

Megazyme

MegaQuantTM

**D-FRUCTOSE plus
D-GLUCOSE
(Reducing sugars)**

ASSAY PROCEDURE

K-FRGLMQ 06/09

(Patent Pending)

(60 Assays per Kit)

*This Data Booklet will soon be available at
www.megazyme.com in the following languages
French-German-Italian-Spanish-Portuguese*



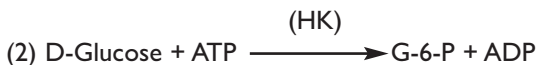
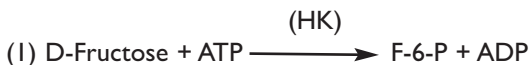
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INTRODUCTION:

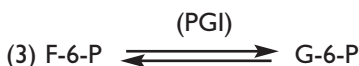
D-Fructose and D-glucose are found in most plant products. In foods, they are present in significant quantities in honey, wine and beer, and a range of solid foodstuffs such as bread and pastries, chocolate and candies. In the wine industry, the D-fructose and D-glucose content is one of the most important parameters and is monitored at each stage of the wine making process. The specific aim of the current assay format is to provide a simple, rugged, reliable and accurate method for the measurement of D-fructose plus D-glucose employing an inexpensive colorimeter (the MegaQuant™ Meter).

PRINCIPLE:

D-Fructose and D-glucose are phosphorylated by the enzyme hexokinase (HK) and adenosine-5'-triphosphate (ATP) to fructose-6-phosphate (F-6-P) and glucose-6-phosphate (G-6-P), with the simultaneous formation of adenosine-5'-diphosphate (ADP) (1), (2).



F-6-P is converted to G-6-P by phosphoglucose isomerase (PGI) (3).

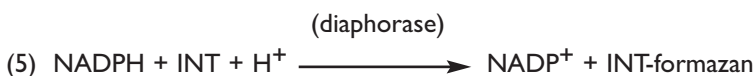


In the presence of the enzyme glucose-6-phosphate dehydrogenase (G6P-DH), G-6-P is oxidised by nicotinamide-adenine dinucleotide phosphate (NADP^+) to gluconate-6-phosphate with the formation of reduced nicotinamide-adenine dinucleotide phosphate (NADPH) (4).



The amount of NADPH formed in this reaction is stoichiometric with the amount of D-fructose + D-glucose.

In the fifth reaction, in the presence of diaphorase, NADPH reduces idonitrotetrazolium chloride (INT) to an INT-formazan compound which absorbs in the range of 480-520 nm (5).



The amount of INT-formazan formed in this reaction is stoichiometric with the amount of D-fructose and/or D-glucose. It is the INT-

formazan which is measured by the increase in absorbance at 505 nm.

SPECIFICITY, SENSITIVITY, LINEARITY AND PRECISION:

The assay is specific for D-fructose and/or D-glucose. The smallest differentiating absorbance for the assay is 0.01 absorbance units. This corresponds to 11.6 mg/L of sample solution with a sample volume of 20 μ L. The detection limit is 23.2 mg/L, which is derived from an absorbance difference of 0.02 and a sample volume of 20 μ L.

The assay is linear over the range of 0.23 to 24 μ g of D-fructose and/or D-glucose per assay (Fig. 3, p. 13). In duplicate determinations using one sample solution, an absorbance difference of 0.01 to 0.02 may occur. With a sample volume of 20 μ L, this corresponds to a D-fructose and/or D-glucose concentration of approx. 11.6 to 23.2 mg/L of sample solution. If the sample is diluted during sample preparation, the result is multiplied by the dilution factor, F. If in sample preparation, the sample is weighed, e.g. 10 g/L, a difference of 0.02 to 0.05 g/100 g can be expected.

INTERFERENCE:

Phenolics, especially in red wine, interfere with the assay as they react with INT causing a “creep” reaction. For red wines, this reaction is so significant that if undiluted wine is to be analysed, phenolics must first be removed with polyvinylpyrrolidone (PVPP) [see sample preparation, example (d), page 12, red wine]. A “creep” reaction is also seen when analysing some undiluted white wines, however, this is so slow that it can either be ignored, or accounted for in one of the recommended assay formats (see Assay Format B) and the associated calculations.

In the measurement of D-fructose plus D-glucose using the MegaQuant™ procedure, it is essential to recognise and account for three potential sources of minor error, namely:

1. While PVPP removes most of the phenolics from red wine, sufficient remains to cause a slight “creep” reaction. Since this reaction is very slow in PVPP treated wines (leading to a potential overestimation of D-fructose plus D-glucose of only 0.00 to 0.01 g/L for white wine, or 0.0 to 0.03 g/L for red wine), it can be ignored (see Assay Format A). However, if desired, this can be accurately accounted for by using assay format B).
2. Using the PVPP tablets as recommended in these assay formats (i.e. 1 tablet per 5 mL of wine) results in a 2 % (v/v) dilution of the sample concentration. This is allowed for in the calculations (see PVPP treated samples; pages 8 and 10).

3. The reagent tablets used in this assay contain trace levels of D-glucose ($\sim 0.3 \mu\text{g}/\text{tablet}$), which yields an absorbance increase of 0.01 in the assay. This equates to just 0.008 g/L in the test, and thus is ignored in the calculations in Assay Format A (page 8), but is included in the more detailed Assay Format B (page 10).

Reducing substances such as sulphite and ascorbate can interfere with the assay, but only at levels not experienced in wine e.g. sulphite at a concentration of 4 g/L gives a slight creep reaction [which can be accounted for (see Assay Format B, page 9)]. Samples containing very high levels of ascorbate ($> 400 \text{ mg/L}$) cannot be assayed with this procedure.

The significance of other interferences can be checked by adding D-fructose ($10 \mu\text{g}$ in $20 \mu\text{L}$) to the cuvette on completion of the reaction. A substantial increase in the absorbance should be observed. Interfering substances in the sample being analysed can be identified by including an internal standard. Quantitative recovery of this standard should be achieved. Interfering substances in the sample being analysed can be identified by including an internal standard. Quantitative recovery of this standard would be expected.

SAFETY:

The reagents used in the determination of D-fructose and/or D-glucose using the MegaQuant™ procedure are not hazardous materials in the sense of the Hazardous Substances Regulations. However, the buffer contains sodium azide (0.02 % w/v) as a preservative. The general safety measures that apply to all chemical substances should be adhered to.

REFERENCES:

1. McCleary, B.V. and Charnock, S. J. (2005). Reagent components in tablet form for colorimetric assays of food and beverage analytes. *Patent submitted*.
2. Kunst, A., Draeger, B. & Ziegenhorn, J. (1988). D-Glucose. In *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), 3rd ed., **Vol. VI**, pp. 163-172, VCH Publishers (UK) Ltd., Cambridge, UK.

KITS:

Kits suitable for performing 60 determinations are available from Megazyme. The kits contain the full assay method plus:

Bottle 1: Glycylglycine buffer (100 mL, 100 mM, pH 10.0) plus magnesium chloride (10 mM), Triton X-100 (1.0 % v/v) and sodium azide (0.02 % w/v) as a preservative. Stable for > 2 years at 4°C.

NOTE: This buffer contains sodium azide (0.02 % w/v) as a preservative, and thus should **not** be dispensed using a mouth pipette.

Bottle 2: Test tablets (60) containing NADP⁺, ATP, INT and FAD. These are supplied in a special plastic vial containing impregnated dessicant. Allow this container to warm to room temperature (preferably in the presence of a dessicant) before opening to remove tablets. This will ensure that the remaining tablets will not absorb moisture and thus guarantee maximum stability. Stable for > 2 years when stored at -20°C in a domestic freezer (preferably in a sealed container with a drying agent such as silica gel).

Bottle 3: Hexokinase (425 U/mL), glucose-6-phosphate dehydrogenase (212 U/mL), phosphoglucose isomerase (1,000 U/mL) and diaphorase (200 U/mL) suspension, 1.3 mL. Stable for > 2 years at 4°C.

Bottle 4: D-Fructose standard solution (5 mL, 0.50 mg/mL) in 0.02 % w/v sodium azide. Stable for > 2 years at 4°C.

Bottle 5: Tablets (65) containing PVPPP. Stable for > 5 years at room temperature.

PREPARATION OF REAGENT SOLUTIONS/SUSPENSIONS:

1. Use the contents of bottle 1 as supplied (1.5 mL per assay); or **alternatively**, dilute all of this solution (100 mL) with an equal volume of purified water (see note 1, bottom of page 5) and then use 3.0 mL per assay. Stable for > 2 years at 4°C.
2. Use the contents of bottle 2 as supplied. Stable for > 2 years when stored dry at -20°C in a domestic freezer.
3. Use the contents of bottle 3 as supplied. Before opening for the first time, shake the bottle to remove any enzyme that may have settled on the rubber stopper. Subsequently, store the bottle in an upright position. **Swirl the bottle to mix contents before use.** Stable for > 2 years at 4°C.

4. Use the contents of bottle 4 as supplied (20 μ L per test).
Warm to room temperature before dispensing.
Stable for > 2 years at 4°C.

NOTE: The D-fructose standard solution is only assayed where there is some doubt about the accuracy of the colorimeter or where it is suspected that inhibition is being caused by substances in the sample. The concentration of D-fructose is determined using the equations on page 8 or 10 (that were derived from the standard curve on page 13).

5. Use the contents of bottle 5 as supplied.
Stable for > 5 years at room temperature.

EXTRA EQUIPMENT/REAGENTS REQUIRED:

1. Special glass colorimeter test tubes (flat bottomed; 16 x 100 mm) as supplied with the instrument. Extra tubes are available from Megazyme (cat. no. G-MQTB25).
2. Disposable, graduated 10 mL polypropylene tubes e.g. Sarstedt cat. no. 62.551.201 PP (www.sarstedt.com).
3. Micro-pipettor, e.g. Gilson Pipetman® (20 μ L) to dispense 20 μ L of sample, standard and hexokinase/glucose-6-phosphate dehydrogenase/phosphoglucose isomerase/diaphorase suspension.
4. Eppendorf Multipette® positive displacement pipettor
- with 25 mL Combitip® (to dispense 1.5 mL aliquots of solution I and 1.5 mL aliquots of purified water); **or**:
5. Glass graduated pipette (5 mL) and Pipette Pump II (10 mL, green; Sigma cat. no. P-7924); or Pipette filler (10 mL, green; Merck Eurolab/VWR International, cat. no. 241/3983/03) for use with glass pipettes.
6. MegaQuant™ colorimeter (reading at 505 nm).
7. Stop clock or watch.
8. Whatman No. 1 (9 cm) filter circles and filter funnels.

NOTES:

1. Purified water (distilled water or good quality, commercially available, filtered drinking water) will be required in the assay and for dilution of grape juice or wine samples. If using drinking water, first check the suitability of this by running a test with the D-fructose standard.
2. Colorimeter tubes must be clean and dry before use. The tubes should be polished clean with tissue paper.

PROCEDURE:

I. REMOVAL OF PHENOLICS. (generally required only for red wines)

1. Transfer approx. 5 mL of red wine to a graduated 10 mL polypropylene tube (equipment 2), using the graduations on the tube as a guide.
2. Add one PVPP tablet (bottle 5), cap the tube and mix the contents by continuous inversion over **5 min**, Filter the contents of the tube through a Whatman No. 1 (9 cm) filter circle or allow the PVPP to settle over approx 1 hour. Collect approx. 1 mL of filtrate. The filtrate must be a light pink colour (which is generally the case). However, intensely coloured wines may require the addition of two PVPP tablets per 5 mL of wine. In such cases, to obtain sufficient filtrate (approx. 1 mL), it is necessary to carefully squeeze the folded filter circle with a spatula.
3. Analyse 20 μ L of the filtrate (or the supernatant, i.e. if the PVPP suspension was allowed to settle over approx. 1 hour).

II. ASSAY OF SAMPLES.

Two assay formats are detailed below. In most cases, Assay Format A (simple format) can be used and is recommended. This procedure can be used when analysing all samples where a sample dilution of > 10-fold is required. It can also be used to analyse undiluted, PVPP treated red or white wine or non-PVPP treated white wine. Alternatively, to allow for the slight creep reaction in PVPP treated red wine and non-PVPP treated white wine, Assay Format B should be employed.

A. Assay Format A (simple format - see Figure 1)

1. Transfer **1.5 mL** of Solution 1 (at $\sim 23^{\circ}\text{C}$ or higher) and 1.5 mL of purified water [using an Eppendorf Multipipette[®] with 25 mL Combitip[®] (equipment 4) or a 5 mL graduated glass pipette with pipette filler (equipment 5)] to a colorimeter test tube.
Alternatively, transfer **3.0 mL** of Solution 1 that has been diluted with an equal volume of purified water (see Preparation of Reagent Solutions/Suspensions, point 1, page 4) to the test tube.
2. Using the supplied forceps, add a reagent test tablet (bottle 2) and allow this to dissolve over approx. 1-2 min. Swirl the tube intermittently to ensure **complete** dissolution.
3. Add 20 μ L of wine sample [PVPP treated (undiluted red or white wine), non-PVPP treated (undiluted white wine sample) or diluted wine or grape juice sample] and swirl the tube to ensure thorough mixing.

4. After approx. 1 min, insert the tube into the MegaQuant™ colorimeter and press the **ON** key until the '---' symbol appears on the display (2 seconds).
5. Release the ON key when the display shows '0.00' (this is **A₁**).
6. Remove the tube from the colorimeter and add 20 µL of the contents of bottle 3 (HK/G6P-DH/PGI/diaphorase). Swirl the tube to ensure complete mixing. At the same time, press the start button on a stop clock timer, or record the time on a wrist watch.
7. Push the tube back into the colorimeter, and after 5 min, press the **ON** key until the display appears (1 second). Record the reading in a records book as **A₂**.
8. To ensure that the reaction has come to completion, take extra readings at 1 min intervals beyond the 5 min reading. The reading should remain the same over a 1 min interval (see Figure 1 for results obtained for different levels of D-fructose using this assay format; reaction followed in a recording spectrophotometer).

NOTE:

1. If the value of **A₂** is > 1.00, dilute the sample 10-fold more and repeat the test. If the value of **A₂** is < 0.10, use a less diluted sample (e.g. 10-fold diluted instead of 100-fold).
2. The colour of the assay solution **will** increase over longer periods of time due to the light sensitivity of INT (the chromogen in the tablet). It is thus necessary to take readings within the time frame recommended in the method.
3. Before running the test, bottle 1 (page 4) should be removed from the refrigerator and allowed to warm to room temperature. If the temperature is < 23°C, the reaction proceeds less rapidly.
4. Perform a **reagent blank** by adding a test tablet to 1.5 mL of solution 1 (in bottle 1) plus 1.52 mL of purified water. Zero the colorimeter after the tablet has completely dissolved. Then add 20 µL of HK/G6P-DH/PGI/diaphorase mixture (bottle 3) and measure the absorbance increase (**A₂**) after 5 min. The absorbance increase (**A₂**) should be approx. 0.01 (which is due to trace levels of D-glucose in the tablet formulation). These assays are only performed when a new source of purified water is used (see note 1, page 5).

CALCULATION:

$\Delta A_{(D\text{-fru} + D\text{-glu})}$ = the absorbance increase (A_2).

The value of $\Delta A_{(D\text{-fru} + D\text{-glu})}$ should as a rule be at least 0.10 absorbance units to achieve sufficiently accurate results.

The concentration of D-fructose plus D-glucose in the sample being analysed is determined using the equations below (derived from the standard curve in Figure 3; page 13):

Concentration of D-fructose plus D-glucose (reducing sugars) for non-PVPP treated samples.

with 20 μL sample volume and no sample dilution:

$$c = 1.160 \times \Delta A_{(D\text{-fru} + D\text{-glu})} \quad [\text{g/L}]$$

with 20 μL sample volume and with sample dilution:

$$c = 1.160 \times \text{dilution factor} \times \Delta A_{(D\text{-fru} + D\text{-glu})} \quad [\text{g/L}]$$

Concentration of D-fructose plus D-glucose (reducing sugars) for PVPP treated samples.

with 20 μL sample volume and no sample dilution:

$$c = 1.160 \times \Delta A_{(D\text{-fru} + D\text{-glu})} \times 102/100 \quad [\text{g/L}]$$

$$= 1.183 \times \Delta A_{(D\text{-fru} + D\text{-glu})} \quad [\text{g/L}]$$

with 20 μL sample volume and with sample dilution:

$$c = 1.183 \times \text{dilution factor} \times \Delta A_{(D\text{-fru} + D\text{-glu})} \quad [\text{g/L}]$$

where:

102/100 = allowance for the dilution of wine by PVPP treatment.

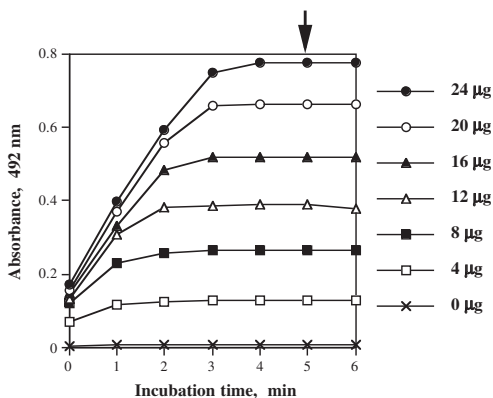


Figure 1. Time course of colour formation in the MegaQuant™ D-Fructose/ D-Glucose Test, performed in a recording spectrophotometer (25°C, 1 cm light path). The spectrophotometer was zeroed before addition of the trigger enzyme mixture (HK/G6P-DH/PGI/diaphorase). D-Fructose standard solution (4-24 µg) was added as indicated.

ASSAY FORMAT B (“creep” corrected)

1. Transfer 1.5 mL of Solution I (at 23°C or higher) and 1.5 mL of purified water [using an Eppendorf Multipipette® with 25 mL Combitip® (equipment 4) or a 5 mL graduated glass pipette with pipette filler (equipment 5)] to a colorimeter test tube.

Alternatively, transfer **3.0 mL** of Solution I that has been diluted with an equal volume of purified water (see Preparation of Reagent Solutions/Suspensions, point 1, page 4) to the test tube.

2. Using the supplied forceps, add a reagent test tablet (bottle 2) and allow this to dissolve over approx. 1-2 min. Swirl the tube intermittently to ensure **complete** dissolution.
3. Add **20 µL** of PVPP treated red or white wine sample and swirl the tube to ensure thorough mixing.
4. After approx. 1 min, insert the tube into the MegaQuant™ colorimeter and press the ON key until the ‘---’ symbol appears on the display (2 seconds).
5. Release the ON key when the display shows ‘0.00’ (this is **A₀**). At the same time, press the start button on a stop clock timer, or record the time on a wrist watch.
6. Leave the tube in the colorimeter for exactly 5 min and then press the ON key until the display appears (1 second). Do not hold the key down or the instrument will blank on the sample and cause errors in readings. Record the reading in a records book as **A₁** (this is the increase in colour due to creep reaction over 5 min).

- Remove the tube from the colorimeter and immediately add **20 μL** of the contents of bottle 3 (HK/G6P-DH/PGI /diaphorase). Swirl the tube to ensure complete mixing. At the same time, press the start button on a stop clock timer, or record the time on a wrist watch.
- Push the tube back into the colorimeter, and after 5 min, press the ON key to display the reading (absorbance) for the sample, **A_2** . Record this value.
- To ensure that the reaction has come to completion, take extra readings at 1 min intervals beyond the 5 min reading. The reading should remain the same over a 1 min interval.

CALCULATION:

Determine the absorbance difference $[(A_2 - A_1) - (A_1 - A_0)]$ and **subtract 0.010** from this value (this value of 0.010 is the colour increase due to the presence of trace amounts of D-glucose in the test tablet components).

Since the meter is zeroed on A_0 , the equation becomes

$A_2 - (2 \times A_1) - 0.010$; this is $\Delta A_{(\text{D-fru} + \text{D-glu})}$ for the sample (see Figure 2).

For example with values of $A_2 = 0.80$ and $A_1 = 0.02$;

$$\Delta A_{(\text{D-fru} + \text{D-glu})}$$

$$= 0.80 - (2 \times 0.02) - 0.010 = 0.80 - 0.04 - 0.010 = 0.75.$$

The value of $\Delta A_{(\text{D-fru} + \text{D-glu})}$ should as a rule be at least 0.10 absorbance units to achieve sufficiently accurate results.

The concentration of D-fructose plus D-glucose in the sample being analysed is determined using the equations below (derived from the standard curve in Figure 3, page 13):

Concentration of D-fructose plus D-glucose (reducing sugars) for PVPP treated samples.

with 20 μL sample volume and no sample dilution:

$$c = 1.183 \times \Delta A_{(\text{D-fru} + \text{D-glu})} \quad [\text{g/L}]$$

with 20 μL sample volume and with sample dilution:

$$c = 1.183 \times \text{dilution factor} \times \Delta A_{(\text{D-fru} + \text{D-glu})} \quad [\text{g/L}]$$

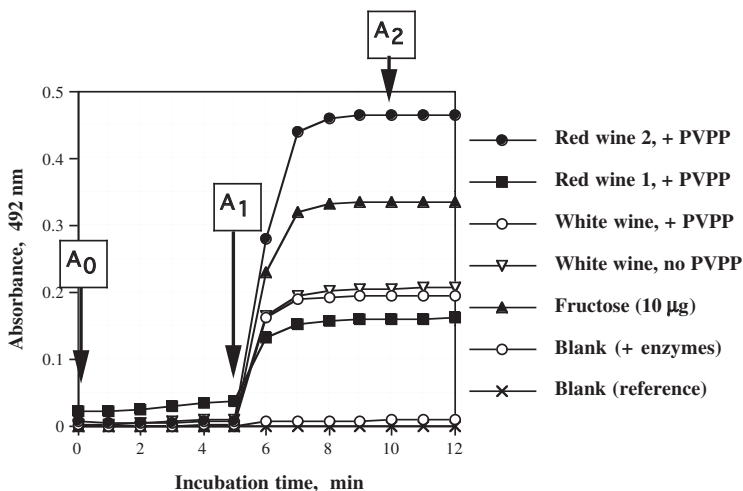


Figure 2. Time course of colour formation in the MegaQuant™ D-Fructose/D-Glucose Test, performed in a recording spectrophotometer (25°C, 1 cm light path). The initial reaction (A_1-A_0) shows the creep rate for the different samples. At 5 min, the enzyme mixture (HK/G6P-DH/PGI/diaphorase) was added and the reaction was allowed to proceed for a further 5 min to obtain A_2 . For wine samples, 20 μL of undiluted wine was analysed.

SAMPLE PREPARATION:

Sample dilution.

The amount of D-fructose plus D-glucose present in the cuvette (i.e. in the 20 μL of sample being analysed) should range between 2.4 and 24 μg . The sample solution must therefore be diluted sufficiently to yield a D-fructose plus D-glucose concentration between 0.12 and 1.00 g/L.

Dilution Table

Estimated concentration of D-fructose + D-glucose (g/L)	Dilution with water	Dilution factor (F)
< 1.00	No dilution required	1
1.00-10.0	1 + 9	10
10.0-100	1 + 99	100
100-400	1 + 999	1000

If the value of $\Delta A_{(D-fru + D-glu)}$ is too low (e.g. < 0.100), dilute less strongly.

SAMPLE PREPARATION EXAMPLES:

(a) Determination of D-fructose plus D-glucose in grape juice.

This can generally be determined without any sample treatment (except dilution according to the dilution table). *Typically, a dilution of 1:400 and sample volume of 20 μ L are satisfactory.*

(b) Determination of D-fructose + D-glucose in grapes.

Crush several single grapes taken from the bunch with a garlic press and recover the juice. To 10 mL of this juice in a 25 mL flask, add 10 mL of distilled water. Mix well and filter. Dilute the filtrate as required. *Typically, a further dilution of 1:200 (i.e total sample dilution of 400-fold) and a sample volume of 20 μ L are satisfactory.* For larger samples of grapes, extract as described in the data booklet for L-Malic Acid (MegaQuant™ Format) (K-LMALMQ).

(c) Determination of D-fructose + D-glucose in white wine.

White wines can generally be analysed without any sample treatment (except dilution according to the dilution table). *Typically, for dry white wine, no dilution is required and a sample volume of 20 μ L is satisfactory. For a sweet, white port, a dilution of 1:100 and sample volume of 20 μ L are satisfactory.*

(d) Determination of D-fructose + D-glucose in red wine.

Transfer approx. 5 mL of red wine to a 10 mL graduated polypropylene tube (Equipment 2), using the graduations on the tube as a guide. Add one PVPP tablet (bottle 5), cap the tube and invert the contents continually over 5 min. The tablet will disintegrate after 1-2 min, but inversion of the tube must be continued intermittently for the full 5 min to get optimal binding of the phenolics. Filter the tube contents through a Whatman No. 1 (9 cm) filter circle and collect approx. 1 mL of filtrate. This solution must be a light pink colour (which it will be for most red wine samples). However, if the filtrate is still a deep red colour, repeat the treatment of the wine, but use two PVPP tablets. In such cases, to ensure that sufficient filtrate is collected, it is necessary to carefully squeeze the folded filter paper in the filter funnel with a metal spatula or tea spoon. An essentially clear, light pink coloured solution should be obtained, and this is suitable for analysis. *Typically, for dry red wine, no dilution and a sample volume of 20 μ L are satisfactory. For a sweet, red port, a dilution of 1:50 and sample volume of 20 μ L are satisfactory.*

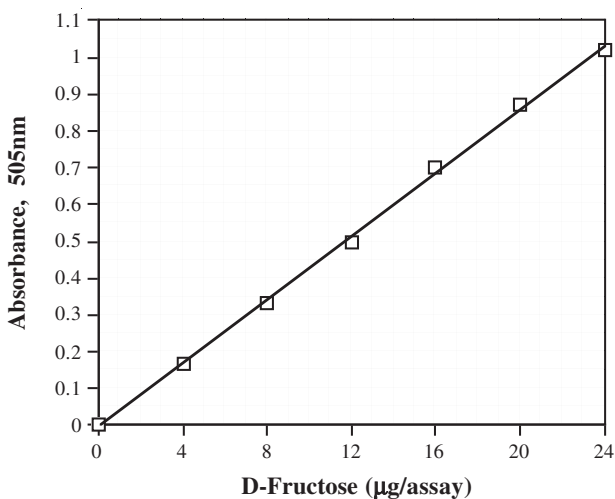


Figure 3. Standard curve relating D-fructose concentration ($\mu\text{g}/\text{test}$; final assay volume of 3.04 mL) to absorbance measured with the MegaQuant™ colorimeter (at ambient temperature and 505 nm). An identical curve was obtained with D-glucose.

CHECKING THE ACCURACY AND RELIABILITY OF THE MegaQuant™ COLORIMETER AND THE D-FRUCTOSE PLUS D-GLUCOSE TEST:

1. Certified colour standards are available from Megazyme for checking the instrument calibration. However, instrument calibration should not change in use.
2. Accuracy of the MegaQuant™ D-Fructose/D-Glucose Test is determined using the provided D-fructose standard. Perform an assay exactly as described on pages 6 and 7, using 20 μL of D-fructose standard (0.50 mg/mL) in place of the diluted sample. Allow the reaction to proceed to completion. The expected absorbance value ($A_2 - A_1$) is 0.44. Values significantly different to this may be due to pipetting inaccuracies. If this possibility is excluded, contact Megazyme for advice.

GENERAL POINTS ON USE AND MAINTENANCE OF THE MegaQuant™ COLORIMETER:

Error Messages:

Various error messages may appear on the instrument display panel. The cause of the problem and the appropriate action is as follows:

Display	Cause	Action
E1	Coloured, dirty or scratched blank tube used.	Re-zero on a correct blank in a clean, dry tube.
E2 or E3	a) Incorrect blank used to zero instrument. b) Electrical fault in the instrument.	a) Re-zero instrument on a correct blank. b) Return instrument for repair.
E4	Battery voltage too low for correct reading.	Change batteries.
E5	Stray light has affected the reading.	a) Remove instrument from vicinity of spotlights or other bright light source. b) Check instrument case for damage.

Care and Maintenance:

Keep the test tubes clean. Place the plastic screw caps on the tubes before taking a reading to prevent test solutions being spilt into the instrument. Wipe off spillages and moisture immediately with a dry cloth. On no account should solvents or abrasive materials be used to clean the instrument.

Replace the batteries when the 'B' symbol appears on the display. To remove the battery compartment cover press gently on the sides of the cover and pull downwards. Use 2 x 1.5 V alkaline 'AA' batteries, MN 1500, LR6, E91, AM3 or equivalent. Remove batteries from the instrument if it is to be stored or left unused for a long period of time.

The MegaQuant™ colorimeter is guaranteed for a period of one year from the time of purchase excluding accidental damage or damage caused by unauthorised repair or misuse. Should repair be necessary, contact the Technical Services Department of Palintest Ltd. at www.palintest.com, alternatively, phone: +44 (0) 191 491 0808, fax: +44 (0) 191 482 5372 or write to Palintest House, Kingsway, Team Valley, Tyne and Wear, NE11 0NS, UK, quoting the serial number shown in the battery compartment. This guarantee does not affect statutory rights.

Technical Specifications:

Instrument Type	Single beam colorimeter pre-programmed for absorbance readings at 505 nm.
Optics	Palintest M2 optical system with pulsed blue green LED, wavelength filter and photodetector.
Operating Range	0.00 - 1.20 absorbance units.
Operating Temperature	0-40°C / 32-104°F.
Display	10 mm LCD.
Test Cells	For use with round, flat-bottomed, test tubes, 1.60 cm OD, 1.415 cm light path.
Operation	Single button.
Blank/Zero Setting	Optionally held in memory or reset for each reading.
Internal Calibration	Factory set. Recalibration through internal software.
Instrument Case	Splash-proof case with membrane key pad.
Power Supply	2 x 1.5V batteries. Power management system with auto-switch off.
Size	Instrument only 17.3 x 7.5/4.4 x 4.1 cm.



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