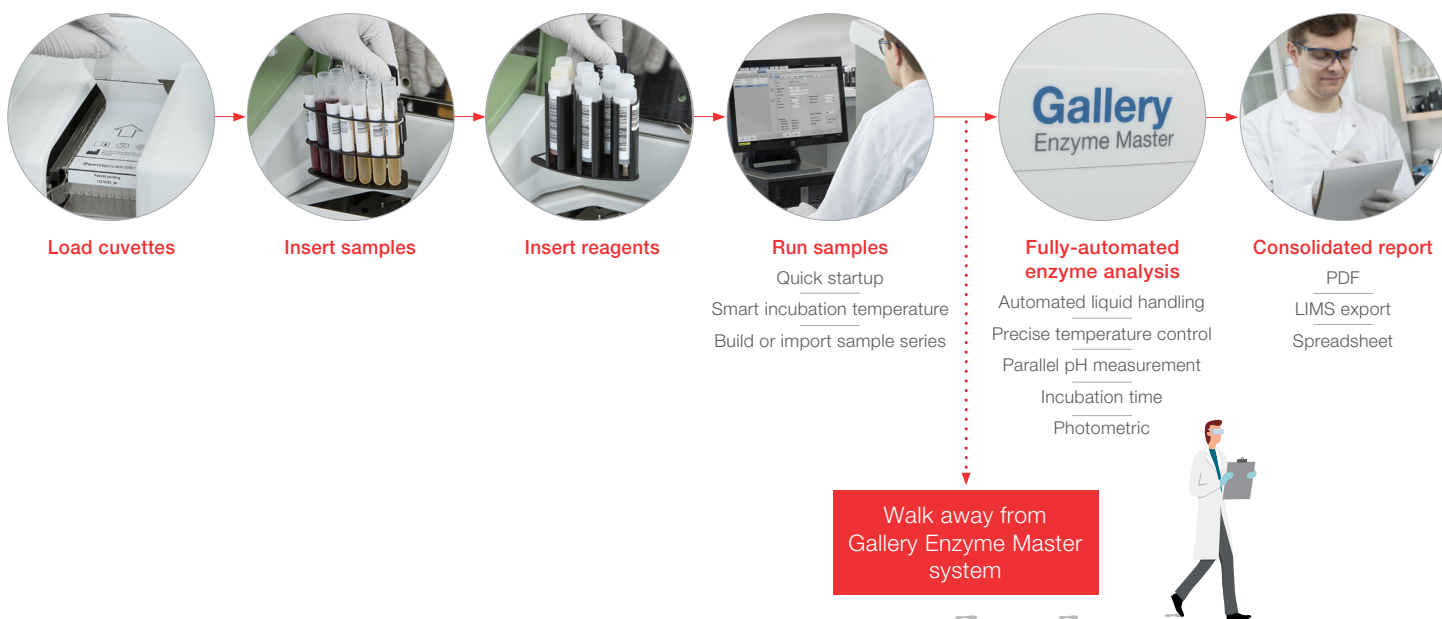


Figure 5. Gallery Enzyme Master system workflow.

5 Traceability

Combining robust hardware and custom designed software, these best-in-class solutions deliver fully-automated incubation settings, reagent additions, and precise measurement calculations—all with a touch of a button. Gallery Enzyme Master systems offer the smartest (and fastest) way to streamline method development and deliver reliable results from enzyme assay analysis. Traceability is delivered via time-stamped raw data, audit trail, user administration, and confident certificate of analysis (CoA).

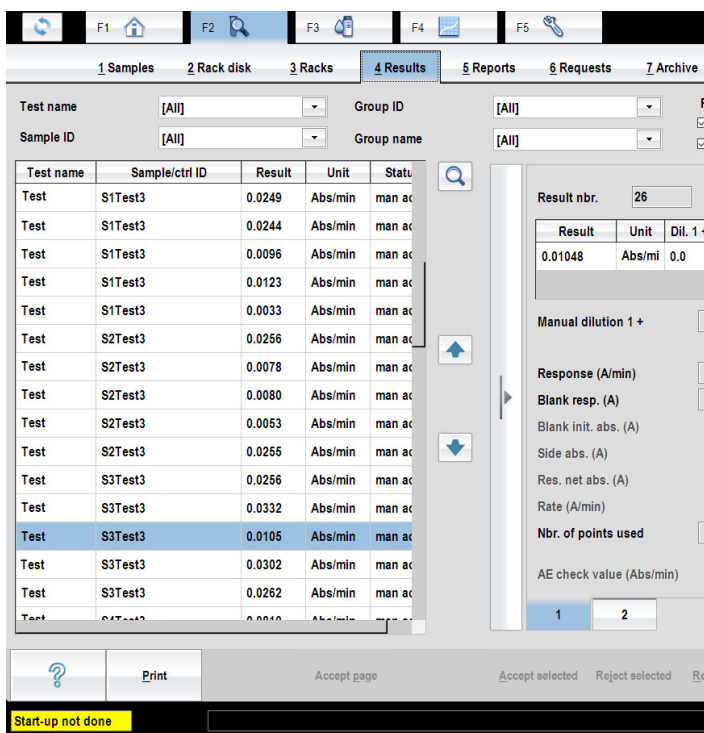


Figure 6. Audit trail and time stamped raw data.