Megazyme

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D-FRUCTOSE and D-GLUCOSE (*Liquid Stable Format*)

ASSAY PROCEDURE

K-FRGLQR 02/17

(1100 Auto-Analyser Assays of each per Kit) or
(1100 Microplate Assays of each per Kit)

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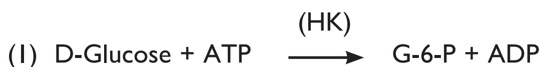
INTRODUCTION:

D-Glucose and D-fructose 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 sum of D-glucose and D-fructose, termed "total residual sugars", is a key parameter, as this represents the amount of sugar that is available to the yeast for the conversion into ethanol. Total residual sugar levels are monitored throughout fermentation and, after fermentation is complete, are adjusted to achieve the desired taste profile. For the vast majority of measurements taken during the wine making process it is unnecessary to differentiate between the D-glucose and D-fructose, allowing them to be quantified simultaneously.

This kit (**K-FRGLQR**) is suitable for the specific measurement of D-glucose and D-fructose in wines, beverages, foodstuffs and other materials. The procedure formats provided in this booklet allow for the individual or simultaneous measurement of D-glucose and D-fructose.

PRINCIPLE:

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



$$(1)\quad \text{D-Glucose} + \text{ATP} \xrightarrow{\text{(HK)}} \text{G-6-P} + \text{ADP}$$

$$(2)\quad \text{D-Fructose} + \text{ATP} \xrightarrow{\text{(HK)}} \text{F-6-P} + \text{ADP}$$

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) (3).

$$(3)\quad \text{G-6-P} + NADP^+ \xrightleftharpoons{\text{(G6P-DH)}} \text{gluconate-6-phosphate} + \text{NADPH} + H^+$$

The amount of NADPH formed in this reaction is stoichiometric with the amount of D-glucose. It is the NADPH which is measured by the increase in absorbance at 340 nm.

On completion of reaction (3), F-6-P is converted to G-6-P by phosphoglucose isomerase (PGI) (4).

The G-6-P formed reacts in turn with $NADP^+$ forming gluconate-6-phosphate and NADPH, leading to a further rise in absorbance that is stoichiometric with the amount of D-fructose.

SAFETY:

The general safety measures that apply to all chemical substances should be adhered to.

For more information regarding the safe usage and handling of this product please refer to the associated SDS that is available from the Megazyme website.

KITS:

Kits suitable for performing 1100 assays of each test in auto-analyser format or microplate format are available from Megazyme. The kits contain the full assay method plus:

Bottle 1: **Reagent 1 (44 mL)**
Contains sodium azide (0.08% w/v) as a preservative.
Ready to use.
Stable for > 2 years at 4°C.

Bottle 2: **Reagent 2 (22 mL)**
Contains sodium azide (0.05% w/v) as a preservative.
Ready to use.
Stable for > 2 years at 4°C.

Bottle 3: **Reagent 3 (22 mL)**
Contains sodium azide (0.05% w/v) as a preservative.
Ready to use.
Stable for > 2 years at 4°C.

Bottle 4: **D-Glucose plus D-Fructose Standard (2 mL)**
(3 g/L of each sugar). Ready to use.
Stable for > 2 years at room temperature.

AUTO-ANALYSER ASSAY PROCEDURES:

NOTES:

1. To obtain individual measurements of D-glucose and D-fructose use Auto-Analyser Format A (page 3). Alternatively, to obtain a single combined measurement of D-glucose and D-fructose (e.g. total residual sugars) use Auto-Analyser Format B (page 4).
2. For each batch of samples that is applied to the determination of D-glucose or D-fructose **either a single point standard or a calibration curve must be performed concurrently using the same batch of reagents.**

A. AUTO-ANALYSER FORMAT: D–Glucose & D–Fructose

Wavelength: 340 nm
Calculation: End-point
Temperature: ~ 25°C or 37°C
Reaction: Absorbance increase
Final volume: 0.223 mL (for D-glucose)
0.243 mL (for D-fructose)
Linearity: 0.4-20 µg of D-glucose plus D-fructose per cuvette (in 0.003 mL sample volume)
(up to 6,000 mg/L of original sample)

Pipette into cuvettes	Sample	Standard
reagent 1 distilled water sample standard	0.040 mL 0.160 mL 0.003 mL -	0.040 mL 0.160 mL - 0.003 mL
Mix, read the absorbances of the solutions (A_1) after 3 min and start the reactions by addition of:		
reagent 2	0.020 mL	0.020 mL
Mix and read the absorbances of the solutions (A_2) after 5 min. Then add:		
reagent 3	0.020 mL	0.020 mL
Mix and read the absorbances of the solutions (A_3) after 10 min.		

CALCULATION FORMULA:

D-Glucose

A_2 - (A_1 x 203/223)

D-Fructose

A_3 - (A_2 x 223/243)

B. AUTO-ANALYSER FORMAT: Total Residual Sugars

Wavelength: 340 nm
Calculation: End-point
Temperature: ~ 25°C or 37°C
Reaction: Absorbance increase
Final volume: 0.243 mL
Linearity: 0.4-20 µg of D-glucose plus D-fructose per cuvette (in 0.003 mL sample volume) (up to 6,000 mg/L of original sample)

Pipette into cuvettes	Sample	Standard
reagent 1 distilled water sample standard	0.040 mL 0.160 mL 0.003 mL -	0.040 mL 0.160 mL - 0.003 mL
Mix, read the absorbances of the solutions (A_1) after 3 min and start the reactions by addition of:		
reagent 2 reagent 3	0.020 mL 0.020 mL	0.020 mL 0.020 mL
Mix and read the absorbances of the solutions (A_{total}) after 10 min.		

CALCULATION FORMULA: Total Residual Sugars

A_{total} - (A_1 x 203/243)

MICROPLATE ASSAY PROCEDURES:

NOTES:

1. To obtain individual measurements of D-glucose and D-fructose use Microplate Format A (page 5). Alternatively, to obtain a single combined measurement of D-glucose and D-fructose (e.g. total residual sugars) use Microplate Format B (page 6).
2. For each batch of samples that is applied to the determination of D-glucose or D-fructose **either a single point standard or a calibration curve must be performed concurrently using the same batch of reagents.**

A. MICROPLATE FORMAT: D-Glucose & D-Fructose

Wavelength: 340 nm
Microplate: 96-well (e.g. clear flat-bottomed, glass or plastic)
Temperature: ~ 25°C or 37°C
Final volume: 0.223 mL (for D-glucose)
0.243 mL (for D-fructose)
Linearity: 0.4-20 µg of D-glucose plus D-fructose per cuvette (in 0.003 mL sample volume)
(up to 6,000 mg/L of original sample)

Pipette into well	Sample	Standard	Blank
reagent 1 distilled water sample standard	0.040 mL 0.160 mL 0.003 mL -	0.040 mL 0.160 mL - 0.003 mL	0.040 mL 0.163 mL - -
Mix* and read absorbances of the solutions (A_1) after exactly 3 min. Then add:			
reagent 2	0.020 mL	0.020 mL	0.020 mL
Mix* and read the absorbances of the solutions (A_2) at the end of the reaction (approx. 5 min). If the reaction has not stopped after 5 min, continue to read the absorbances at 2 min intervals until the absorbances remain the same over 2 min. Then add:			
reagent 3	0.020 mL	0.020 mL	0.020 mL
Mix* and read the absorbances of the solutions (A_3) at the end of the reaction (approx. 10 min). If the reaction has not stopped after 10 min, continue to read the absorbances at 2 min intervals until the absorbances remain the same over 2 min.			

* for example using microplate shaker, shake function on a microplate reader or repeated aspiration using a pipettor.

B. MICROPLATE FORMAT: Total Residual Sugars

Wavelength: 340 nm
Microplate: 96-well (e.g. clear flat-bottomed, glass or plastic)
Temperature: ~ 25°C or 37°C
Final volume: 0.243 mL
Linearity: 0.4-20 µg of D-glucose plus D-fructose per cuvette (in 0.003 mL sample volume) (up to 6,000 mg/L of original sample)

Pipette into well	Sample	Standard	Blank
reagent 1	0.040 mL	0.040 mL	0.040 mL
distilled water	0.160 mL	0.160 mL	0.163 mL
sample	0.003 mL	-	-
standard	-	0.003 mL	-
Mix* and read absorbances of the solutions (A_1) after exactly 3 min. Then add:			
reagent 2	0.020 mL	0.020 mL	0.020 mL
reagent 3	0.020 mL	0.020 mL	0.020 mL
Mix* and read the absorbances of the solutions (A_{total}) at the end of the reaction (approx. 10 min). If the reaction has not stopped after 10 min, continue to read the absorbances at 2 min intervals until the absorbances remain the same over 2 min.			

* for example using microplate shaker, shake function on a microplate reader or repeated aspiration using a pipettor.

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