Wine Analysis

Megazyme

www.megazyme.com

Dear Reader,

Welcome to Megazyme's Wine Product Guide for 2019. We seek to provide the very best service to our customers worldwide, supported by our international distributor network.

As your trusted partner for quality control, we look forward to supporting your wine business in the coming season.

- The Megazyme Team

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Wine Analysis: The Basics

As viticulture has modernised and grown as a global industry, wineries have looked deeper into the art of winemaking to discover the science behind it.

Why test wine?

Today's winemakers can understand the flavour, style, and quality of their wines right down to the molecular level. Wineries and co-operatives can ensure consistency and quality through rapid, cost-effective in-house testing for sugars, acids, alcohols, and more at any stage of production.

Three reasons why every winery needs to analyse in-house

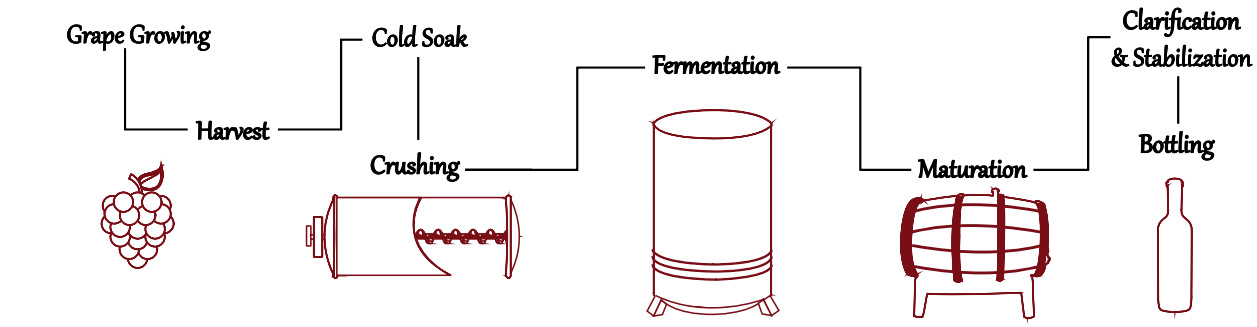
On-site testing - as opposed to sending samples to an analytical laboratory - has considerable Features:

- Cost saving: the cost of running a test in-house is around 88% cheaper (in terms of cost per test) than sending it to an external laboratory; see below for more details.
- Rapid turnaround: our wine assay kits produce a result in as little as 3 minutes, allowing you to respond to the results in real time - unlike the 2-3 day wait for results from a laboratory.
- Flexibility: with a testing facility in your own winery, you can test as frequently as needed, not just at crucial stages of production, to analyse samples within the same vintage.

In-house testing means cheaper, quicker test results available at a moment's notice - without compromising on precision.

Even accounting for the investment in laboratory equipment, in-house analysis with Megazyme generates savings by the 100th assay - for wineries of any size. Our loyal customers include some of the biggest names in winemaking, and it's easy to see why: Megazyme kits and equipment give you access to internationally-approved, industry-standard methods at an extremely competitive price.

Analysis from Grape to Wine



Grape Maturity (Juice)

Analyte	Product Code
L-Arginine	K-LARGE
Fructose/Glucose	K-FRUGL, K-FRGLQR
Nitrogen (YAN)	*multiple options*
Sulphites	*multiple options*

Organic Acid Analyte	Product Code
Acetic acid	*multiple options*
Gluconic acid	K-GATE
Malic acid	*multiple options*
Tartaric acid	K-TART

Alcoholic & Malolactic

Analyte	Product Code
Nitrogen (YAN)	*multiple options*
Ethanol	K-ETOH
Fructose/Glucose	K-FRUGL,K-FRGLQR
Sulphites	*multiple options*

Organic Acid Analyte	Product Code
Acetic acid	*multiple options*
L-Lactic acid	K-LATE
Malic acid	*multiple options*
Tartaric acid	K-TART

Finished Wine

Analyte	Product Code
Acetaldehyde	K-ACHYD
Ethanol	K-ETOH
Fructose/Glucose	K-FRUGL, K-FRGLQR
Glycerol	K-GCROL
Sucrose	K-SUFRG
Sulphites	*multiple options*

Acid Analyte	Product Code
Acetic acid	*multiple options*
Ascorbic acid	K-ASCO
Citric acid	K-CITR
D-Gluconic acid	K-GATE
Malic acid	*multiple options*
Succinic acid	K-SUCC
Tartaric acid	K-TART

Megazyme has developed assay kits for a wide range of components and substances found in beverage and food, including 15 analytes that are of particular interest to the wine industry. The graphic above shows the major analytes that should be measured at key points during the vinification process, which will be explained in more detail later in the guide.

In this product guide

The **Beginner's Guide to Enzymatic Assay Kits** will walk you though the testing process, including how the equipment is able to measure substances, how the assay kits work with the machines, and how to perform the calculations. The laboratory techniques employed are straightforward, and can be performed by anyone with minimal laboratory experience.

In the **Assay Kits** section, each analyte (measured substance) is covered in turn, explaining how they appear in wine, when they should be tested, and the implications for the winemaker. The Megazyme assay kit (or kits) for that analyte are also listed. We outline the cost per test and the type of equipment needed to use the kit. We even explain what chemical reaction is taking place during the test so that you can understand the differences between our products - and what sets Megazyme's product apart from those available from competitors.

Finally, the **Equipment** section looks at our range of analysers suitable for use in the wine laboratory. We offer options for wine businesses of any size, from analysers that can process 200 samples per hour right down to simple equipment suitable for wineries that test just a few samples per week.

The Megazyme Difference

Research is at the core of Megazyme's product development. Continual innovation has allowed us to introduce new developments and improvements to accepted, industry-standard methods of analysis. Megazyme test kits – researched and manufactured in-house – have attracted worldwide acclaim for their novel methodologies and for the exceptional purity of their enzymes. Backed by best-in-class customer support, Megazyme products offer:

- reduced reaction times
- improved enzyme stability (resulting in a longer 'shelf-life')
- alternative biochemical reactions, which have been evaluated and implemented successfully
- extended cofactor stability (offered in a stable tablet form, or as improved formulations that last longer in solution)

A Beginner's Guide to Enzymatic Assay Kits

Megazyme's enzymatic assay kits are used to measure the quantities of specific analytes (substances) in a sample (e.g. wine).

In general terms, enzymes are catalytic proteins that convert one compound into another. Such reactions frequently occur without any visible sign that they have taken place. However, (as with Megazyme kits) certain enzymatic reactions result in an increase or decrease in the *absorbance* of the reaction solution.

Absorbance

The absorbance of a solution dictates the amount of light that can pass through the solution. Absorbance can be measured by absorption spectroscopy, which uses a *spectrophotometer* (e.g. the MegaQuant™ Wave, pictured below), auto-analyser or microplate reader.

When a sample is tested for a specific analyte using a Megazyme assay kit, the enzyme(s) used in the kit act specifically on that analyte. The resulting absorbance change measured in the reaction solution is directly proportional to the amount of the analyte present. The absorbance value is then used to calculate the amount of analyte in the original sample.

The MegaQuant™ Wave spectrophotometer.

The Spectrophotometer

The spectrophotometer is a powerful analytical instrument because it can measure changes in absorbance very accurately and quickly. Modern models are often pre-programmed with software to lead the user through enzymatic assays step by step, making the spectrophotometer user-friendly as well as accurate.

How the Spectrophotometer Works

The enzymatic analysis reaction itself is performed in a plastic or glass cuvette that sits between the source of light and the light detector inside the spectrophotometer. A known amount of light is passed through the cuvette and the amount that emerges is quantified by the detector.

The change in intensity as the light passes through the reaction solution in the cuvette is recorded as an *absorbance reading*, used in later calculations.

Using an Enzymatic Assay Kit

Megazyme enzymatic analysis assay kits generally contain all reagents necessary to perform the assay, e.g. buffer, cofactor(s), trigger enzyme and standard solution. The reagents are clearly labelled as shown in Figure 1, and are shipped in an easy-to-use form that is stable for over 2 years (even while in regular use).

Comprehensive, step-by-step instructions for sample preparation, set-up, and assay procedure are enclosed in the kit data booklet. Megazyme also publishes video tutorials demonstrating how to use our kits and equipment.

Figure 1. *Left to right: buffer solution, enzyme, cofactor, enzyme, standard solution..*

Building a Basic Winery Laboratory

Some basic laboratory equipment is needed to carry out an assay. For example, for the Ethanol Assay Kit (K-ETOH) pictured above, the wine analyst would require access to the following:

1. Spectrophotometer and disposable cuvettes
2. Vortex mixer
3. Analytical balance (highly sensitive weighing scale)
4. Pipettor and micro-pipettor
5. Volumetric flasks
6. Filter papers and distilled water

All of these components are widely available, with very low running costs once the initial investment in equipment is made.

Performing an Enzymatic Assay with a Spectrophotometer

During a typical enzymatic analysis, deionised water is mixed with buffer, cofactor and sample. An absorbance reading (A_1) is taken just before addition of the "trigger" enzyme (specific for the analyte in question), after which the reaction takes place (see figure 2).

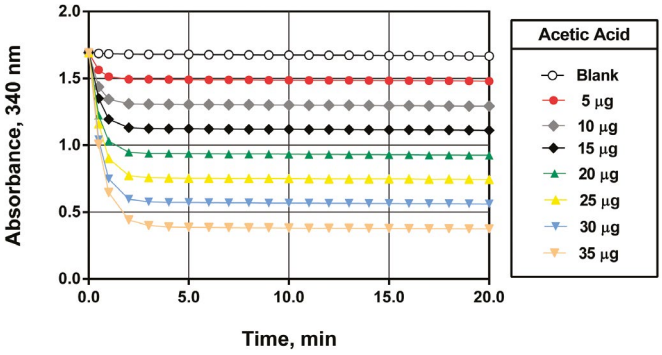


Figure 2. Decrease in absorbance at 340 nm on incubation of 0-35 µg of acetic acid with acetate kinase in the acetic acid AK/PTA format.

When the reaction has finished (i.e. the "endpoint" has been achieved), a second absorbance reading is taken (A_2).

The difference between these two absorbance readings (i.e. A_1 - A_2) is called the *change in absorbance* (or ΔA) and is directly related to analyte content.

It is this value, after correction with a blank reading (reaction containing no sample), that is used to calculate the concentration of the analyte in the sample. using a simple factor (e.g. 0.2535 in the case of the acetic acid AK/PTA format).

Results are typically expressed as g/L.

Completing the Calculation

Some spectrophotometers (e.g. the MegaQuant™ Wave) will perform the calculations automatically. When using Megazyme assay kits with other analysers, calculations can either be performed manually as illustrated below or by using our free and convenient "Mega-Calc™" (Excel™-based calculator), downloadable from the product page on the Megazyme website (www.megazyme.com). A sample manual calculation is shown below.

Typical acetic acid calculation

A_1 (blank) = 1.400 A_2 (blank) = 1.398
A_1 (sample) = 1.420 A_2 (sample) = 0.650

$^{\Delta A}$acetic acid = (A_1 (sample) - A_2 (sample)) - (A_1 (blank) - A_2 (blank))
$^{\Delta A}$acetic acid = (1.420 - 0.650) - (1.400 - 1.398)
= 0.768

Thus the concentration of acetic acid
= 0.768 x 0.2535
= 0.1947 g/L

The Science Behind Megazyme Assay Kits

Our enzymatic assay kits are designed to be accessible for any user, including those without a specialised scientific background.

A full understanding of the principles behind our products is not required in order to generate meaningful, accurate results at your winery. However, for the interested user, the underlying chemistry behind our enzymatic assay kits is described below.

Enzymatic Assays Based on NAD^+/NADH/ $NADP^+$/NADPH

Megazyme assay kits usually involve enzymes that either directly, or indirectly (via other enzymes), produce or consume a compound called NADH (or NADPH).

NADH, although invisible to the human eye, absorbs light strongly at a wavelength of 340 nm (extinction coefficient [ε] = 6300 M^{-1} cm^{-1}). This means that any changes in the quantity of available NADH can be detected using a spectrophotometer.

Figure 3 depicts the various types of enzymatic reactions that are employed in many enzymatic assay kits, either consuming or producing NADH (Reaction 1). As can be seen from Figure 3, it is sometimes necessary to include an additional reaction in order to obtain quantitative results (Reaction 2).

Reaction 2 is catalysed by the enzyme *diaphorase*, in the presence of a compound called *INT*, which converts the NADH (or NADPH) produced in the first reaction into a red coloured compound called *INT-formazan*.

The resulting change in absorbance at 492 nm can be used to quantify the analyte of interest, as discussed in the Beginner's Guide in the previous pages.

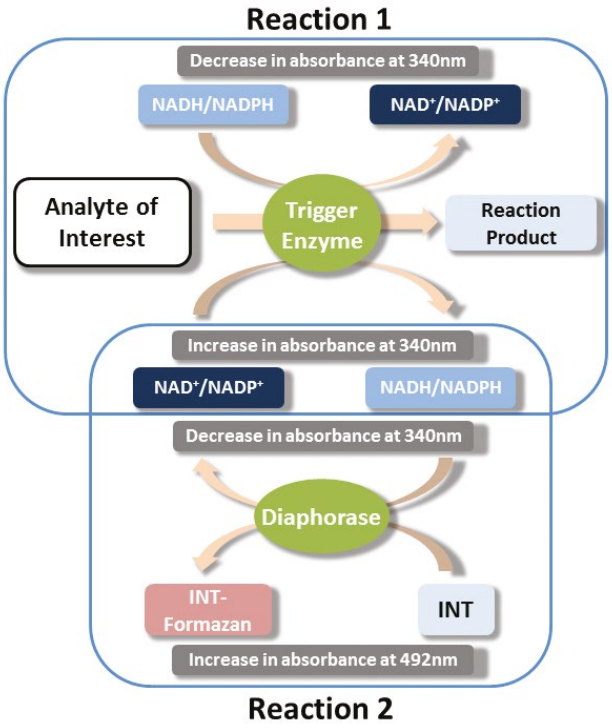


Figure 3. Enzymatic Assays based on NAD^+ / NADH / $NADP^+$ / NADPH.

Enzymatic Assays Based on GOPOD Determination

A number of our test procedures are based on the ability to quantify glucose using Megazyme's own Glucose Oxidase/Peroxidase system (GOPOD).

The GOPOD method can be applied directly to measure glucose in a sample or to measure analytes that can be stoichiometrically converted to glucose.

The principle of the GOPOD system is shown in Figure 4. In Reaction 1, glucose is converted to glucono-δ-lactone by glucose oxidase with the production of H_2O_2. In Reaction 2, H_2O_2 is used by peroxidase to form a quinoneimine that absorbs at 510 nm.

The resulting change in absorbance at 510 nm can be used to quantify the analyte of interest as discussed previously.

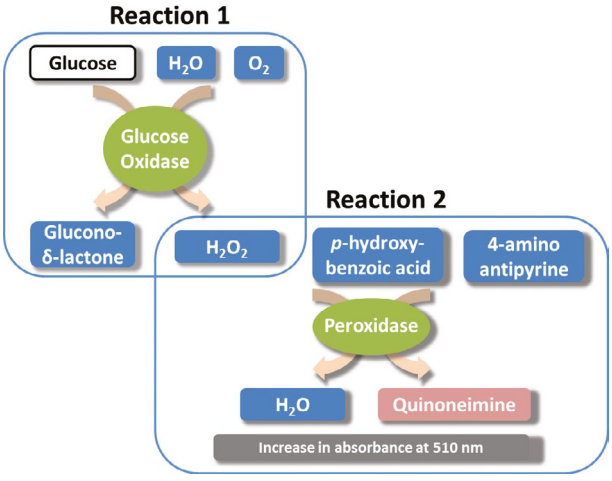


Figure 4. Enzymatic assays based on GOPOD determination

Kit Formats and Equipment Explained

Megazyme is proud to offer complete laboratory solutions, by not only supplying enzymatic assay kits but also the associated equipment required to perform the analyses.

The Megazyme range caters to wine businesses of all sizes, from small vineyards with minimal scientific equipment to specialised, high-throughput analytical laboratories carrying out thousands of tests per day.

Laboratory Equipment

Enzymatic assays can be carried out on three main types of analyser: the manual spectrophotometer (described above), the microplate reader, and the auto-analyser. If you wish to find out more about which analyser meets the needs of your wine business, you can find in-depth product details in our **Equipment** section (page 48).

Assay Kit Formats

Our standard kits are designed for use with a manual spectrophotometer as described in the Beginner's Guide, but most kits in the range can also be used with microplate readers and/or auto-analysers. For some of our most popular products, we offer specialised kits that are optimised for use with auto-analyser formats.

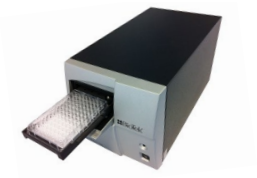
Manual Spectrophotometer

Manual format for use with cuvettes/tubes and a standard UV/Vis spectrophotometer.

One of the most widely-used formats, Features of the manual spectrophotometer include:

- lowest equipment cost
- simple assay format
- step-by-step instructions built in (on the MegaQuant™ Wave)

Microplate



Automated or manual format for use with standard 96-well microplates and microplate reader.

Microplate readers work in a similar way to manual spectrophotometers, but can measure the absorbance of a greater number of samples at once.

- measures absorbance of one microplate (up to 96 assays) in ~ 1 min
- uses ~ 10-fold lower assay volume than manual spectrophotometer format

Auto-analyser

Automated format for use with any auto-analyser model.

Auto-analysers are designed for laboratories carrying out a high volume of tests per day.

- suited for high-throughput processes
- uses ~ 10-fold lower assay volume than manual spectrophotometer format
- assays hundreds of samples per hour

Method Validation and Accreditation

Many of the analytical methods developed by Megazyme have been validated as official standard methods, recommended by the relevant regulatory bodies and scientific associations following rigorous inter-laboratory evaluations.

These stamps of approval demonstrate that these methods deliver accurate, reliable, quantitative and easy-to-use test methods that meet our customers' exacting specifications.

The following official bodies have approved one or more Megazyme test procedures for wine. Also shown are their abbreviations which are used in the individual assay kit descriptions, where applicable.

Beverage Industry Standards

AIJN
Association of the Industry of Juices and Nectars

BAWB
Austrian Federal Office for Viticulture

ASBC
American Society of Brewing Chemists

EBC
European Brewery Convention

IFU
International Federation of Fruit Juice Producers

MEBAK
Central European Committee for Brewing Analysis

OIV
International Organisation of Vine and Wine

UV
Université du Vin de Suze-la-Rousse

Other National and International Standards

AOAC
Association of Official Analytical Chemists

DIN
German Norms

EN / EEC
European Norms

GOST
Russian Federation Standards

ICUMSA
International Commission for Uniform Methods of Sugar Analysis

IDF
International Dairy Federation

IOCCC
International Office of Cocoa and Chocolate

ISO
International Standard Organisation

NBN
Belgian Norms

NEN
Dutch Norms

NF
French Norms

NMKL
Nordic Committee on Food Analysis

Precision of Megazyme Kits

'Precision' refers to the capability of an assay to produce the same result from the same sample measured under the same conditions, that is, the repeatability or reproducibility of its results.

This characteristic is of vital importance for winemakers comparing outcomes within batches or across vintages.

Quantifying Precision

The precision of the individual values generated by a specific assay kit can be measured in terms of the *Coefficient of Variation* (%CV).

The tables below show repeatability performance data for assays performed at Megazyme laboratories. The results demonstrate that Megazyme assay kits provide for extremely precise biological assays, generating %CVs well within the target %CV of < 3 for both red and white wine samples.

White Wine Samples

Assay Kit and Analyte		*n*	Mean	Standard Deviation	%CV
K-ETOH:	Ethanol (%v/v)	6	12.87	0.28	**2.21**
K-ACETRM:	Acetic Acid (g/L)	6	0.39	0.01	**1.66**
K-TSULPH:	Total Sulphite (mg/L)	6	111.06	1.64	**1.48**
K-FSULPH:	Free Sulphite (mg/L)	6	31.16	0.18	**0.59**
K-FRUGL:	D-Glucose (g/L)	6	1.10	0.02	**2.16**
K-FRUGL:	D-Fructose (g/L)	6	2.64	0.01	**0.22**
K-TART:	Tartaric Acid (g/L)	6	0.81	0.02	**1.85**
K-LMAL:	L-Malic Acid (g/L)	6	1.21	0.01	**1.04**
K-LATE:	Lactic Acid (g/L)	6	1.29	0.01	**1.14**
K-SUCC:	Succinic Acid (g/L)	6	0.39	0.00	**0.87**

Red Wine Samples

Assay Kit and Analyte		*n*	Mean	Standard Deviation	%CV
K-ETOH:	Ethanol (%v/v)	6	12.21	0.22	**1.77**
K-ACETRM:	Acetic Acid (g/L)	6	0.61	0.00	**0.76**
K-TSULPH:	Total Sulphite (mg/L)	6	64.21	1.43	**2.23**
K-FSULPH:	Free Sulphite (mg/L)	6	33.64	0.36	**1.07**
K-FRUGL:	D-Glucose (g/L)	6	0.98	0.01	**1.03**
K-FRUGL:	D-Fructose (g/L)	6	0.87	0.01	**1.25**
K-TART:	Tartaric Acid (g/L)	6	1.11	0.01	**1.09**
K-CITR:	Citric Acid (g/L)	6	0.11	0.00	**1.51**
K-LATE:	Lactic Acid (g/L)	6	1.29	0.02	**1.53**
K-SUCC:	Succinic Acid (g/L)	6	1.02	0.01	**0.59**

Accuracy of Megazyme Kits

Accuracy refers to the agreement between results from one form of analysis with the results of other analyses of the same sample.

Quantifying Accuracy

The tables below show accuracy performance data for assays performed by a number of laboratories around the world. Accuracy is measured in terms of the *Bias* (*b*) of the assay or method. Here, a mean is calculated from the analyses performed by all laboratories. The *Bias* (*b*) for the Megazyme kit is the relative percentage recovery of the analyte in comparison with the mean. The data below demonstrate the excellent performance of Megazyme kits relative to other enzymatic assay kits and to alternative methods of analysis.

Ethanol Assays

Laboratory ID	White Wine (% v/v)	Red Wine (% v/v)	Method Type
2	13.2	12.8	**Distillation/specific gravity**
3	13.3	12.9	**FOSS WineScan**
4	13.4	12.7	**Ebulliometer**
Megazyme	13.4	13.3	**Enzymatic kit: K-ETOH**
11	13.6	13.2	**Alcolyzer**
17	13.7	13.1	**ANIR**
No. labs	26	26	
Mean	13.592	13.154	
***b* (%)**	**-1.67**	**0.93**	

L-Malic Acid Assays

Laboratory ID	White Wine (g/L)	Red Wine (g/L)	Method Type
10	2.73	0.05	**Enzymatic Kit**
11	2.80	0.06	**Enzymatic Kit**
Megazyme	2.77	0.06	**Enzymatic Kit: K-LMAL**
14	2.89	0.03	**FOSS WineScan**
15	2.90	0.06	**EasyLab Reflectometry**
No. labs	18	18	
Mean	2.765	0.055	
***b* (%)**	**0.24**	**4.14**	

Reducing Sugars (Fructose/Glucose) Assays

Laboratory ID	White Wine (g/L)	Red Wine (g/L)	Method Type
2	3.18	2.14	**FOSS WineScan**
3	3.25	3.29	**Enzymatic Kit**
5	3.49	2.66	**Enzymatic Kit**
Megazyme	3.64	2.67	**Enzymatic Kit: K-FRUGL**
10	3.74	2.56	**HPLC**
12	3.80	2.70	**HPLC**
No. labs	13	13	
Mean	3.526	2.635	
***b* (%)**	**3.08**	**1.49**	

ASSAY KITS FOR WINE ANALYSIS

List of Assay Kits

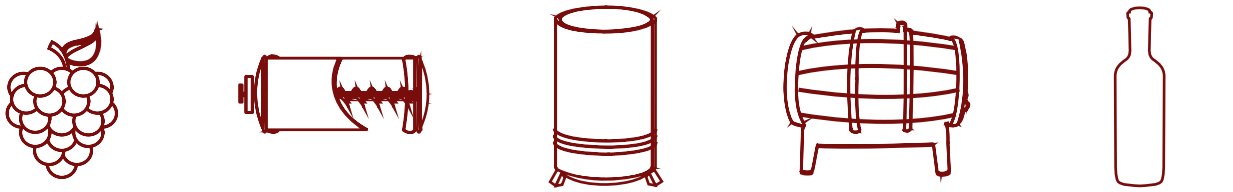
Analyte	Product Name	Catalogue No.	Page
Assay Kits for Organic Acids			
Acetic Acid (for Volatile Acidity)			16
	Acetic Acid (acetate kinase)	K-ACETRM, K-ACETAK	
	Acetic Acid (ADP-GK)	K-ACETGK	
	Acetic Acid (acetyl-CoA synthetase)	K-ACET, K-ACETAF	
Ascorbic Acid	L-Ascorbic Acid	K-ASCO	20
Citric Acid		K-CITR	21
Gluconic Acid	D-Gluconic Acid / D-Glucono-δ-lactone	K-GATE	22
Lactic Acid			23
	D-Lactic Acid	K-DATE	
	D-/L-Lactic Acid	K-DLATE	
	L-Lactic Acid	K-LATE	
Malic Acid			25
	D-Malic Acid	K-DMAL	
	L-Malic Acid	K-LMAL, K-LMALAF, K-LMALQR	
Succinic Acid		K-SUCC	28
Tartaric Acid (for Titratable Acidity)		K-TART	29
Assay Kits for Other Analytes			
Acetaldehyde		K-ACHYD	30
Ethanol		K-ETOH, K-ETOHLQR	31
Glycerol		K-GCROL, K-GCROLGK	33
Reducing Sugars and Sucrose			35
	D-Fructose/D-Glucose	K-FRUGL, K-FRGLQR	
	Sucrose/D-Fructose/D-Glucose	K-SUFRG	
Sulphites			38
	Total Sulphite	K-ETSULPH, K-TSULPH	
	Total & Free Sulphite	K-SULPH	
	Free Sulphite	K-FSULPH	
Yeast Assimilable Nitrogen (YAN)			41
	Primary Amino Nitrogen	K-PANOPA	
	L-Arginine/Urea /Ammonia (Rapid)	K-LARGE	
	Ammonia (Rapid)	K-AMIAR	

Guide to Symbols

The following icons, symbols and graphics are used in this catalogue.

'When to test' graphic

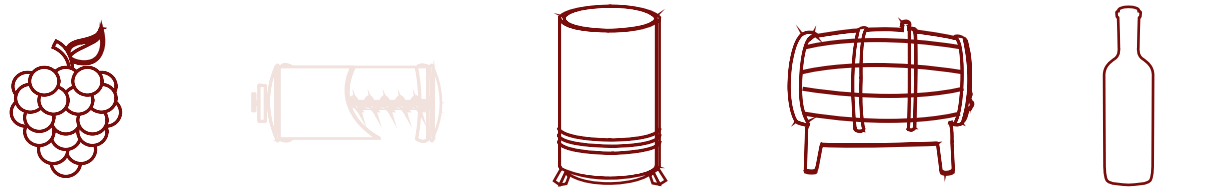
Each analyte guide includes a graphic showing when that analyte is most commonly tested during the vinification process. The steps in the vinification process that are considered are as follows (also used on our wine infographic on page 5):



Mature Grapes *Juicing* *Fermentation* *Maturation* *Finished Wine*

Every winery and wine will have its own unique testing requirements. However we have aimed to indicate the typical stages during winemaking when measurement of an analyte is likely to provide useful results that will direct further action. If we recommend testing at a specific step, it is displayed in a dark red colour and labelled as follows:

Test Acetic Acid in...



Mature Grapes *Juicing* *Fermentation* *Maturation* *Finished Wine*

*In this example, we recommend measuring Acetic Acid in **Mature Grapes**, during **Fermentation**, during **Maturation**, and in **Finished Wine**.*

Icons for product features

The data listing for each Megazyme product is shown with a sidebar that summarises the product features. The following symbols are used where appropriate:

Mega-Calc™

Developed by Megazyme, **Mega-Calc™** is an Excel-based spreadsheet tool which automatically completes calculations for our assay kits for hassle-free raw data processing. Each Mega-Calc™ is freely available from the relevant kit's product page on the Megazyme website.

MegaQuant™ Wave Pre-installed Protocol

The MegaQuant™ Wave spectrophotometer is supplied with a **pre-installed software protocol** for this kit, giving the user step-by-step instructions while completing the assay.

Standard Included

Kit includes **assay standard** sample to assess assay performance.

Enzymes Available

Enzymes used in this kit are **available separately**. See page 45 for our list of wine enzymes.

Acetic Acid (for Volatile Acidity)

Acetic Acid is the main component of a wine's volatile acidity (VA), and is responsible for causing "vinegar taint." VA refers to gaseous acids found in the wine, with acetic acid accounting for around 85% of VA.

Where does it come from?

Acetic acid is produced by certain yeasts as a natural part of the wine-making process including *Brettanomyces* and, to a lesser extent, *Saccharomyces.* However, it is also produced in larger quantities by spoilage organisms such as *Acetobacter aceti,* which may be present between juicing and bottling, and by lactic acid bacteria (if there are high sugar levels in the wine during malolactic fermentation).

What does acetic acid/VA mean for my wine?

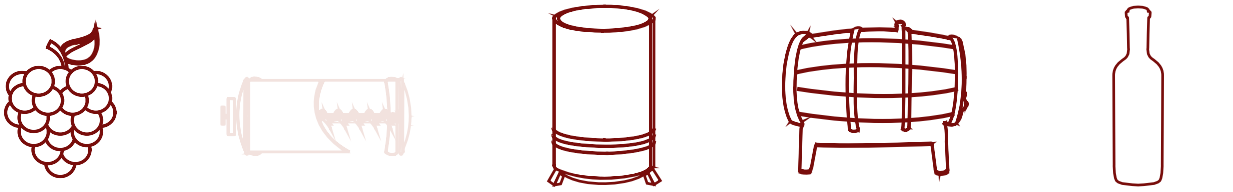
An unpleasant vinegar taste becomes noticeable when acetic acid concentration reaches 600-900 mg/L. However there is also a regulatory dimension.

What can I do with the acetic acid result?

Winemakers can limit the growth of acetic acid micro-organisms depending on the source. For example, *Brettanomyces* yeast contamination can be prevented by inoculating wine with another type of yeast. Growth of acetic acid bacteria during maturation can be limited by correcting the oxygen levels in wine containers (usually by minimising headspace); or SO_2 may be added at various points during vinification.

Megazyme also offers a range of kits for measuring **Sulphites**.
See page 38 for more.

Test Acetic Acid in...



Mature Grapes *Juicing* *Fermentation* *Maturation* *Finished Wine*

Legal Limits

In some important markets, there is a legal limit to the level of volatile acid permitted in a table wine.

Around 15% of actual volatile acidity comes from the presence of lactic, butyric, and other acids, but typically, winemakers are asked to report the acetic acid concentration only.

However, notably, the legal limit is higher than the sensory threshold for acetic acid, i.e. the point at which VA begins to affect the wine's flavour.

Country	Red Wine	White Wine
Australia	1.5 g/L	1.5 g/L
Argentina	*no limit*	*no limit*
Chile	1.5 g/L	1.5 g/L
China	1.2 g/L	1.2 g/L
European Union	1.07 g/L	1.2 g/L
New Zealand	*by certification*	*by certification*
South Africa	*no limit*	*no limit*
United States	1.4 g/L	1.2 g/L

Which acetic acid kit is right for me?

At Megazyme, we understand that different wine laboratories have different priorities when selecting an assay format. Megazyme offers five kits for measuring acetic acid in order to cater for laboratories of every size. The features of our five acetic acid options are summarised in the following table.

	K-ACETRM	K-ACETAK	K-ACETGK	K-ACET	K-ACETAF
Suitable for manual	✔	✖	✖	✔	✖
Suitable for microplate	✔	✖	✖	✖	✖
Suitable for auto-analyser	✖	✔	✔	✖	✔
Biochemistry of assay	Acetate kinase			Acetyl-CoA synthetase	
Reaction time	~ 4 min	~ 10 min	~ 8 min at 25°C ~ 5 min at 37°C	~ 14 min	~ 15 min
Limit of detection	0.254 mg/L	~ 10 mg/L	~ 10 mg/L	0.14 mg/L	~ 10 mg/L
Method recognition		UV		EN, ICUMSA, IFU, ISO, MEBAK	EN, ICUMSA, IFU, ISO, MEBAK, UV
Shelf life	> 2 years	> 2 years	> 2 years	> 2 years	> 2 years
Assays per kit	72 / 720	550	500	53	456

For advice on which Megazyme solution is best for your laboratory, contact our team of experts via the Megazyme website.

Acetic Acid (Acetate kinase - rapid manual format) Cat. No. K-ACETRM

	Manual	Microplate	Auto-analyser
Kit size	72	720	n/a

Detection method: Spectrophotometric at 340 nm.
Reaction time: ~ 4 min
Detection limit: 0.254 mg/L
Range: up to 0.25 g/L

The Megazyme Difference:
All of our Acetic Acid kits contain PVP to prevent tannin inhibition. K-ACETRM is a very rapid acetate kinase (AK) based kit for manual formats, with excellent linearity.

Principle:

(1) **Acetic acid + ATP** —(acetate kinase)→ **acetyl-phosphate + ADP**

(2) **Acetyl-phosphate + CoA** —(phosphotransacetylase)→ **acetyl-CoA + P_i**

(3) **ADP + PEP** —(pyruvate kinase)→ **ATP + pyruvate**

(4) **Pyruvate + NADH + H^+** —(D-lactate dehydrogenase)→ **D-lactic acid + NAD^+**

Method recognition: Improved method

Features

- Improved assay format (only two absorbance readings required)
- All reagents stable for > 2 years after preparation
- Extended cofactor stability
- Linear range 0.3 to 25 µg
- When used with MegaQuant™ Wave, the number of manual tests per kit can be doubled by halving all reagent volumes.

Enzymes Available | Standard Included | Mega-Calc™ | MegaQuant™ Wave Pre-installed Protocol

Acetic Acid (Acetate kinase analyser format) — Cat. No. K-ACETAK

	Manual	Microplate	Auto-analyser
Kit size	n/a	n/a	550

Detection method: Spectrophotometric at 340 nm
Reaction time: ~ 10 min
Detection limit: ~ 10 mg/L
Range: up to ~ 1.8 g/L

The Megazyme Difference:
All of our Acetic Acid kits contain PVP to prevent tannin inhibition. K-ACETAK is a very rapid acetate kinase-based auto-analyser kit with excellent linearity.

Principle:

(1) **Acetic acid + ATP** —(acetate kinase)→ **acetyl-phosphate + ADP**

(2) **ADP + PEP** —(pyruvate kinase)→ **ATP + pyruvate**

(3) **Pyruvate + NADH + H$^+$** —(D-lactate dehydrogenase)→ **D-lactic acid + NAD$^+$**

Method recognition:

UV

UV

Features

- Very stable reagent when prepared for auto-analyser applications (> 7 days at 4°C)
- All reagents stable for > 2 years
- Linear calibration (R^2 ~ 0.9995) up to 30 µg/mL of acetic acid in final reaction solution
- Very rapid reaction

Enzymes Available

Standard Included

Acetic Acid (ADP-GK analyser format) — Cat. No. K-ACETGK

	Manual	Microplate	Auto-analyser
Kit size	n/a	n/a	500

Detection method: Spectrophotometric at 340 nm
Reaction time: 8 min at 25°C or 5 min at 37°C
Detection limit: ~ 10 mg/L
Range: up to ~ 1.8 g/L

The Megazyme Difference:
All of our Acetic Acid kits contain PVP to prevent tannin inhibition. K-ACETGK is a new rapid auto-analyser assay kit employing AK and phosphotransacetylase.

Principle:

(1) **Acetic acid + ATP** —(acetate kinase)→ **acetyl-phosphate + ADP**

(2) **Acetyl-phosphate + CoA** —(phosphotransacetylase)→ **acetyl-CoA + P_i**

(3) **ADP + D-glucose** —(ADP-GK)→ **glucose-6-phosphate + AMP**

(4) **Glucose 6-phosphate + NAD$^+$** —(G6P-DH)→ **6-phosphogluconate + NADH + H$^+$**

Method recognition: Improved method

Features

- Excellent reagent stability: > 2 years as supplied
- When prepared, reagents stable for 7 days at 4°C or > 2 years at below -10°C
- Very rapid reaction (~ 5 min at 37°C)
- Linear calibration (R^2 ~ 0.997 up to 1.8 g/L sample)

Enzymes Available

Standard Included

Acetic Acid (Acetyl-CoA synthetase manual format) — Cat. No. K-ACET

	Manual	Microplate	Auto-analyser
Kit size	53	n/a	n/a

Detection method: Spectrophotometric at 340 nm
Reaction time: ~ 14 min
Detection limit: 0.14 mg/L
Range: up to 0.2 g/L

The Megazyme Difference:
All of our Acetic Acid kits contain PVP to prevent tannin inhibition. K-ACET contains a stable ACS solution, meaning no enzyme is wasted when performing the assay.

Principle:

(acetyl-CoA synthetase)

(1) **Acetic acid + ATP + CoA ⟶ acetyl-CoA + AMP + pyrophosphate**

(citrate synthase)

(2) **Acetyl-CoA + oxaloacetate + H_2O ⟶ citrate + CoA**

(L-malate dehydrogenase)

(3) **L-Malate + NAD^+ ⟷ oxaloacetate + NADH + H^+**

Method recognition:

EN ICUMSA IFU ISO MEBAK

Features

- No wasted ACS solution (stable suspension supplied)
- All reagents stable for > 2 years after preparation
- Extended cofactor stability
- Linear range 0.3 to 20 µg
- When used with MegaQuant™ Wave, the number of manual tests per kit can be doubled by halving all reagent volumes.

Enzymes Available · Standard Included · Mega-Calc™ · MegaQuant™ Wave Pre-installed Protocol

Acetic Acid (Acetyl-CoA synthetase analyser format) — Cat. No. K-ACETAF

	Manual	Microplate	Auto-analyser
Kit size	n/a	n/a	141.6 ml (R1 and R2)

Detection method: Spectrophotometric at 340 nm
Reaction time: ~ 15 min
Detection limit: ~ 10 mg/L
Range: up to ~ 1.8 g/L

The Megazyme Difference:
All of our Acetic Acid kits contain PVP to prevent tannin inhibition. K-ACETAF is used to prepare very stable R1 and R2.

Principle:

(acetyl-CoA synthetase)

(1) **Acetic acid + ATP + CoA ⟶ acetyl-CoA + AMP + pyrophosphate**

(citrate synthase)

(2) **Acetyl-CoA + oxaloacetate + H_2O ⟶ citrate + CoA**

(L-malate dehydrogenase)

(3) **L-Malate + NAD^+ ⟶ oxaloacetate + NADH + H^+**

Method recognition:

EN ICUMSA IFU ISO MEBAK UV

Features

- No wasted ACS solution (stable suspension supplied)
- Very stable reagent when prepared for auto-analyser formats (> 3 days at 4°C)
- Linear calibration up to 30 µg/mL of acetic acid in final reaction solution
- All reagents stable for > 2 years after preparation

Enzymes Available · Standard Included

Ascorbic Acid

Present naturally in grapes and can be added at bottling as an antioxidant.

Where does it come from?

Ascorbic acid, also known as Vitamin C, is found naturally in young grapes before they ripen. It is also added by winemakers at bottling.

What does ascorbic acid mean for my wine?

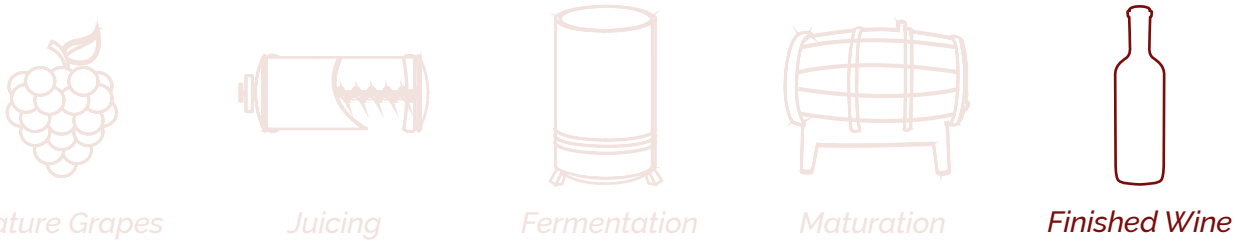
Ascorbic acid is used alongside sulphur dioxide as an antioxidant, i.e. to bind to substances in the wine that could cause oxidation. Ascorbic acid is not typically added to red wine due to its interaction with red wine pigments, resulting in a yellowish colour.

Where an influence on taste is noted, ascorbic acid is said to impart a "fruity" flavour to wine.

What can I do with the ascorbic acid result?

Limits set by the OIV state that wine sold in the European Union may not contain more than 300 mg/L of ascorbic acid.

Test Ascorbic Acid in...



L-Ascorbic Acid

Cat. No. **K-ASCO**

	Manual	Microplate	Auto-analyser
Kit size	40	400	400

Detection method: Spectrophotometric at 578 nm
Reaction time: ~ 8 min
Detection limit: 0.175 mg/L
Range: up to 0.3 g/L

The Megazyme Difference:
Rapid reaction and stable reagents.

Principle:

(5-methylphenazinium methosulphate)

(1) L-Ascorbic acid + R-H_2 + MTT ⟶ dehydroascorbate + MTT-formazan + H^+

(ascorbic acid oxidase)

(2) L-Ascorbic acid + ½O_2 ⟶ dehydroascorbate + H_2O

Method recognition:

MEBAK

Features

- All reagents stable for > 6 months after preparation
- When used with MegaQuant™ Wave, the number of manual tests per kit can be doubled by halving all reagent volumes.

Standard Included

Mega-Calc™

MegaQuant™ Wave Pre-installed Protocol

Citric Acid

Naturally present in small amounts. High concentrations indicate addition for acidification.

Where does it come from?

Citric acid is found in very low concentrations in grapes. However, it may be added by winemakers for a variety of reasons.

What does citric acid mean for my wine?

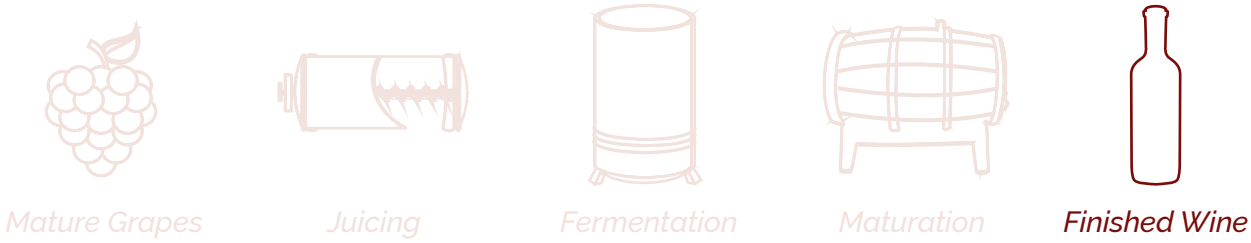
Citric acid has an important role in a number of biochemical processes that take place during vinification. Most notably, citric acid slows the growth of *Oenococcus oeni*, a lactic acid bacterium that breaks down the desirable compound diacetyl during malolactic fermentation. Addition of citric acid means that diacetyl persists into the finished wine at higher concentrations, lending wine a "buttery" mouthfeel.

Other applications of citric acid include addition to reduce "browning" of white wines, to clear any ferric haze caused by high iron concentration, or to increase a wine's acidity and impart a "fresh" flavour.

What can I do with the citric acid result?

In some jurisdictions, there are regulations surrounding the concentration of citric acid that is permitted in wine offered commercially. For example, wine sold in the European Union may not contain more than 1 g/L of citric acid (per OIV rules).

Test Citric Acid in...



Citric Acid — Cat. No. K-CITR

	Manual	Microplate	Auto-analyser
Kit size	72	720	840

Detection method: Spectrophotometric at 340 nm
Reaction time: ~ 5 min
Detection limit: 0.491 mg/L
Range: up to 0.5 g/L

The Megazyme Difference:
Ideal for both manual and auto-analyser applications. Reconstituted citrate lyase stable for 4 weeks at 4°C or > 6 months below -10°C.

Principle:

(1) Citrate —(citrate lyase)→ oxaloacetate + acetate

(2) Oxaloacetate + NADH + H^+ —(L-malate dehydrogenase)→ L-malate + NAD^+

(3) Pyruvate + NADH + H^+ —(D-lactate dehydrogenase)→ D-lactate + NAD^+

Method recognition:
AOAC, EN, IFU22, ISO2963, MEBAK, OIV

Features

- Buffer/cofactor/enzyme tablets for efficient use of kit components
- PVP incorporated to prevent tannin inhibition
- Extended cofactors stability
- When used with MegaQuant™ Wave, number of tests per kit can be doubled by halving all reagent volumes.

Enzymes Available | Standard Included | Mega-Calc™ | MegaQuant™ Wave Pre-installed Protocol

Gluconic Acid

An indicator of *Botrytis cinerea* presence in the harvest.

Where does it come from?

Glucose is oxidised to gluconic acid during ripening, but this process is accelerated when *Botrytis cinerea* (grey mould fungus) is present on grapes.

What does gluconic acid mean for my wine?

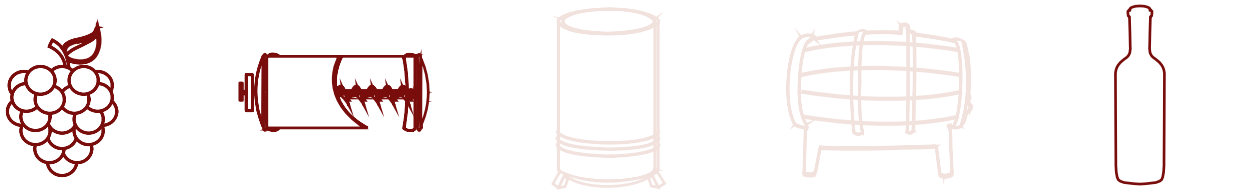
Gluconic acid is most important as an indicator of *Botrytis cinerea* infection. If weather conditions are favourable, *Botrytis* will draw water out of colonised grapes, leaving behind a higher concentration of sugars and acids ('noble rot'). However, in cool or damp conditions, *Botrytis* may spread as highly damaging 'grey rot'.

In addition to introducing oxidative spoilage bacteria to the wine, grapes infected with *Botrytis* mould can also impart an "earthy" flavour. A finished wine should contain no more than 200-300 mg/L of gluconic acid.

What can I do with the gluconic acid result?

The oxidative effect of laccase can be addressed by ensuring minimal contact of the maturing wine with oxygen.

Test Gluconic Acid in...



Mature Grapes *Juicing* *Fermentation* *Maturation* *Finished Wine*

D-Gluconic Acid / D-Glucono-δ-lactone — Cat. No. K-GATE

	Manual	Microplate	Auto-analyser
Kit size	60	600	600

Detection method: Spectrophotometric at 340 nm
Reaction time: ~ 6 min
Detection limit: 0.792 mg/L
Range: up to 0.5 g/L

The Megazyme Difference:
Rapid reaction, stable reagents.

Principle:

(1) D-Gluconate + ATP —(gluconate kinase)→ gluconate-6-phosphate + ADP

(2) Gluconate-6-phosphate + $NADP^+$ —(gluconate-6-phosphate dehydrogenase)→ ribulose-5-phosphate + NADPH + CO_2 + H^+

(3) D-Glucono-δ-lactone + H_2O —(pH 11)→ D-gluconate

Method recognition:

DIN GOST ISO

Features

- All reagents stable for > 2 years after preparation
- Extended cofactors stability
- When used with MegaQuant™ Wave, the number of manual tests per kit can be doubled by halving all reagent volumes.

Enzymes Available | Standard Included | Mega-Calc™ | MegaQuant™ Wave Pre-installed Protocol

Lactic Acid

Normal product of malolactic fermentation: a step in winemaking which converts 'tart' malic acid to milder lactic acid.

Where does it come from?

During fermentation, pyruvic acid is converted into one of two forms of lactic acid. D-Lactic acid is generated through yeast fermentation while L-lactic acid is mainly produced by bacteria in malolactic fermentation (which can be intentional or incidental), replacing malic acid with the milder lactic acid.

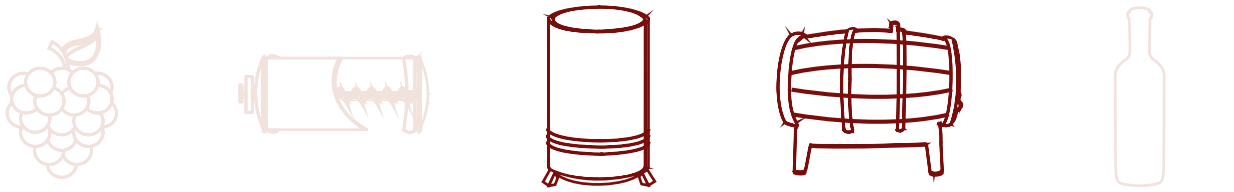
What does lactic acid mean for my wine?

The presence of lactic acid confers a "creamy" mouthfeel in lower concentrations but may cause spoilage at higher concentrations. A barrel colonised by lactic acid bacteria will induce malolactic fermentation in any wine matured in it.

What can I do with the lactic acid result?

The course of malolactic fermentation is monitored by following the falling level of L-malic acid, and the increasing level of L-lactic acid. The production of D-lactic acid can indicate wine spoilage.

Test Lactic Acid in...



D-Lactic Acid — Cat. No. K-DATE

	Manual	Microplate	Auto-analyser
Kit size	50	500	450

Detection method: Spectrophotometric at 340 nm
Reaction time: ~ 5 min
Detection limit: 0.214 mg/L
Range: up to 0.3 g/L

The Megazyme Difference:
Rapid reaction, stable reagents.

Features

- Very rapid reaction with most samples (~ 5 min)
- All reagents stable for > 2 years after preparation
- Extended cofactors stability
- When used with MegaQuant™ Wave, the number of manual tests per kit can be doubled by halving all reagent volumes.

Enzymes Available | Standard Included | Mega-Calc™ | MegaQuant™ Wave Pre-installed Protocol

Principle:

(D-lactate dehydrogenase)

(1) **D-Lactic acid + NAD^+ ⟷ pyruvate + $NADH + H^+$**

(glutamate-pyruvate transaminase)

(2) **Pyruvate + D-glutamate ⟶ D-alanine + 2-oxoglutarate**

Method recognition:

AIJN DIN EN GOST IDF IFU ISO MEBAK OIV

D- / L-Lactic Acid

Cat. No. K-DLATE

	Manual	Microplate	Auto-analyser
Kit size	100	n/a	n/a

Detection method: Spectrophotometric at 340 nm
Reaction time: ~ 10 min (L-lactic acid) and ~ 5 min (D-lactic acid)
Detection limit: 0.214 mg/L
Range: up to 0.3 g/L

The Megazyme Difference:
Rapid total analysis time (concurrent/flexible D- and L-lactic acid reaction format), stable reagents.

Principle:

(D-lactate dehydrogenase)

(1) **D-Lactic acid + NAD^+ ⟷ pyruvate + NADH + H^+**

(glutamate-pyruvate transaminase)

(2) **Pyruvate + D-glutamate ⟶ D-alanine + 2-oxoglutarate**

(L-lactate dehydrogenase)

(3) **L-Lactic acid + NAD^+ ⟶ pyruvate + NADH + H^+**

Method recognition:

AIJN DIN EN GOST IDF IFU ISO MEBAK OIV

Features

- D-lactate dehydrogenase reaction very rapid with most samples (~ 5 min)
- All reagents stable for > 2 years after preparation
- Extended cofactors stability
- When used with MegaQuant™ Wave, the number of manual tests per kit can be doubled by halving all reagent volumes.

Enzymes Available · Standard Included · Mega-Calc™ · MegaQuant™ Wave Pre-installed Protocol

L-Lactic Acid

Cat. No. K-LATE

	Manual	Microplate	Auto-analyser
Kit size	50	500	450

Detection method: Spectrophotometric at 340 nm
Reaction time: ~ 10 min
Detection limit: 0.214 mg/L
Range: up to 0.3 g/L

The Megazyme Difference:
Rapid reaction, stable reagents.

Principle:

(L-lactate dehydrogenase)

(1) **L-Lactic acid + NAD^+ ⟷ pyruvate + NADH + H^+**

(glutamate-pyruvate transaminase)

(2) **Pyruvate + D-glutamate ⟶ D-alanine + 2-oxoglutarate**

Method recognition:

AIJN DIN EN GOST IDF IFU ISO MEBAK OIV

Features

- All reagents stable for > 2 years after preparation
- Rapid reaction
- Extended cofactors stability
- When used with MegaQuant™ Wave, the number of manual tests per kit can be doubled by halving all reagent volumes.

Enzymes Available · Standard Included · Mega-Calc™ · MegaQuant™ Wave Pre-installed Protocol

Malic Acid

Sour-tasting organic acid which is converted to less acidic (milder-tasting) lactic acid during malolactic fermentation.

Where does it come from?

L-Malic acid occurs naturally in grape must and is used as an indicator of ripeness. D-malic acid is primarily found in fruit juices and is not a natural by-product of vinification.

What does malic acid mean for my wine?

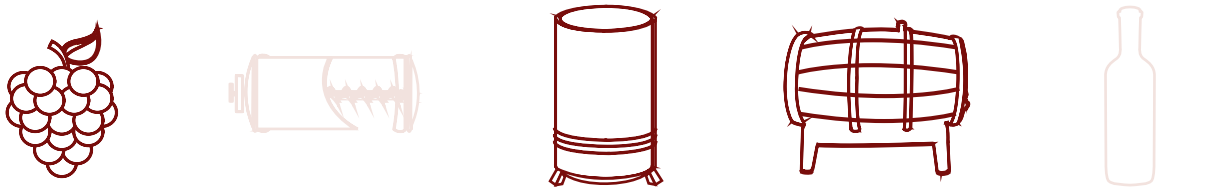
Malic acid contributes a sour, 'tart' flavour to the wine's overall taste, which is addressed by converting malic acid to the milder lactic acid in malolactic fermentation.

The detection of D-malic acid indicates that it has been added artificially to boost the wine's acidity.

What can I do with the malic acid result?

L-Malic acid is the most commonly-assayed acid in winemaking, allowing the winemaker to assess grape ripeness and to quantify the progress of malolactic fermentation.

Test Malic Acid in...



Mature Grapes *Juicing* *Fermentation* *Maturation* *Finished Wine*

D-Malic Acid

Cat. No. **K-DMAL**

	Manual	Microplate	Auto-analyser
Kit size	100	1000	1100

Detection method: Spectrophotometric at 340 nm
Reaction time: ~ 6 min
Detection limit: 0.26 mg/L
Range: up to 0.4 g/L

The Megazyme Difference:
D-Malate dehydrogenase is supplied as a stabilised suspension rather than a lyophilised powder, thus less wasted enzyme.

Principle:

$$\text{D-Malic acid} + NAD^+ \xrightarrow{\text{(D-malate dehydrogenase)}} \text{pyruvate} + CO_2 + NADH + H^+$$

Method recognition:

AIJN DIN EN IFU OIV

Features

- All reagents stable for > 2 years after preparation
- Rapid reaction (even with difficult samples)
- Extended cofactors stability
- When used with MegaQuant™ Wave, the number of manual tests per kit can be doubled by halving all reagent volumes.

Enzymes Available | Standard Included | Mega-Calc™ | MegaQuant™ Wave Pre-installed Protocol

L-Malic Acid (Manual Format) — Cat. No. K-LMAL

	Manual	Microplate	Auto-analyser
58A: Kit size	58	580	n/a
116A: Kit size	116	1160	n/a

Detection method: Spectrophotometric at 340 nm
Reaction time: ~ 3 min
Detection limit: 0.25 mg/L
Range: up to 0.3 g/L

The Megazyme Difference:
Very rapid reaction (~3 min). Our L-malic acid kits also incorporate PVP to prevent tannin inhibition.

Principle:

(L-malate dehydrogenase)

(1) **L-Malic acid + NAD^+** ⟷ **oxaloacetate + NADH + H^+**

(glutamate-oxaloacetate transaminase)

(2) **Oxaloacetate + L-glutamate** ⟶ **L-aspartate + 2-oxoglutarate**

Method recognition:

AOAC AIJN DIN EN GOST IFU ISO MEBAK NBN NEN NF OIV

Features

- Both enzymes supplied as stable suspensions
- All reagents stable for > 2 years after preparation
- Extended cofactors stability
- When used with MegaQuant™ Wave, the number of manual tests per kit can be doubled by halving all reagent volumes.

Enzymes Available | Standard Included | Mega-Calc™ | MegaQuant™ Wave Pre-installed Protocol

L-Malic Acid (Analyser format) — Cat. No. K-LMALAF

	Manual	Microplate	Auto-analyser
Kit size	n/a	n/a	1116

Detection method: Spectrophotometric at 340 nm
Reaction time: ~ 3 min
Detection limit: ~ 20 mg/L
Range: up to ~ 6 g/L

The Megazyme Difference:
Very stable reagent when prepared for auto-analyser applications. Our L-malic acid kits also incorporate PVP to prevent tannin inhibition.

Principle:

(L-malate dehydrogenase)

(1) **L-Malic acid + NAD^+** ⟷ **oxaloacetate + NADH + H^+**

(glutamate-oxaloacetate transaminase)

(2) **Oxaloacetate + L-glutamate** ⟶ **L-aspartate + 2-oxoglutarate**

Method recognition:

AOAC AIJN DIN EN GOST IFU ISO NBN MEBAK NEN NF OIV

Features

- Linear calibration range (R^2 ~ 0.9994) up to 80 µg/mL of L-malic acid in final reaction solution.
- Both enzymes supplied as stable suspensions.
- Competitive price per mL of reagent
- Extended cofactor stability

Enzymes Available | Standard Included

L-Malic Acid (Liquid Ready Reagents) Cat. No. K-LMALQR

	Manual	Microplate	Auto-analyser
Kit size	n/a	1100	1100

Detection method: Spectrophotometric at 340 nm
Reaction time: ~ 3 min
Detection limit: ~ 166 mg/L
Range: up to ~ 6 g/L

The Megazyme Difference:
"Ready to use" liquid stable formulation, with PVP incorporated to prevent tannin inhibition.

Principle:

(L-malate dehydrogenase)

(1) **L-Malic acid + NAD^+ ⟷ oxaloacetate + NADH + H^+**

(glutamate-oxaloacetate transaminase)

(2) **Oxaloacetate + L-glutamate ⟶ L-aspartate + 2-oxoglutarate**

Method recognition:

AOAC AIJN DIN EN GOST IFU ISO NBN MEBAK NEN NF OIV

Features

- All reagents stable for > 18 months
- Extended cofactor stability

Enzymes Available

Standard Included

Succinic Acid

'Salty' and bitter-tasting acid produced during fermentation.

Where does it come from?

Succinic acid is produced from glucose as a normal byproduct of yeast fermentation.

What does succinic acid mean for my wine?

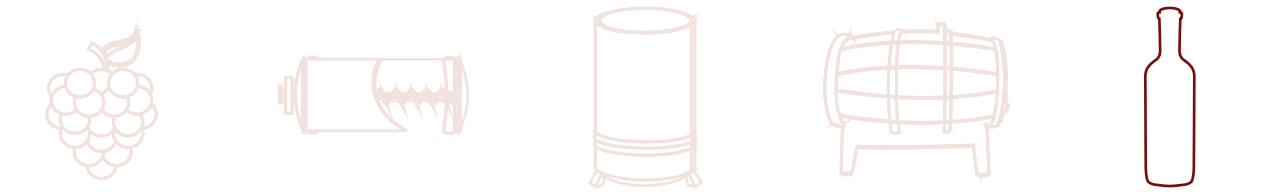
The intense bitter taste of succinic acid causes salivation and can accentuate a wine's flavour. Concentrations are typically higher in white wine than in red, and tend to differ between vintages.

What can I do with the succinic acid result?

Careful management of succinic acid concentration can have a significant impact on the final taste of the wine.

Measuring succinic acid allows winemakers to quantify the results of decisions around fermentation temperatures, yeast strains, aeration, and other factors that promote production of succinic acid.

Test Succinic Acid in...



Succinic Acid — Cat. No. K-SUCC

	Manual	Microplate	Auto-analyser
Kit size	20	200	270

Detection method: Spectrophotometric at 340 nm
Reaction time: ~ 6 min
Detection limit: 0.256 mg/L
Range: up to ~ 0.4 g/L

The Megazyme Difference:
Rapid reaction (~ 6 min even at room temperature), stable reagents.

Principle:

(succinyl-CoA synthetase)

(1) **Succinate + ATP + CoA** $\longrightarrow$ **ADP + succinyl-CoA + P_i**

(pyruvate kinase)

(2) **ADP + PEP** $\longrightarrow$ **ATP + pyruvate**

(L-lactate dehydrogenase)

(3) **Pyruvate + NADH + H^+** $\longrightarrow$ **NAD^+ + L-lactic acid**

Method recognition:

EN

Features

- All reagents stable for > 2 years as supplied
- Extended cofactors stability
- When used with MegaQuant™ Wave, the number of manual tests per kit can be doubled by halving all reagent volumes.

Enzymes Available

Standard Included

Mega-Calc™

MegaQuant™ Wave Pre-installed Protocol

Tartaric Acid

Occurs naturally in grapes and is one of the most prevalent organic acids. Key indicator of total (titratable) acidity (TA).

Where does it come from?

Tartaric acid occurs naturally in the developing grape, with higher concentrations persisting right through to harvest in cooler climates.

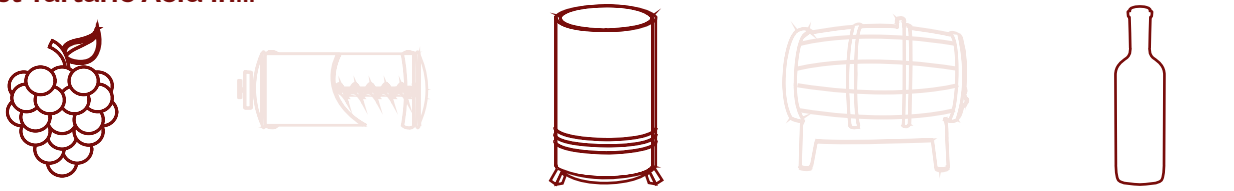
What does tartaric acid mean for my wine?

Tartaric acid has an important role in the wine's ability to tolerate changes in acid levels without an impact on flavour or quality (that is, in its 'buffer capacity'). The tartaric acid concentration is therefore indicative of a wine's chemical stability.

What can I do with the tartaric acid result?

Tartaric acid should be measured in order to gain a baseline tartaric acid concentration ahead of tartration (the process of adding tartaric acid to correct flavour in wines that taste "flat"). Furthermore, tartaric acid is a major component in the titratable acidity (total acidity) of a wine, which may need to be reported along with volatile acidity (see **Acetic Acid**, page 16).

Test Tartaric Acid in...



Mature Grapes *Juicing* *Fermentation* *Maturation* *Finished Wine*

Tartaric Acid — Cat. No. K-TART

	Manual	Microplate	Auto-analyser
Kit size	200	2000	2000

Detection method: Spectrophotometric at 505 nm
Reaction time: ~ 5 min
Detection limit: ~ 108 mg/L
Range: up to ~ 11 g/L

The Megazyme Difference:
Simple, rapid chemical reaction for all formats. Supplied with stable liquid ready reagents.

Principle:

Based on the reaction principle between tartaric acid and vanadate.

Method recognition: Used widely in the wine industry

Features

- "Ready to use" liquid stable formulation
- All reagents stable for > 1 year
- Very rapid reaction
- When used with MegaQuant™ Wave, the number of manual tests per kit can be doubled by halving all reagent volumes.

Standard Included

Mega-Calc™

MegaQuant™ Wave Pre-installed Protocol

Acetaldehyde

Acetaldehyde (sometimes called ethanal) is a sensory compound that adds flavour and complexity, but spoils wine at high concentrations.

Where does it come from?

Acetaldehyde can form at two different points in the vinification process. During yeast fermentation, by-products of ethanol formation are converted to acetaldehyde by the same micro-organisms responsible for producing acetic acid. Much of this acetaldehyde degrades during malolactic fermentation. However, during the maturation stage, ethanol in the wine may be oxidised to acetaldehyde by the enzyme alcohol dehydrogenase.

What does acetaldehyde mean for my wine?

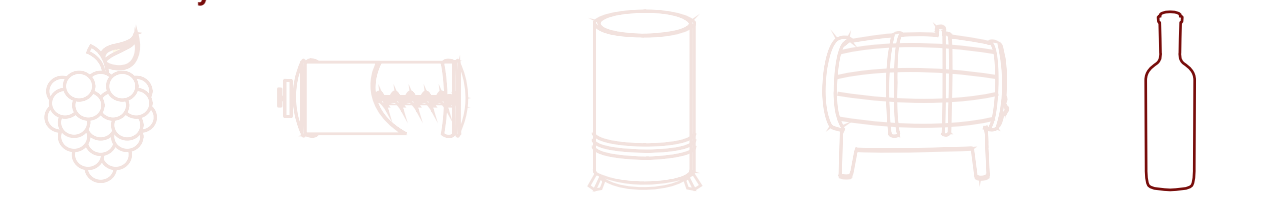
At low levels (< 30 mg/L for red wine and < 80 mg/L for white wine), acetaldehyde imparts a pleasant "fruity" aroma to wine. Above this threshold, acetaldehyde can result in a metallic, "grassy", sherry-like odour.

What can I do with the acetaldehyde result?

High levels of acetaldehyde can be corrected by addition of sulphur dioxide (SO_2). Sulphur dioxide and acetaldehyde bind together to form a compound called hydroxy-sulphonate, which does not influence the flavour and aroma of the wine.

Megazyme also offers a range of kits for measuring **Sulphites**. *See page 38 for more.*

Test Acetaldehyde in...



Acetaldehyde — Cat. No. K-ACHYD

	Manual	Microplate	Auto-analyser
Kit size	50	500	500

Detection method: Spectrophotometric at 340 nm.
Reaction time: ~ 4 min
Detection limit: 0.176 mg/L
Range: up to ~ 0.2 g/L

The Megazyme Difference:
Our aldehyde dehydrogenase enzyme is supplied as a stabilised solution rather than a lyophilised powder (as found in competing products), therefore no enzyme solution is wasted in performing the assay.

Features

- Reagent stability:
  < 2 years (limiting)
  < 2 days (prepared)
- Extended cofactor stability
- When used with MegaQuant™ Wave, the number of manual tests per kit can be doubled by halving all reagent volumes.

Standard Included

Mega-Calc™

MegaQuant™ Wave Pre-installed Protocol

Principle:

$$\text{Acetaldehyde} + NAD^+ + H_2O \xrightarrow{\text{(aldehyde dehydrogenase)}} \text{acetic acid} + NADH + H^+$$

Method recognition:

MEBAK

Ethanol

Produced naturally during fermentation, a wine's alcohol content must be reported on the label in all major markets (see table).

Where does it come from?

Sugars build up naturally in the grape as it ripens, and are converted into ethanol (alcohol) by yeast during fermentation.

What does alcohol mean for my wine?

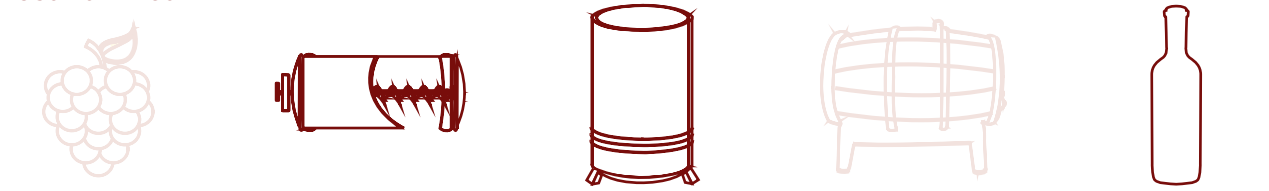
A wine with a higher alcohol level will have a more full-bodied texture. As most yeast species are unable to survive once alcohol concentration reaches 18%, this is typically the natural ceiling for alcohol content unless a wine is fortified.

Country	Reporting	Limit
Australia	% volume. (± 1.5%)	*no limit*
Argentina	% volume.	*Min: 7%*
Chile	Gay-Lusac degrees	
China	% volume	*no limit*
European Union	% volume (± 0.5%)	*no limit*
New Zealand	% volume	16.5%
South Africa	% volume	*Min: 7%*
United States	% volume (± 1%)	*Min: 7%*

What can I do with the alcohol result?

Alcohol content must be stated on the bottle. However, winemakers may also wish to test alcohol levels during the production process to look for variation between harvests and to guide decisions in the fermentation process.

Test Ethanol in...



Ethanol (Liquid Ready Reagents) — Cat. No. K-ETOHLQR

	Manual	Microplate	Auto-analyser
Kit size	60	600	600

Detection method: Spectrophotometric at 340 nm
Reaction time: ~ 7 min
Detection limit: 1.6 mg/L
Range: up to 0.30 g/L

The Megazyme Difference:
'Ready-to-use' liquid stable formulation for maximum convenience. Improves upon our K-ETOH kit with a simplified reaction that results in less interference from environmental ethanol often present in the laboratory. Analyst error is also reduced due to lower sample dilution.

Principle:

(alcohol dehydrogenase)

Ethanol + NAD^+ ⟶ acetaldehyde + NADH + H^+

Method recognition:

AOAC

Features

- Reagent stability: > 12 months (prepared)
- Extended cofactor stability
- When used with MegaQuant™ Wave, the number of manual tests per kit can be doubled by halving all reagent volumes.

Enzymes Available | Standard Included | Mega-Calc™

Ethanol — Cat. No. K-ETOH

	Manual	Microplate	Auto-analyser
Kit size	60	600	600

Detection method: Spectrophotometric at 340 nm
Reaction time: ~ 5 min
Detection limit: 0.093 mg/L
Range: up to 0.12 g/L

The Megazyme Difference:
Rapid reaction, simple format, and stable reagents (AIDH supplied as a stable suspension).

Principle:

(alcohol dehydrogenase)

(1) **Ethanol + NAD^+ ⟶ acetaldehyde + NADH + H^+**

(aldehyde dehydrogenase)

(2) **acetaldehyde + NAD^+ + H_2O ⟶ acetic acid + NADH + H^+**

Method recognition:

ASBC | EBC | IFU | MEBAK

Features

- Reagent stability: < 1 years (limiting) < 2 days (prepared)
- Extended cofactor stability
- When used with MegaQuant™ Wave, the number of manual tests per kit can be doubled by halving all reagent volumes.

Standard Included | Mega-Calc™ | MegaQuant™ Wave Pre-installed Protocol

Glycerol

As a product of fermentation, glycerol occurs at concentrations of approx. 1% (v/v) and is an indicator of quality.

Where does it come from?

During yeast fermentation of sugars, glycerol is produced alongside ethanol and carbon dioxide. *Botrytis* fungus, if present, is also metabolised into glycerol during vinification.

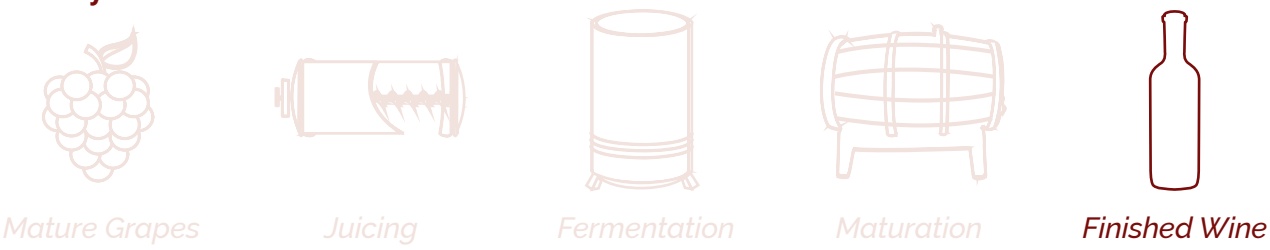
What does glycerol mean for my wine?

Glycerol confers a sweeter flavour to wine. At concentrations above 26 g/L, it is associated with a more full-bodied mouthfeel. Some winemakers use overall glycerol as an indicator of the all-round quality of a wine.

What can I do with the glycerol result?

Winemakers seeking to add body to their wine can experiment with the use of different yeast strains during fermentation, measuring glycerol in order to quantify the impact of changes made.

Test Glycerol in...



Glycerol

Cat. No. K-GCROL

	Manual	Microplate	Auto-analyser
Kit size	70	700	n/a

Detection method: Spectrophotometric at 340 nm
Reaction time: ~ 5 min
Detection limit: 0.342 mg/L
Range: up to 0.35 g/L

The Megazyme Difference:
Extended cofactor stability. Very rapid reaction.

Principle:

(1) **Glycerol + ATP** —(glycerokinase)→ **L-glycerol-3-phosphate + ADP**

(2) **ADP + PEP** —(pyruvate kinase)→ **ATP + pyruvate**

(3) **Pyruvate + NADH + H^+** —(L-lactate dehydrogenase)→ **NAD^+ + L-lactic acid**

Method recognition:

MEBAK OIV

Features

- Reagent stability:
  < 2 years (limiting)
  < 1 year (prepared)
- Extended cofactor stability
- When used with MegaQuant™ Wave, the number of manual tests per kit can be doubled by halving all reagent volumes.

Enzymes Available | Standard Included | Mega-Calc™ | MegaQuant™ Wave Pre-installed Protocol

Glycerol (ADP-GK format)

Cat. No. K-GCROLGK

	Manual	Microplate	Auto-analyser
Kit size	70	700	600

Detection method: Spectrophotometric at 340 nm
Reaction time: ~ 7 min
Detection limit: 0.373 mg/L
Range: up to 0.35 g/L

The Megazyme Difference:
Our Glycerol ADP-GK assay kit represents an advance on the standard kit by using a positive reaction, i.e. the assay proceeds with an increase in absorbance, offering greater ease of use.

Principle:

(1) **Glycerol + ATP** —(glycerokinase)→ **L-glycerol-3-phosphate + ADP**

(2) **ADP + D-glucose** —(ADP-GK)→ **G-6-P + AMP**

(3) **G-6-P + NAD^+** —(glucose-6-phosphate dehydrogenase)→ **gluconate-6-phosphate + NADH + H^+**

Method recognition: Novel method

Features

- Reagent stability:
  < 1 year (limiting)
  < 14 days (prepared)
- Extended cofactor stability
- When used with MegaQuant™ Wave, the number of manual tests per kit can be doubled by halving all reagent volumes.

Enzymes Available | Standard Included | Mega-Calc™ | MegaQuant™ Wave Pre-installed Protocol

Reducing Sugars (Glucose/Fructose) and Sucrose

Sugar influences alcohol content, flavour, and mouthfeel, and is measured throughout the production process.

Where does it come from?

Generated through photosynthesis, D-glucose and D-fructose occur naturally in grapes both as free sugars and the form of sucrose.

What does sugar mean for my wine?

The D-glucose and D-fructose content, often referred to as **total reducing sugars**, is considered a key quality parameter ahead of fermentation as it reflects the amount of sugar available to yeast for conversion into ethanol. By testing glucose and fructose in juice, winemakers can estimate the potential alcohol concentration of the finished wine.

The total glucose and fructose concentration that remains after fermentation - the **residual sugar** - indicates how "dry" the finished wine is likely to be.

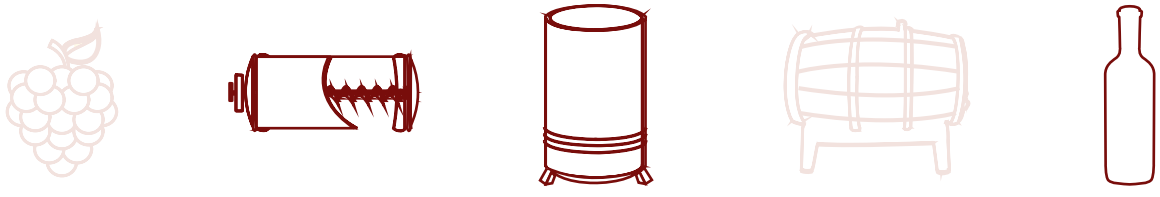
The addition of sucrose is only permitted in a few situations, for example in the production of champagne. The practice of 'chaptalisation' involves adding sucrose to grape must in order to increase the amount of alcohol in the finished wine.

What can I do with the sugar result?

Monitoring of sugar levels at each stage of the winemaking process helps producers to make decisions that will influence the final composition and texture of the wine, for example around grape ripeness, whether to chaptalise, and when to stop fermentation.

Note: Sucrose is not a 'reducing sugar' but must be factored in when calculating a wine's total sugar content.

Test Reducing Sugars and Sucrose in...



D-Fructose / D-Glucose

Cat. No. K-FRUGL

	Manual	Microplate	Auto-analyser
Kit size	110	1100	1100

Detection method: Spectrophotometric at 340 nm
Reaction time: ~ 13 min
Detection limit: 0.663 mg/L
Range: up to 0.8 g/L

The Megazyme Difference:
Contains PVP to prevent tannin inhibition. Stable reagents.

Principle:

(1) **D-Glucose + ATP** —(hexokinase)→ **G-6-P + ADP**

(2) **D-Fructose + ATP** —(hexokinase)→ **F-6-P + ADP**

(3) **G-6-P + $NADP^+$** —(glucose-6-phosphate dehydrogenase)→ **gluconate-6-phosphate + NADPH + H^+**

(4) **F-6-P** ⟷ **G-6-P** (phosphoglucose isomerase)

Method recognition:

AOAC AIJN DIN EN GOST IFU IOCCC MEBAK NEN NF OIV UV

Features

- All reagents stable for > 2 years after preparation (manual analysis applications)
- Rapid reaction at either 25°C or 37°C
- Extended cofactors stability
- When used with MegaQuant™ Wave, the number of manual tests per kit can be doubled by halving all reagent volumes.

Enzymes Available | Standard Included | Mega-Calc™ | MegaQuant™ Wave Pre-installed Protocol

D-Fructose / D-Glucose (Liquid Ready Reagents)

Cat. No. K-FRGLQR

	Manual	Microplate	Auto-analyser
Kit size	n/a	1100	1100

Detection method: Spectrophotometric at 340 nm
Reaction time: ~ 15 min
Detection limit: ~ 133 mg/L
Range: up to ~ 6 g/L

The Megazyme Difference:
Contains PVP to prevent tannin inhibition. Stable reagents.

Principle:

(1) **D-Glucose + ATP** —(hexokinase)→ **G-6-P + ADP**

(2) **D-Fructose + ATP** —(hexokinase)→ **F-6-P + ADP**

(3) **G-6-P + $NADP^+$** —(glucose-6-phosphate dehydrogenase)→ **gluconate-6-phosphate + NADPH + H^+**

(4) **F-6-P** ⟷ **G-6-P** (phosphoglucose isomerase)

Method recognition:

AOAC AIJN DIN EN GOST IFU IOCCC MEBAK NEN NF OIV UV

Features

- PVP incorporated to prevent tannin inhibition
- 'Ready-to-use' liquid stable formulation
- All reagents stable for > 2 years

Enzymes Available | Standard Included

Sucrose / D-Fructose / D-Glucose

Cat. No. K-SUFRG

	Manual	Microplate	Auto-analyser
Kit size	300 (100 of each)	n/a	n/a

Detection method: Spectrophotometric at 340 nm
Reaction time: ~ 23 min
Detection limit: 1.38 mg/L
Range: up to 0.8 g/L

The Megazyme Difference:
Stable reagents.

Principle:

(β-fructosidase)
(1) **Sucrose + H_2O ⟶ D-glucose + D-fructose**

(hexokinase)
(2) **D-Glucose + ATP ⟶ G-6-P + ADP**

(hexokinase)
(3) **D-Fructose + ATP ⟶ F-6-P + ADP**

(glucose-6-phosphate dehydrogenase)
(4) **G-6-P + $NADP^+$ ⟶ gluconate-6-phosphate + NADPH + H^+**

(phosphoglucose isomerase)
(5) **F-6-P ⟷ G-6-P**

Method recognition:
AIJN, EN, NF, NEN, DIN, GOST, IFU, IOCCC, MEBAK

Features

- All reagents stable for > 2 years after preparation
- Rapid reaction
- Stabilised D-glucose/ D-fructose standard solution included
- Extended cofactors stability
- When used with MegaQuant™ Wave, the number of manual tests per kit can be doubled by halving all reagent volumes.

Enzymes Available
Standard Included
Mega-Calc™

Sulphites

Sulphites are used as an essential additive in the control of microbial contamination during aging and also to protect the wine against detrimental "oxidative and enzymatic browning".

Where do they come from?

Sulphites occur naturally as a by-product of yeast fermentation. However, wine producers have also added sulphur dioxide (SO_2) to wine for thousands of years due to its antimicrobial and antioxidant properties.

What do sulphites mean for my wine?

Sulphites enhance the microbial stability of a wine (discouraging the growth of spoilage bacteria) and interact with reactive molecules like acetaldehyde, which can cause oxidation if left untreated.

At concentrations of 100 mg/L, sulphites begin to affect taste, imparting a "burnt" flavour.

Most wines have SO_2 added just before bottling in order to kill any remaining spoilage bacteria, and to bind to molecules in the wine that could affect its flavour as it ages. Some winemakers also find applications for SO_2 at other points, for example to halt malolactic fermentation or to kill any wild yeasts present at the time of harvest/crushing.

SO_2 is only active as an antimicrobial and antioxidant preservative in the unbound "free" form. Given that SO_2 becomes "inactive" when it binds the colour pigments of wine, and with legal restrictions on SO_2 levels in wine, it has become valuable to wine producers to measure both the Free SO_2 (FSO_2) and Total SO_2 (TSO_2).

What can I do with the sulphite result?

Any wine with a sulphur dioxide concentration over 10 mg/L is required to state on the label that it "contains sulphites." It is not uncommon for wines to exceed this threshold even without the artificial addition of sulphur dioxide.

Winemakers test FSO_2 to ensure that SO_2 previously added has not entirely reacted away (indicating that more sulphur dioxide needs to be added).

TSO_2 needs to be monitored to check how much SO_2 has been added so far, in order to ensure that the wine remains compliant with local regulations.

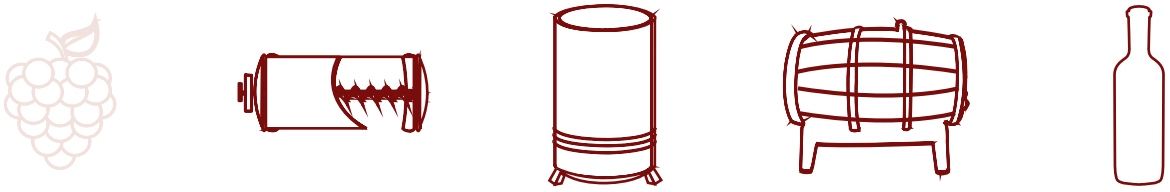
Many countries have set restrictions on the Total Sulphite concentration of wines made or sold within their jurisdiction. The limit may be different for dessert wines or other wines with a particularly high sugar content (typically defined as over 35 g/L). The table shows national regulations at the time of printing.

Legal Limits

Country	Red Wine (mg/L)	White Wine (mg/L)
Australia	250-400	250-400
Argentina	150-400	200-400
Canada	250-350	250-350
Chile	250-400	250-400
China	250-400	250-400
European Union	150-400	200-400
India	450	450
Japan	350	350
New Zealand	250-400	250-400
South Africa	150-400	160-400
United States	250-350	250-350

Maximum concentration of TSO_2 permitted in table wines. Where a range is shown, different thresholds are in place depending on the sugar content of a wine.

Test Sulphites in...



Total Sulfite (Enzymatic) — Cat. No. K-ETSULPH

	Manual	Microplate	Auto-analyser
Kit size	50	500	588

Detection method: Spectrophotometric at 340 nm
Reaction time: ~ 30 min
Detection limit: 0.34 mg/L
Range: up to 0.5 g/L

The Megazyme Difference:
Recognised method and simple format based on enzymatic reaction.

Principle:

(1) $SO_3^{2-} + O_2 + H_2O \xrightarrow{\text{(sulphite oxidase)}} SO_4^{2-} + H_2O_2$

(2) $H_2O_2 + NADH + H^+ \xrightarrow{\text{(NADH-peroxidase)}} 2H_2O + NAD^+$

Method recognition:

DIN EN NMKL MEBAK

Features

- Very cost effective
- All reagents stable for > 2 years during use
- Simple format
- Extended cofactors stability
- When used with MegaQuant™ Wave, the number of manual tests per kit can be doubled by halving all reagent volumes.

Enzymes Available · Standard Included · Mega-Calc™ · MegaQuant™ Wave Pre-installed Protocol

Total Sulfite — Cat. No. K-TSULPH

	Manual	Microplate	Auto-analyser
Kit size	80	800	800

Detection method: Spectrophotometric at 405 nm
Reaction time: ~ 6 min
Detection limit: ~ 5 mg/L
Range: up to ~ 400 mg/L

The Megazyme Difference:
Simple format based on liquid ready reagent chemical reaction.

Principle:

Based on the reaction principle between thiol groups and Ellman's reagent

Method recognition:

BAWB

Features

- "Ready to use" liquid stable formulation
- All reagents stable for > 18 months
- Very rapid reaction
- When used with MegaQuant™ Wave, the number of manual tests per kit can be doubled by halving all reagent volumes.

Standard Included · Mega-Calc™ · MegaQuant™ Wave Pre-installed Protocol

Total and Free Sulfite

Cat. No. K-SULPH

	Manual	Microplate	Auto-analyser
Kit size	40 of each	400 of each	400 of each

Detection method: Total sulphite: Spectrophotometric at 405 nm
Free sulphite: Spectrophotometric at 575 nm
Reaction time: Total sulphite: ~ 6 min, Free sulphite: ~ 9 min
Detection limit: Total sulphite: ~ 5 mg/L, Free sulphite: ~ 2 mg/L
Range: Total sulphite: up to ~ 400 mg/L
Free sulphite: up to ~ 150 mg/L

The Megazyme Difference:
Simple format based on liquid ready reagent chemical reaction.

Principle:

Total Sulphite assay:
Based on the reaction principle between thiol groups and Ellman's reagent

Free Sulphite assay:
Based on the reaction principle of SO_2, fuchsin and aldehyde.

Method recognition:

BAWB

Features

- "Ready to use" liquid stable formulation
- All reagents stable for > 18 months
- Very rapid reaction
- When used with MegaQuant™ Wave, the number of manual tests per kit can be doubled by halving all reagent volumes.

Enzymes Available | Standard Included | Mega-Calc™ | MegaQuant™ Wave Pre-installed Protocol

Free Sulfite

Cat. No. K-FSULPH

	Manual	Microplate	Auto-analyser
Kit size	80	800	800

Detection method: Spectrophotometric at 575 nm
Reaction time: ~ 9 min
Detection limit: ~ 2 mg/L
Range: up to ~ 150 mg/L

The Megazyme Difference:
Simple format based on liquid ready reagent chemical reaction.

Principle:

Free Sulphite assay:
Based on the reaction principle of SO_2, fuchsin, and aldehyde.

Method recognition:

BAWB

Features

- "Ready to use" liquid stable formulation
- All reagents stable for > 18 months
- Very rapid reaction
- When used with MegaQuant™ Wave, the number of manual tests per kit can be doubled by halving all reagent volumes.

Standard Included | Mega-Calc™ | MegaQuant™ Wave Pre-installed Protocol

Yeast Assimilable Nitrogen

Nitrogen influences the activity of yeast before and during fermentation. Nitrogen-based components, e.g. proteins, also contribute to the mouthfeel of the finished wine.

Where does it come from?

Nitrogen is part of the amino acids and proteins found naturally in grapes and micro-organisms. Nitrogen compounds are important nutrients for yeast involved in fermentation. For this reason, the form of nitrogen most assayed in the wine industry is Yeast Assimilable Nitrogen (YAN), i.e. nitrogen available to yeast cells.

What does YAN mean for my wine?

Too little YAN can result in sluggish or "stuck" fermentation.

Too much nitrogen will mean there are nutrients available for spoilage organisms as well as yeast. Excess nitrogen can also lead to the formation of the carcinogenic compound ethyl carbamate (especially where starting levels of L-arginine in the juice are high).

What can I do with the result?

The result can be used to determine whether extra YAN should be added in the form of diammonium phosphate (DAP), and if so, how much can be added prudently.

Wines sold in the European Union may contain up to 1 g/L of ammonium salts.

Measuring YAN with Megazyme

Total Yeast Available Nitrogen (YAN_T) is comprised of three components:

(a) primary amino nitrogen (PAN, from free amino acids) ,

(b) free ammonium ions, and

(c) the contribution from the side chain of L-arginine (after hydrolysis by yeast arginase that creates ornithine and urea).

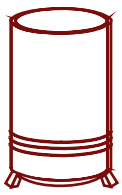
YAN component:	**K-PANOPA**	**K-AMIAR**	**K-LARGE**
Primary amino nitrogen	✔	✖	✖
Free ammonium	✖	✔	✔
L-Arginine	✔	✖	✔

Total YAN is determined using a combination of kits. If using the **K-PANOPA** and **K-LARGE** kits together, please note that both measure the primary amino group of L-arginine and this must be accounted for in the calculations.

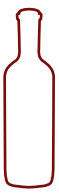
Test Yeast Assimilable Nitrogen in...

Mature Grapes


Juicing


Fermentation

Maturation


Finished Wine

Primary Amino Nitrogen (NOPA) — Cat. No. K-PANOPA

	Manual	Microplate	Auto-analyser
Kit size	100	1000	1100

Detection method: Spectrophotometric at 340 nm
Reaction time: ~ 15 min
Detection limit: 2.59 mg N/L
Range: up to 200 mg N/L

The Megazyme Difference:
Novel kit, rapid reaction, stable reagents and simple format.

Principle:

Method recognition: Novel method

Features

- Simple format (absorbances read at 340 nm)
- All reagents stable for > 2 years after preparation
- When used with MegaQuant™ Wave, the number of manual tests per kit can be doubled by halving all reagent volumes.

Standard Included

Mega-Calc™

MegaQuant™ Wave Pre-installed Protocol

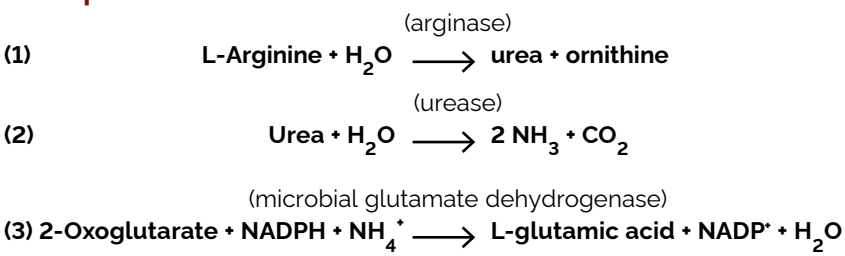
L-Arginine / Urea / Ammonia (Rapid) — Cat. No. K-LARGE

	Manual	Microplate	Auto-analyser
Kit size	150 (50 of each)	n/a	n/a

Detection method: Spectrophotometric at 340 nm
Reaction time: ~ 20 min [ammonia 2 min, urea 6 min, L-arginine 7 min]
Detection limit: ammonia 0.07 mg/L, urea 0.13 mg/L, L-arginine 0.37 mg/L
Range: ammonia up to 70 mg/L, urea up to 140 mg/L, L-arginine up to 350 mg/L

The Megazyme Difference:
Simple and rapid assay kit gives sequential values for ammonia, urea and L-arginine. No tannin inhibition.

Principle:



Method recognition: Improved method

Features

- Improved assay format
- All enzymes supplied as stabilised suspensions
- All reagents stable for > 2 years after preparation
- Extended cofactor stability
- When used with MegaQuant™ Wave, the number of manual tests per kit can be doubled by halving all reagent volumes.

Standard Included

Mega-Calc™

MegaQuant™ Wave Pre-installed Protocol

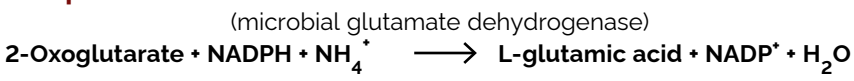
Ammonia (Rapid) Cat. No. K-AMIAR

	Manual	Microplate	Auto-analyser
Kit size	96	960	960

Detection method: Spectrophotometric at 340 nm
Reaction time: ~ 5 min
Detection limit: 0.07 mg/L
Detection limit: up to 70 mg/L

The Megazyme Difference:
Novel enzyme employed is not inhibited by tannins. Very rapid reactions (endpoint reaction time ~ 5 min) due to use of uninhibited glutamate dehydrogenase.

Principle:



Method recognition:

MEBAK

Features

- Enzyme supplied as stabilised suspension
- Reagent stability:
  < 1 year (limiting)
  < 7 days (prepared)
- When used with MegaQuant™ Wave, the number of manual tests per kit can be doubled by halving all reagent volumes.
- Reaction type: endpoint
- Reaction direction: decrease

Standard Included

Mega-Calc™

MegaQuant™ Wave Pre-installed Protocol

Assay Kits: Other Analytes

ENZYMES FOR WINE ANALYSIS

Enzymes for High-Throughput Wine Laboratories

Megazyme is proud to offer unparalleled purity in our enzyme products. A wide array of enzymes is available for manufacturing and research, including 15 enzymes of particular relevance in wine analysis.

At Megazyme, we have developed optimised analytical methods based on the enzymes listed below. These methods are commercially available in the form of our Assay Kits, which include our signature ultra-pure enzymes plus our top-quality reagents, cofactors and buffer solutions.

Customers also have the option to purchase selected enzymes separately - a popular solution for high-throughput laboratories and large wineries who wish to perform their own in-house assay development and optimisation.

The table below lists our enzymes for wine analysis, including the analytes each enzyme may be used to measure.

Enzyme	Acetic Acid	Citric Acid	Glucon-ic Acid	Lactic Acid	Malic Acid	Succinic Acid	Ethanol	Glucose/ Fructose	Sulphites
Acetyl-CoA synthetase Cat. No. E-ACSBS	✔								
Alcohol dehydrogenase Cat. No. E-ADHEC							✔		
Citrate synthase Cat. No. E-CITEC									
Gluconate kinase Cat. No. E-GLUKEC			✔						
Glutamate oxaloacetate transaminase Cat. No. E-GOTEC					✔				
Hexokinase/Glucose-6-phosphate dehydrogenase Cat. No. E-HKGDH								✔	
D-Lactate dehydrogenase Cat. No. E-DLDHLM	✔	✔		✔					
L-Lactate dehydrogenase Cat. No. E-LLDHP				✔					
L-Malate dehydrogenase Cat. No. E-LMDHEC	✔	✔			✔				
Phosphoglucose isomerase Cat. No. E-PGISC								✔	
Phosphotransacetylase Cat. No. E-PTABS	✔								
Pyruvate kinase Cat. No. E-PKRM	✔								
Succinyl CoA synthetase Cat. No. E-SCOAS						✔			

Popular Wine Enzymes

Megazyme enzymes are internationally renowned for their exceptional purity, which means accurate results and total reliability every time.

Full details for our entire range of wine enzymes can by found on our website, **www.megazyme.com**, with bulk pack sizes available on request.

Three of our most popular enzymes for wine analysis are outlined below.

Glutamate Oxaloacetate Transaminase (*E. coli*) — Cat. No. E-GOTEC

Specificity: Catalyses the reversible transfer of an α-amino group between aspartate and glutamate.

Specific activity: ~ 200 U/mg (25°C, pH 8.5 on α-ketoglutaric acid).

The Megazyme Difference:
Exceptional purity: single band on SDS-gel electrophoresis (MW = 45,737) and one major band on isoelectric focusing (pI = 6.1)

Pack size: 5000 Units (~ 2,500 U/mL)

Applications: Can be used in the measurement of L-malic acid

EC: 2.6.1.1
CAS: 9000-97-9

Features

- Stable for > 4 years at 4°C
- Supplied in 3.2 M ammonium sulphate
- Recombinant enzyme from *E. coli*

L-Malate Dehydrogenase (*E. coli*) — Cat. No. E-LMDHEC

Specificity: Catalyses the reaction between L-malate and NAD^+.

Specific activity: ~ 1,700 U/mg (25°C, pH 7.5 on oxaloacetic acid).

The Megazyme Difference:
Exceptional purity: single band on SDS-gel electrophoresis (MW = 34,501) and single major band on isoelectric focusing (pI = 6.5)

Pack size: 50,000 Units (~ 15,000 U/mL)

Applications: Can be used in the measurement of acetic acid, citric acid and L-malic acid

EC: 1.1.1.37
CAS: 9001-64-3

Features

- Stable for > 4 years at 4°C
- Supplied in 3.2 M ammonium sulphate
- Recombinant enzyme from *E. coli*

Phosphoglucose Isomerase (*Saccharomyces cerevisiae*) — Cat. No. E-PGISC

Specificity: Catalyses the conversion of D-glucose-6-phosphate into D-fructose-6-phosphate.

Specific activity: ~ 350 U/mg of protein at pH 7.6 and 25°C
~ 570 U/mg of protein at pH 7.6 and 40°C

The Megazyme Difference:
Exceptional purity: single band on SDS-gel electrophoresis (MW = 62,400) and one major band on isoelectric focusing (pI = 6.1), minor band pI 6.9.

Pack size:
E-PGISC-5KU 5000 Units (~ 1,000 U/mL)
E-PGISC-50KU 50,000 Units (~ 5,000 U/mL)

Applications: Can be used in the measurement of fructose, glucose and sucrose

EC: 5.3.1.9
CAS: 9001-41-6

Features

- Stable for > 4 years at 4°C
- Supplied in 3.2 M ammonium sulphate
- Recombinant enzyme from *Saccharomyces cerevisiae*

EQUIPMENT FOR WINE ANALYSIS

Spectrophotometers

MegaQuant™ Wave Spectrophotometer — Cat. No. D-MQWAVE-1

A compact, standalone benchtop, spectrophotometer supplied with exclusive software providing on-board step-by-step instruction and automated calculation of results.

It can also be used as a standard spectrophotometer and is ideally suitable for low sample numbers or small wineries

WINES & VINES

As featured in Wines and Vines magazine, January 2018 issue.

Our revolutionary MegaQuant™ Wave is the perfect investment for any wine laboratory, and is available either as a standalone spectrophotometer or as part of our Starter Pack for laboratories.

Unique and Exclusive Software

The MegaQuant™ Wave's built-in software - engineered exclusively for Megazyme - is designed with advanced functionality.

The MegaQuant Wave™ also offers ease of use for the analyst via the following key features:

- Capable of up to 4 absorbance readings per sample to accommodate sequential reactions
- On-board step-by-step instructions provided by pre-installed test protocols
- Pre-installed test protocols optimised for use with over 60 Megazyme assay kits
- Fully automated calculation of results accounting for individual sample dilution
- Use as a standard spectrophotometer in absorption mode (MegaCalc™ compatible)
- Option to use half-volumes assays with Megazyme kits, thereby doubling the number of assays per kit

User experiences with the MegaQuant Wave

The wine laboratory is as important to the success of a winery as any other element.... In my laboratory I have run acetic acid, L-malic acid, glucose, fructose, SO_2 free and total tests on [the MegaQuant Wave]. Every one of the test results came well within the standards expected for any wine analysis. The precision of the tests was excellent.

- Richard Carey PhD, writing in Wines and Vines

For our wine customers, the MegaQuant™ Wave is supplied with protocols for the following kits:

Acetic acid:	K-ACETRM, K-ACET	**Acetaldehyde:**	K-ACHYD
Ascorbic acid:	K-ASCO	**Ethanol:**	K-ETOH
Citric acid:	K-CITR	**Fructose/Glucose:**	K-FRUGL
Gluconic acid:	K-GATE	**Glycerol:**	K-GCROL, K-GCROLGK
Lactic acid:	K-DATE, K-DLATE, K-LATE	**Nitrogen:**	K-AMIAR, K-LARGE, K-PANOPA
Malic acid:	K-DMAL, K-LMAL	**Succinic Acid:**	K-SUCC
Tartaric acid:	K-TART	**Sulphites:**	K-SULPH, K-FSULPH, K-TSULPH, K-ETSULPH

MegaQuant™ Wave Starter Pack

Cat. No. D-MQWAVE-2

- Everything your winery needs for in-house analysis.
- Analysis-ready straight out of the box: all associated equipment supplied.
- Perfect for use with Megazyme test kits.
- Includes the MegaQuant™ Wave spectrophotometer.

The MegaQuant™ Wave Starter Pack includes everything you need to start using Megazyme's bestselling assay kits at your winery.

Key features

Megazyme brings you a complete wine laboratory straight out of the box - ideal for wineries that want to establish a comprehensive, high-quality analytical capability in-house and want a quick, all-in-one solution.

The Starter Pack is based around our MegaQuant™ Wave spectrophotometer and is designed to help small and medium wineries get started with enzymatic testing.

Starter Pack Contents

- MegaQuant™ Wave Spectrophotometer
- Top-of-the-range Gilson Pipetman Kit (P20, P200, P1000)
- Test tubes for MegaQuant™ Wave
- Test tube rack
- PVPP tablets
- Polypropylene tubes (13 mL & 30 mL)
- 100 mL Volumetric flasks
- Filter papers
- Plastic funnels
- Megazyme timer

In addition to shipping to 220 territories, Megazyme also has a partner network of International Distributors serving over 50 countries. Contact details can be found online at www.megazyme.com

Megazyme

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e cs@megazyme.com

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Megazyme

At a Glance: Popular Assay Kits for Wine Analysis

Analyte	Product Name	Product Code	Enzymes Available	When to Test
Acetic Acid (for Volatile Acidity) Page 16	Acetic Acid (acetate kinase)	K-ACETRM K-ACETAK (analyser)	Enzymes Available	Mature Grapes, Fermentation, Maturation, Finished Wine
	Acetic Acid (ADP-GK)	K-ACETGK (analyser)	Enzymes Available	Mature Grapes, Fermentation, Maturation, Finished Wine
	Acetic Acid (acetyl-CoA synthetase)	K-ACET K-ACETAF (analyser)	Enzymes Available	Mature Grapes, Fermentation, Maturation, Finished Wine
Acetaldehyde Page 30	Acetaldehyde	K-ACHYD		Finished Wine
Ethanol Page 31	Ethanol	K-ETOH	Enzymes Available	Juicing, Fermentation, Finished Wine
Glycerol Page 33	Glycerol	K-GCROL K-GCROLGK (analyser)		Finished Wine
Lactic Acid Page 23	L-Lactic Acid	K-LATE	Enzymes Available	Fermentation, Maturation
Malic Acid Page 25	L-Malic Acid	K-LMAL K-LMALAF (analyser) K-LMALQR (analyser)	Enzymes Available	Mature Grapes, Fermentation, Maturation
Reducing Sugars and Sucrose Page 35	D-Fructose/D-Glucose	K-FRUGL K-FRGLQR (analyser)	Enzymes Available	Juicing, Fermentation, Finished Wine
	Sucrose/D-Fructose/D-Glucose	K-SUFRG	Enzymes Available	Finished Wine
Sulphites Page 38	Total Sulphite	K-ETSULPH K-TSULPH		Juicing, Fermentation, Finished Wine
	Total & Free Sulphite	K-SULPH		Juicing, Fermentation, Finished Wine
	Free Sulphite	K-FSULPH		Juicing, Fermentation, Finished Wine
Yeast Assimilable Nitrogen (YAN) Page 41	Primary Amino Nitrogen	K-PANOPA		Juicing, Fermentation, Finished Wine
	L-Arginine/Urea /Ammonia	K-LARGE		Juicing, Fermentation, Finished Wine
	Ammonia (Rapid)	K-AMIAR		Juicing, Fermentation, Finished Wine

Learn about our range of assay kits, enzymes and equipment for the wine industry inside the Product Guide, or discover more on our website: **www.megazyme.com**

Key: When to Test — *Mature Grapes*, *Juicing*, *Fermentation*, *Maturation*, *Finished Wine*

Megazyme

www.megazyme.com

Megazyme *Think Quality • Expect Precision • Choose Megazyme*