Megazyme

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

ASSAY PROCEDURE

K-FRGLQR 07/12

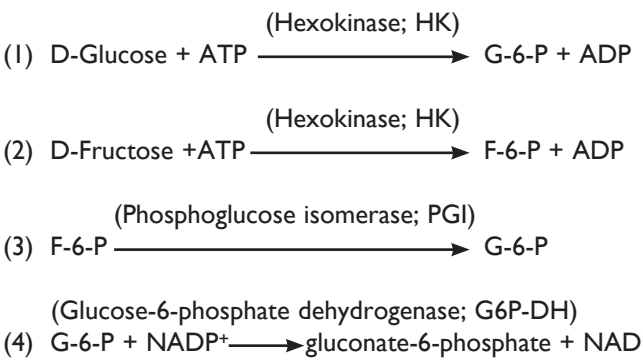
(1100 Auto-Analyser Assays of each per Kit)

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 the 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:



SAFETY:

The reagents used in the determination of D-glucose and D-fructose are not hazardous materials in the sense of the Hazardous Substances Regulations. However, the buffer concentrates contain sodium azide (up to 0.08% w/v) as a preservative. The general safety measures that apply to all chemical substances should be adhered to.

KITS:

Kits suitable for performing 1100 assays of each test in auto-analyser 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 6000 mg/L of original sample)

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

CALCULATION FORMULA:

D-Glucose

$A_2 - (A_1 \times 203 / 223)$

D-Fructose

$A_3 - (A_2 \times 223 / 243)$

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 6000 mg/L of original sample)

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

CALCULATION FORMULA: Total Residual Sugars

$A_{total} - (A_1 \times 203 / 243)$

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 6000 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*, 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*, 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*, 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 6000 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*, 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*, 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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