NZYTECH ANALYTICAL ENZYMES AND KITS

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INTRODUCTION

NZYTech - Genes and Enzymes, Ltd. is an ISO 9001:2008 certified company dedicated to research & development, production and commercialization of biotech products and services to the community. One of its activity areas is the development, manufacture, supply and support of enzymatic bio-analysis test kits, with applications mainly in the food, feed, dairy and beverage industries.

Enzymes are now finding many and widespread industrial applications. In general, the use of enzymes is considered to be safe, cost effective, and is associated with environmentally friendly technologies. Although research scientists realised the great potential for the application of enzymes in medical diagnostics and industrial fermentation, presently, most applications for analytical enzymes are in food industry.

During the last years, taking advantage of our expertise in the production of highly active and pure recombinant enzymes, NZYTech has developed a wide variety of convenient analytical enzymes and kits, with a large range of applications, including in the food, feed, wine, brewing and dairy industries (Table 1). With the ability to rapidly develop new test kits in response to new legislation or developments in the food industry, such as in the labelling, the future of NZYTech in enzymatic bioanalysis is very promising.

Table 1. Applications and benefits of NZYTech analytical kits.

Kit	Food industry	Feed Industry	Wine Industry	Brewing Industry	Dairy Industry	Other Fermentation Industries	Benefits of NZYTech Analytical kits
Acetaldehyde			•	•	•	•	- Easy to use - Stable AlDH suspension, no waste - Stable reagents - Standard included
Acetic acid	•	•	•	•	•	•	- Stable ACS suspension, no waste - Prevention of tannin inhibition - Stable reagents - Standard included
Ammonia	•	•	•		•	•	- Very rapid reaction - Stable GDH suspension - Tablet format for higher stability - Standard included
L-Arginine/Urea/ Ammonia			•			•	- Very rapid reaction - Stable enzyme suspensions - Tablet format for higher stability - Standard included
Ethanol	•		•	•		•	- Rapid reaction - Stable AlDH suspension, no waste - Stable reagents - Standard included
D-Fructose/D-Glucose	•	•	•	•	•	•	- Rapid reactions - Prevention of tannin inhibition - Stable reagents - Stable standard included
D-Glucose GOD-POD	•	•	•	•	•	•	- Simple colorimetric method - Stable reagents - Standard included
D-Glucose HK	•	•	•	•	•	•	- Rapid reactions - Prevention of tannin inhibition - Stable reagents

							- Stable standard included
L-Glutamine/ Ammonia	•					•	- Very rapid reaction - Stable enzyme suspensions - Tablet format for higher stability - Standard included
D-/L-Lactic acid	•		•		•	•	- Rapid reaction - Stable reagents - Stable standard included
D-Lactic acid	•		•		•	•	- Rapid reaction - Stable reagents - Stable standard included
L-Lactic acid	•		•		•	•	- Rapid reaction - Stable reagents - Stable standard included
D-Malic acid	•		•				- Very rapid reaction - Stable D-MDH suspension - Stable reagents - Standard included
L-Malic acid	•		•			•	- Very rapid reaction - Prevention of tannin inhibition - Stable enzyme suspensions - Standard included
L-Malic acid colorimetic	•		•			•	- Simple colorimetric method - Stable enzyme suspension - Standard included
Pyruvic acid				•	•	•	- Very rapid reaction - Stable D-LDH suspension - Stable reagents - Standard included
Sulfite	•		•	•			- Stable enzyme suspensions - Tablet format for higher stability - Standard included
Urea/Ammonia	•	•	•		•	•	- Very rapid reaction - Stable enzyme suspensions - Tablet format for higher stability - Standard included

ANALYTICAL ENZYMES

Enzymes are biological macromolecules responsible for the thousands of metabolic processes that sustain life. They are highly selective catalysts, greatly accelerating the rate of metabolic reactions, from the digestion of food to the synthesis of DNA. Most of enzymes are proteins, although some catalytic RNA molecules have been identified. Enzymes adopt a specific three-dimensional structure, and may employ organic (*e.g.* biotin) and inorganic (*e.g.* magnesium ion) cofactors to assist in catalysis. In enzymatic reactions, the molecules at the beginning of the process, called substrates, are converted into different molecules, called products. Almost all chemical reactions in a cell need enzymes in order to occur at rates sufficient for life. Since enzymes are selective for their substrates and speed up only a few reactions from among many possibilities, the set of enzymes made in a cell determines which metabolic pathways occur in that cell.

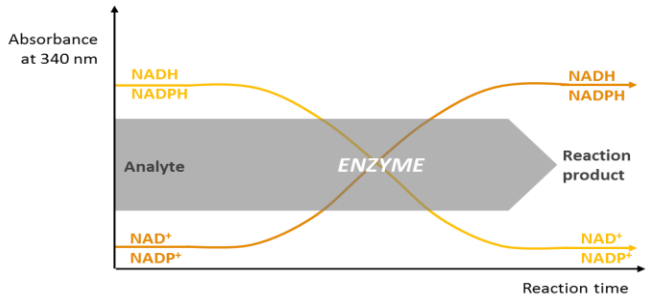
The industrial application of enzymes began in the 1960 decade with the use of glucoamylase to produce in the degradation of starch into glucose. The next major development was the implementation of immobilised glucose isomerase in 1970s. Nowadays, enzymes are used in the industry when extremely specific catalysts are required. However, enzymes in general are limited in the number of reactions they have evolved to catalyse and also by their lack of stability in organic solvents and at high temperatures. As a consequence, protein engineering is an active area of research and involves attempts to create new enzymes with novel properties, either through rational design or *in vitro* evolution.

For an enzyme to be used for analytical purposes, it must be of exceptional purity as individual enzymes are generally found along with many other molecules and these impurities must be removed before the enzyme is of any use. Moreover, analytical enzymes are often required in large amounts, thus requiring cheap and rapid purification processes. The core expertise of NZYTech is the production of highly pure and active recombinant enzymes, by using different strategies, including High-Throughput (HTP) cloning, purification and the detailed analysis of their catalytic properties. Therefore, NZYTech has developed cutting edge technologies to identify and characterize the most appropriate enzymes for a specific application, as well as the most efficient purification protocols in order to ensure its most competitive price/quality ratio.

ANALYTICAL KITS

A test kit contains all the reagents in a convenient form and amount, including the analytical enzymes, required to perform a set number of analyses, in the optimal conditions for the enzymes used. In addition to a spectrophotometer, very little laboratory equipment is required to perform an enzymatic analysis, including the common micropipettes with disposable plastic tips (200-1000 µl) and cuvettes.

The high specificity of enzymes enables the analysis in complex sample matrixes without complicated sample preparation techniques. This makes enzymatic food analysis a highly valuable tool because it saves time, reduces costs and gives reliable results independent of the sample matrix. Additionally, enzymatic methods use non-hazardous reagents, are environmentally friendly and can be automated for in-line process monitoring. NZYTech offers a wide variety of convenient kits for rapid and reliable enzymatic food analysis. NZYTech analytical kits are based on enzymatic reactions and performed using spectrophotometric methods, either in the UV region (UV tests) or in the visible region (colorimetric tests). The main features of these tests are specificity, sensitivity, easy to use, based on simple protocols and endpoint analysis, safe to the operator and the fact that include standards.



■ **Figure 1. Principle of enzymatic tests based on the NAD(P)+/NAD(P)H system.**

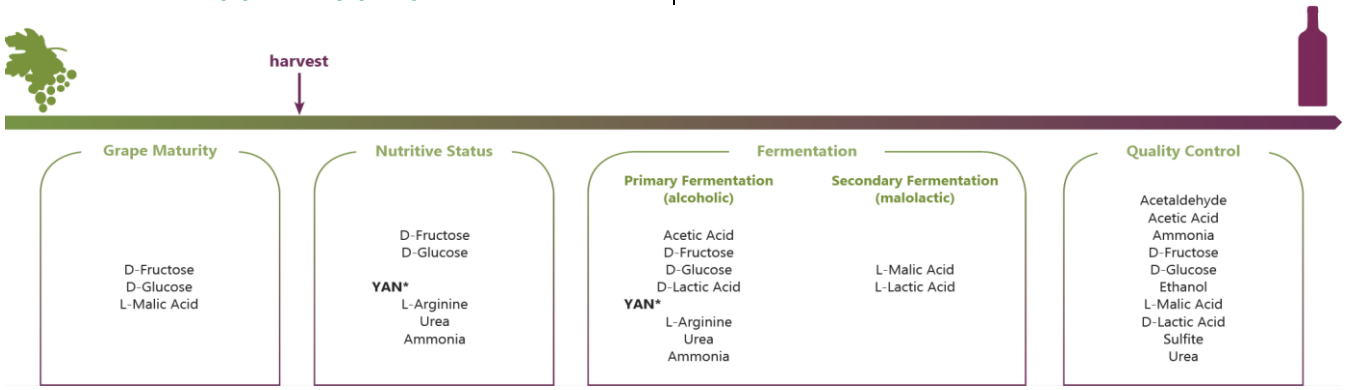
The principle of UV tests is based on the NAD(P)+/NAD(P)H system. The enzymes used in these analytical kits produce or consume NAD(P)H, which strongly absorbs the UV radiation at 340 nm (extinction coefficient of 6300 $M^{-1}cm^{-1}$) (Figure 1). The principle of colorimetric tests is based on a chromogenic reaction with the formation of a coloured compound, absorbing in the visible region of the electromagnetic spectrum. The coloured compound results from the interaction between the product of a first enzymatic reaction and a chromogenic compound. In this case, the concentration of the analyte must be determined by using a standard curve.

NZYTech kits are based on rapid and simple stereo-specific enzymatic reactions that could be used for the determination of analytes in foodstuffs such as wine, beer, bread, fruit and vegetable products, fruit juice, as well as in cosmetics, pharmaceuticals and biological samples. In spite of this potential, most of their applications are in food industry, in particular in wine industry.

Application in wine Industry

Chemical analysis plays a main role throughout all the vinification process, from grape to wine. From before harvesting of the grapes until bottling of the wine, step by step testing allows minimizing process problems to achieve the best quality wine.

NZYTech provides a comprehensive range of analytical kits intending to cover all enological processes, with applications in the evaluation of grape maturity, nutritive status, monitoring of fermentation (primary or alcoholic, and secondary or malolactic) and quality control of wine (Figure 2).



■ **Figure 2. Application of NZYTech analytical kits to wine industry.**

NZYTECH PRODUCTS

The current analytical enzymes and kits commercially available at NZYTech are shown in Table 2. Most of these methods are approved by European Community regulations and recommended by several Food and Beverages International Federations. Additional information is available online at www.nzytech.com.

■Table 2. NZYTech analytical products (kits and enzymes).

Kits	Catalogue No.	No. of tests
Acetaldehyde, UV method	AK00051	50
Acetic acid, UV method	AK00081	53
Ammonia, UV method	AK00091	96
L-Arginine/Urea/Ammonia, UV method	AK00171	50 of each
Ethanol, UV method	AK00061	60
D-Fructose/D-Glucose, UV method	AK00041	110
D-Glucose GOD-POD	AK00161	660
D-Glucose HK, UV method	AK00031	110
L-Glutamine/Ammonia, UV method	AK00111	50 of each
D-/L-Lactic acid, UV method	AK00141	50 of each
D-Lactic acid, UV method	AK00121	50
L-Lactic acid, UV method	AK00131	50
D-Malic acid, UV method	AK00021	100
L-Malic acid, UV method	AK00011	58
L-Malic acid, colorimetic method	AK00191	5 x 10
Pyruvic acid, UV method	AK00151	100
Sulfite, UV method	AK00071	30
Urea/Ammonia, UV method	AK00101	50 of each
Glutathione peroxidase	DG00041	80
Glutathione reductase	DG00051	5 x 10
NZYTech Vintage Pack	AK00181	371 (total)
Enzymes	**Catalogue No.**	**Quantity**
Acetyl-CoA synthetase (EC 6.2.1.1), *Bacillus subtilis*	AE00081	250 U
Alcohol dehydrogenase (EC 1.1.1.1), *Escherichia coli*	AE00131	1000 U
Aldehyde reductase YqhD, *Escherichia coli*	AE00021	2 mg

Arginase (EC 3.5.3.1), *Homo sapiens* liver	AE00211	1950 U
Aspartate aminotransferase (EC 2.6.1.1), *Escherichia coli*	AE00061	5000 U
Citrate synthase (EC 2.3.3.1), *Escherichia coli*	AE00041	2500 U
D-Alanine aminotransferase (EC 2.6.1.21), *Bacillus subtilis*	AE00141	2500 U
Diaphorase (EC 1.8.1.4), *Escherichia coli*	AE00231	1000 U
D-Lactate dehydrogenase (EC 1.1.1.28), *Leuconostoc mesenteroides*	AE00121	22 KU
D-Malate dehydrogenase (EC 1.1.1.83), *Escherichia coli*	AE00151	200 U
Glucokinase (EC 2.7.1.2), *Escherichia coli*	AE00171	1400 U
Glucose-6-phosphate dehydrogenase (EC 1.1.1.49), *Escherichia coli*	AE00111	5000 U
Glucose-6-phosphate isomerase (EC 5.3.1.9), *Escherichia coli*	AE00101	5000 U
Glutamate dehydrogenase (EC 1.4.1.4), *Escherichia coli*	AE00051	3300 U
Glutaminase (EC 3.5.1.2), *Escherichia coli*	AE00071	2500 U
Glutathione reductase (EC 1.8.1.7), *Escherichia coli*	AE00221	500 U
L-Malate dehydrogenase (EC 1.1.1.37), *Escherichia coli*	AE00091	50 kU
Lactaldehyde dehydrogenase (EC 1.2.1.22), *Escherichia coli*	AE00031	4 mg
NADH peroxidase (EC 1.11.1.1), *Streptococcus faecalis* ATCC 11700	AE00201	437 U
Sulfite oxidase Mo centre domain (EC 1.8.3.1), *Homo sapiens*	AE00011	2.25 U

Acknowledgements

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