Megazyme "Advanced" Wine Test Kits
General characteristics and validation

Simon J. Charnock
PhD (Doctor of Philosophy). Megazyme International Ireland Limited - www.megazyme.com
Christine Daverede
Docteur en sciences agronomiques - Centre de Recherche et de Développement Oenoagronomique - LACO - Suze-la-Rousse - France. www.laco-credo.com
Patrick Galant
Directeur de l'Université du Vin - Suze-la-Rousse - France. www.universite-du-vin.com
Barry V. McCleary
PhD, DScAgr (Doctor of Science in Agriculture) - Megazyme International Ireland Limited - www.megazyme.com

Extract from Revue des Œnologues No. 120
www.oeno.tm.fr

TECHNIQUE

With the next vintage now firmly on the horizon, the whole wine industry is readying itself ahead of its busiest time of the year. This is especially true of the analysts in laboratories both large and small, who will soon be responsible for monitoring the process of vinification, often from before the grapes are even crushed, through to the successful completion of fermentation.

Like their colleagues in the rest of the wine industry, it is imperative that the analyst keeps up-to-date with technological developments of significance to them, and adopt more advanced methods where appropriate.

One of the most exciting trends in recent times in terms of analysis within the wine industry has been the increased use of enzymatic bio-analysis, in replacement of older and more traditional methods. This topic has been introduced in general *(see previous issue No. 116)* and discussed in detail with respect to the modern wine industry *(see previous issue 117)*. The current article will build upon this foundation, by focussing on the recent development of a range of advanced wine enzymatic test kits, and the validation of the most relevant ones (i.e. for acetic acid, D-fructose/D-glucose, and L-malic acid), by the Université du Vin, Suze-la-Rousse.

■ **Photo 1: Production of recombinant enzymes for enzymatic test kits via molecular biology.**

Megazyme - an old company, with new wine test kits

Megazyme International Ireland Limited is a biotechnology company that has specialised in the development, manufacture, application, supply, and support of enzymatic bio-analysis test kits and related products since 1989, and has always been at the forefront of this technology in the food, feed, dairy and beverage industries.

Many of the enzymatic test kits are official methods of prestigious organisations such as the Association of Official Analytical Chemists (AOAC) and the American Association of Cereal Chemists (AACC). In response to interest from oenologists, Megazyme decided to use its long history of enzymatic bio-analysis to make a significant contribution to the wine industry, by the development of a range of advanced enzymatic test kits. This task has now been successfully completed through the strategic and comprehensive process of identifying limitations of existing enzymatic bio-analysis test kits where they occurred, and then using advanced techniques, such as molecular biology ***(photo 1)***, to rapidly overcome them. Novel test kits have also been developed for analytes of emerging interest to the oenologist, such as yeast available nitrogen *(YAN; see pages 2-3 of issue 117 article)*, or where previously enzymes were simply either not available, or were too expensive to employ, such as for D-mannitol analysis.

Megazyme currently offers 64 advanced test kits, 30 of which have specific applications in the wine industry ***(table 1)***, and the range is still growing. These new and advanced products now make enzymatic bio-analysis commercially viable for laboratories of practically any size.

The Megazyme advantage

In general, Megazyme test kits are ideal for wine analysis because they have long shelf-lives (spanning at least two vintages), rapid analysis times (often only a few minutes), simple formats, and very competitive pricing. Furthermore, each test

kit is under continual scrutiny and re-evaluation by a very experienced R&D team. As all test kits have been developed by the company, our research scientists are ideally positioned to offer the very best and rapid technical customer support.
Recently, with the customer in mind, a range of calculation aids (MegaCalc™) based on Microsoft Excel were developed, which enable hassle-free and rapid processing of raw absorbance data for all manual analysis test kits *(see page 4 of issue 117 article)*.
These calculators are offered free of charge on the Megazyme website (www.megazyme.com), and can be downloaded for added convenience.

Custom analysis solutions for the wine industry

Most test kit manufacturers offer just one single test kit for each analyte, e.g. for L-malic acid. However, there are actually many different types of user, each with very different requirements and available resources. Megazyme thus developed specific test kits with each type of end-user in mind, leading to simple and easy to use formats that require no adaptation by the customer:

• **Manual format test kits** - this is the most popular method of analysis for the average laboratory possessing a standard spectrophotometer and a test kit is available for each wine analyte ***(table 1)***.

• **MegaQuant™ format test kits** - testing by small wineries and grape growers was until recently very difficult, as such companies generally do not even possess a basic spectrophotometer, and thus must rely either on traditional techniques or central laboratories for their analytical requirements.

However, in response to this pressing requirement, a novel product was recently launched called MegaQuant™, that allows rapid and specific measurement of both L-malic acid and D-glucose plus D-fructose, without the requirement for an expensive spectrophotometer or other specialised laboratory apparatus and skills *(see page 2 of issue 117 article)*.

• **Analyser format test kits** - it is common practice for analysts to purchase standard test kits and adapt them to a particular auto-analyser. However, this takes time and the form and quantities of the individual components is often less than optimal, leading to significant waste or inconvenience. Megazyme thus developed optimised "analyser formats" for existing manual

Table 1: Advantages of Megazyme test kits in the measurement of key wine analytes.

Analyte	Cat. No.	Advantages of Megazyme Test Kit
Acetaldehyde	K-ACHYD	AlDH supplied as a stabilised suspension rather than a lyophilised powder, thus less wasted enzyme
Acetic Acid	K-ACET K-ACETAF K-ACETAK	All kits contain PVP to prevent tannin inhibition 1. K-ACET (manual, efficient) contains stable ACS suspension 2. K-ACETAF (auto) used to prepare very stable R1 and R2 3. K-ACETAK (auto) is a new, stable, and very rapid acetate kinase (AK) based kit with excellent linearity
Ammonia	K-AMIAR	Novel enzyme employed is not inhibited by tannins, endpoint reaction time ~ 2 min. Ideal for manual and auto applications
L-Arginine	K-LARGE	Simple and rapid test kit gives sequential values for ammonia, urea and L-arginine. No tannin inhibition
L-Ascorbic Acid	K-ASCO	Rapid reaction, stable reagents
Citric Acid	K-CITR	Contains PVP to prevent tannin inhibition. Ideal for manual and auto applications
Ethanol	K-ETOH	Rapid reaction, stable reagents (AlDH supplied as a stable suspension)
D-Fructose / D-Glucose	K-FRUGL K-FRGLMQ	Contains PVP to prevent tannin inhibition. Ideal for manual and auto applications. Stable reagents
Formic Acid	K-FORM	Rapid reaction, stable reagents
D-Gluconic Acid	K-GATE	Rapid reaction, stable reagents
D-Glucose	K-GLUC K-GLUHKR/L	Choice of simple formats available, based either on glucose oxidase/peroxidase, or hexokinase/G-6-PDH
Glycerol	K-GCROL	Novel tablet format offers superior stability, rapid reaction
D-Lactic Acid	K-DATE	Rapid reaction, stable reagents
L-Lactic Acid	K-LATE	Rapid reaction, stable reagents. Ideal for manual and auto applications
D-Malic Acid	K-DMAL	D-MDH supplied as a stabilised suspension rather than a lyophilised powder, thus less wasted enzyme
L-Malic Acid	K-LMALR/L K-LMALAF K-LMALMQ	All kits contain PVP to prevent tannin inhibition 1. K-LMALR/L (manual) rapid reaction 2. K-LMALAF (auto) rapid reaction, excellent linearity 3. K-LMALMQ (manual, colorimeter based)
D-Mannitol	K-MANOL	Novel kit, rapid reaction, stable reagents, simple format
Primary Amino Nitrogen (PAN)	K-PANOPA	Novel kit, rapid reaction, stable reagents, simple format
D-Sorbitol	K-SORB	Diaphorase supplied as a stabilised suspension rather than a lyophilised powder, thus less wasted enzyme
Starch	K-STAHK	Rapid reaction, stable reagents
Succinic Acid	K-SUCC	Rapid reaction (even at 25oC), stable reagents
Sucrose	K-SUFRG K-SUCGL	Choice of simple formats available, based either on glucose oxidase/peroxidase, or hexokinase/G-6-PDH
Urea	K-URAMR	Simple and rapid test kit gives sequential values for ammonia and urea. No tannin inhibition

kits that are simple to use and result in no wastage of kit reagents. Where existing kits could not be employed, new kits were developed as necessary.

Analyser formats are now available for acetic acid, ammonia, citric acid, D-fructose and D-glucose, L-lactic acid, and L-malic acid analyses, and offer excellent prepared reagent stability and performance linearity *(see page 5 of issue 117 article for an example)*. In addition to test kits, Megazyme also offers a range of individual kit components for those analysts who wish to formulate their own reagents.

This is becoming increasingly popular by the experienced auto-analyst, and rapid assistance is available from the technical support team at Megazyme for such custom method developments.

Validation: the Université du Vin

The Université du Vin (Suze-la-Rousse) was formed in 1978 and is held in great regard for the success of its mission to both assimilate and disseminate knowledge covering all disciplines fundamental to the wine industry. In addition to formal academic qualifications, this private establishment of higher education also offers short courses for wine industry professionals/enthusiasts, a library, a collection of 70 vine varieties, an approved tasting centre, and a large modern oenological research/services laboratory.

This laboratory possesses both the equipment and knowledge sufficient to perform virtually any traditional or modern testing method, thus making it an ideal choice for validation of new test kits against those already established for wine analysis.

Validation: methodology

Validation was performed according to the second part (comparison with the reference method) of OIV protocol Resolution oeno 6/99, that forms part of the international methods for the analysis for wine and musts, and is specifically designed to validate new methods against established reference procedures.

The experiments were performed on a KONELAB 60 auto-analyser, supplied by Thermo Electron Corporation. Samples were analysed in duplicate and comprised 50 - 80 (depending on the analyte in question) red, white or rosé wines from the Côtes du Rhône region. The concentration of the analytes of interest ranged as follows;

- Acetic acid, 0.1 - 0.9 g/L
- D-fructose + D-glucose, 0 - 10 g/L
- L-malic acid, 0 - 3 g/L.

Standards comprised either synthetic solutions or wines of known analyte concentration (determined by reference methods). Titrivin "AA" series solutions were used as controls. Four Megazyme auto-analyser format kits were validated; an acetic acid kit based on acetyl-CoA synthetase (K-ACETAF), a new acetic acid kit based on acetate kinase (K-ACETAK), a D-fructose / D-glucose kit (K-FRUGL) and an L-malic acid kit (K-LMALAF).

These products were chosen as testing for acetic acid, D-fructose / D-glucose and L-malic acid jointly comprise the majority of all testing performed in the wine industry. The Megazyme kits were validated against the largest competitors supplying

Table 2: Repeatability and accuracy statistical parameters determined using a KONELAB 60 auto-analyser.

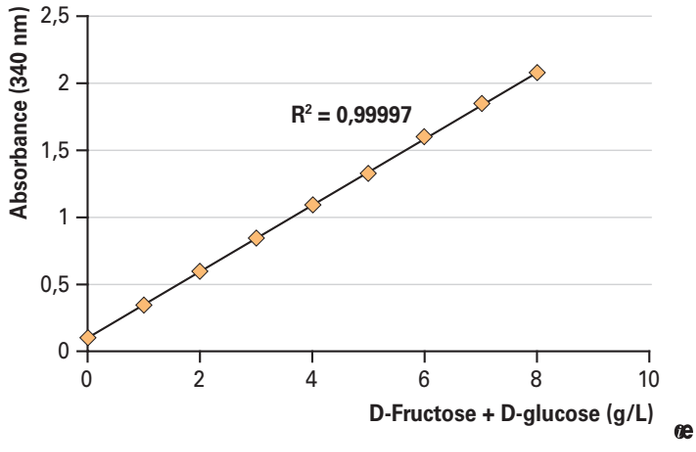
Analyte	Method	Repeatability (g/L)		Correlation between methods	*n*
		Sr	r		
Acetic Acid	Megazyme (K-ACETAF)	0.0077	0.0214	0.99179	69
	Competitor B kit	0.0051	0.0143		
Acetic Acid	Megazyme (K-ACETAK)	0.0100	0.0281	0.98932	50
	Competitor B kit	0.0053	0.0149		
D-Fructose/D-Glucose	Megazyme (K-FRUGL)	0.042	0.117	0.99945	77
	Competitor A kit	0.039	0.111		
L-Malic Acid	Megazyme (K-LMALAF)	0.006	0.017	0.99966	70
	Competitor A kit	0.008	0.022		

Sr *= Standard deviation of repeatability* ***r*** *= Repeatability* ***n*** *= Number of samples analysed in duplicate*

Table 3: Analysis of Titrivin standard solutions using Megazyme K-FRUGL and Competitor A D-fructose / D-glucose kits.

Titrivin standard	Stated value of D-fructose + D-glucose (g/L)	Megazyme (K-FRUGL)	Competitor A
AA1 (Lot A 030412081)	0.73 +/- 0.06	0.9	0.9
AA2 (Lot A 020312072)	3.78 +/- 0.09	3.7	3.8
AA3 (Lot A 020312073)	4.93 +/- 0.15	4.9	5.0
AA4 (Lot A 030412084)	8.49 +/- 0.24	8.6	8.7

Figure 1: Graph to demonstrate the linearity of Megazyme's K-FRUGL kit as determined with a D-fructose + D-glucose concentration range of 0 - 8 g/L.



similar products in France, that are referred to simply as Competitor A (largest competitor), Competitor B and Competitor C (smallest competitor).

Validation: results

Ease of use and general characteristics

Each of the four kits was well presented in a protective polystyrene box and contained an easy to use instruction booklet. The preparation of R1 and R2 did not lead to the wastage of any "spare" reagents. As all four kits are supplied with an optimal amount of polyvinylpyrollidone (PVP) already incorporated (to prevent polyphenolic inhibition by molecules such as tannins in especially red wines), preparation was simplified, and thus required less time.

The long shelf-life of > 2 years (during use) at 4oC ensures efficiency and results in less wastage/expense. It is especially worth noting that the acetyl-CoA synthetase (ACS) of K-ACETAF is supplied as a ready to use ammonium sulphate suspension, and thus requires no preparation; the ACS of Competitor A and Competitor B kits, however, is supplied as a freeze-dried powder, and requires reconstitution.

Moreover, after reconstitution, the resulting ACS solution must be used within 5 days at 4oC, resulting in inefficient use of the kit components during periods of low analysis activity. It is equally noteworthy that no reagents at all need to be prepared and stored for later use with K-ACETAK, as the substrates and cofactors for this kit are supplied in a novel and highly stable tablet form.

Stability of prepared reagent

Reagents for all four kits were prepared (as described by the manufacturer), analysed, and then stored at 4oC until future use. It was found that in common with Competitors A and B, prepared reagents R1 and R2 for Megazyme's D-fructose / D-glucose and L-malic acid kits, were usable for at least 15 days (K-FRUGL), and 7 days (K-LMALAF), respectively. Indeed, reagent for K-FRUGL could be re-used without re-calibration. Reagent for Megazyme's ACS based acetic acid kit (K-ACETAF) could be routinely used for > 3 days, owing to an R1 stabilisation system. This is only occasionally possible for Competitor A and B acetic acid kit products.

Similarly, reagent for Megazyme's acetate kinase based acetic acid kit (K-ACETAK) could be routinely used for > 3 days, and required just a single daily calibration.

Repeatability and accuracy

After linearity was confirmed for each Megazyme method (for example see ***figure 1***), repeatability and accuracy were determined and comparisons made to Competitor A or Competitor B products ***(table 2)***.

• D-Fructose / D-glucose

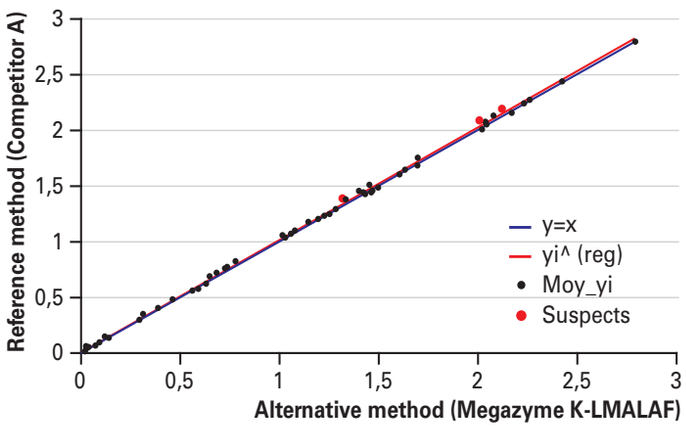
The data in ***table 2*** show that there was no significant difference in repeatability between the Megazyme (K-FRUGL) and Competitor A method, with values of r = 0.117 / Sr = 0.042, and r = 0.111 / Sr = 0.039, being obtained respectively. In terms of accuracy, the data obtained with the Competitor A product is related to that obtained with the Megazyme kit by the equation: y = 1.001x + 0.0269, with a correlation coefficient of 0.99945. A student test shows (at the 5 %

■ Table 4: Comparison of duplicate data for Megazyme'e "analyser format" L-malic acid kit (K-LMALAF) against that supplied by Competitor A.

For the purpose of this figure, only the first 20 duplicate samples of the 70 analysed in total are shown. The data cover an L-malic acid concentration range typically experienced during wine analysis, and show excellent agreement between the two methods.

Sample No.	L-malic acid (g/L)			
	Megazyme		Competitor A	
	(Rep 1)	(Rep 2)	(Rep 1)	(Rep 2)
1	2.35	2.34	2.32	2.30
2	1.59	1.59	1.57	1.57
3	2.05	2.04	2.03	2.04
4	1.48	1.48	1.47	1.45
5	1.64	1.63	1.63	1.63
6	1.18	1.17	1.17	1.16
7	1.49	1.49	1.49	1.48
8	1.24	1.24	1.22	1.23
9	0.75	0.75	0.77	0.77
10	0.75	0.74	0.76	0.76
11	0.87	0.88	0.87	0.88
12	0.89	0.90	0.90	0.89
13	0.54	0.54	0.56	0.56
14	0.57	0.57	0.59	0.59
15	0.92	0.91	0.90	0.91
16	0.09	0.09	0.10	0.10
17	0.10	0.10	0.10	0.10
18	0.23	0.23	0.24	0.25
19	0.02	0.02	0.03	0.03
20	0.02	0.02	0.03	0.03

■ Figure 2: Comparison of the Megazyme (K-LMALAF) and Competitor A L-malic acid methods. The correlation coefficient for this data is 0.99966, i.e. suggesting excellent agreement between the two methods.



confidence level), that the slope is not significantly different from 1, but that there is a skew. In practice this skew, that ranges between - 0.05 and - 0.01 g/L, is completely acceptable taking into account the results obtained for the Titrivin controls ***(table 3)***.

• L-Malic acid

Again, the data in ***table 2*** show that there was no significant difference in repeatability between the Megazyme (K-LMALAF) and Competitor A method, with values of r = 0.017 / Sr = 0.006, and r = 0.022 / Sr = 0.008, being obtained respectively. In terms of accuracy, the data obtained with the Competitor A product is related to that obtained with the Megazyme kit by the equation: y = 0.9983x + 0.0042), with a correlation coefficient of 0.99966. A student test shows that the slope is not significantly different from 1, and that the skew is not significantly different from 0, indicating excellent agreement between the two methods ***(figure 2 and table 4)***.

• Acetic acid (K-ACETAF)

the data in ***table 2*** show that repeatability between the Megazyme and Competitor B method was almost similar, and in both cases significantly less than the internationally accepted limit of 0.04 g/L (accepted for volatile acidity determination). Values of r = 0.0214 / Sr = 0.0077, and r = 0.0143 / Sr = 0.0051, respectively, were determined for the Megazyme and Competitor B test kits.

In terms of accuracy, the data obtained with the Competitor B product is related to that obtained with the Megazyme kit by the equation: y = 1.0042x + 0.0113, with a correlation coefficient of 0.99179. A student test shows (at the 5 % confidence level), that the slope is not significantly different from 1, with a skew of 0.01 being entirely acceptable considering the results presented in ***table 5***.

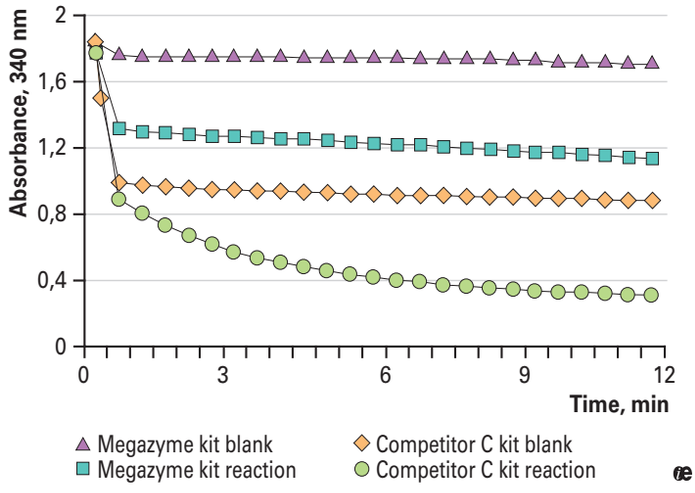
• Acetic acid (K-ACETAK)

The data in ***table 2*** indicate a difference in repeatability between K-ACETAK and the acetic acid kit of Competitor B, as would be expected given these methods are fundamentally different at the biochemical level (the Megazyme kit is based on acetate kinase, while that of Competitor B on ACS). A better comparison would have been to compare K-ACETAK to an acetate kinase based competitor kit, but an equivalent product, based on a similar rapid end-point to that of the Megazyme kit, is not available (see the experiment performed by Megazyme - ***figure 3***). Values of r = 0.0281 / Sr = 0.010, and r = 0.0149 / Sr = 0.0053, respectively, were determined for the acetate kinase based kit of Megazyme, as compared to the ACS based kit from Competitor B. In terms of accuracy, the data obtained with the Competitor B product is related to that obtained with the Megazyme kit by the equation: y = 1.017x - 0.0069, with a correlation coefficient of 0.98932. A student test shows (at the 5 % confidence level), that the slope is not significantly different from 1, and that the skew is not significantly different from 0, thus indicating good agreement between the two methods ***(table 5)***.

■ **Table 5: Analysis of Titrivin standard solutions using Megazyme K-ACETAF, K-ACETAK, and Competitor B acetic acid kits.**

Titrivin standard	Level of volatile acidity determined by distillation (g/L)	Stated value of acetic acid (g/L)	Megazyme (K-ACETAF)	Megazyme (K-ACETAK)	Competitor B
AA1 (Lot A 030412081)	0.23 +/- 0.02	0.21 +/- 0.04	0.23	0.24	0.26
AA2 (Lot A 020312072)	0.37 +/- 0.01	N.D.	0.37	0.38	0.38
AA3 (Lot A 020312073)	0.54 +/- 0.01	N.D.	0.53	0.51	0.51
AA4 (Lot A 030412084)	0.81 +/- 0.02	0.76 +/- 0.05	0.76	0.73	0.75

■ **Figure 3: Comparison of Megazyme's rapid end-point acetate kinase kit (K-ACETAK) with that of a much slower Competitor C product. As can be seen, the Megazyme K-ACETAK reaction finished in approx. 10 min at ambient room temperature, while that of Competitor C was only just approaching the endpoint after approx. 120 min.**



Validation: conclusions

The Megazyme test kits gave results over a diverse range of red, white and rosé wine samples that were not significantly different to those of the two biggest French market competitors. However, the fact the Megazyme kits are simple, easy and efficient to use, all contain optimal levels of PVP (as supplied), and have long shelf-lives (either before or during use), make them especially interesting for wine analysis, and can thus be recommended for this application.

As a result of these validation trials, some, or all, of these methods will now be adopted by the Centre de Recherche et de Development Oenoagronomique, at the Université du Vin, Suze-la-Rousse.

Future wine test kit development

Megazyme remains committed to working with the oenologist to either improve existing methods or develop novel test kit solutions to emerging challenges presented by the modern wine industry. ■

Megazyme Assay Kits for the wine industry…

Experience the next **Revolution**

World leaders in diagnostic kits and reagents for the food and beverage industries

Megazyme

Setting new standards in test technology

SMALLER LABORATORIES
L-Malic Acid and Glucose/Fructose MegaQuant™ Test Kits

- No spectrophotometer required
- Very simple to use
- Cost effective
- Ability to test each barrel
- Enables in-house QC
- Accurate/reliable/rapid

ALL LABORATORIES
Range of 30 Advance Wine Test Kits

- Stability > 2 years
- Novel tests/rapid technical support
- Accurate/reliable
- Optimised for wine analysis
- Very competitively priced
- Next day delivery

LARGER LABORATORIES
Wine Test Kits for Auto-Analysis

- Optimised on-machine stability
- No un-used reagents
- Adaptable to any path-length (5-10mm)
- Optimised linearity
- Very competitively priced
- Accurate/reliable/rapid

Cat. No.	Product	Reagent (mL.)
K-ACETAF	Acetic Acid (AF) Test Kit	170.5
K-ACETAK	Acetic Acid (AK) Test Kit	170.5
K-AMIAR	Ammonia (Rapid) Test Kit	274.6
K-CITR	Citric Acid Test Kit	220.5
K-FRUGL	D-Fructose / D-Glucose Test Kit	254.1
K-GCROL	Glycerol Test Kit	156.8
K-LATE	L-Lactic Acid Test Kit	110.0
K-LMALAF	L-Malic Acid (AF) Test Kit	303.6
K-PANOPA	Primary Amino Nitrogen Test Kit	310.0

Full details on **www.megazyme.com**

Megazyme International Ireland Ltd., Bray Business Park, Bray, Co. Wicklow, Ireland.
T + 353 1 286 1220 F + 353 1 286 1264 E info@megazyme.com