

GLUCOSE+FRUCTOSE
5 x 20 ml

INTENDED USE

Reagent for enzymatic single determination of glucose and fructose in foodstuff and other sample material.

PRINCIPLE OF THE METHOD

D-Glucose determination:

D-glucose, in the presence of the enzyme hexokinase, reacts with ATP to form glucose-6-phosphate. Glucose-6-phosphate in the presence of dinucleotide, is oxidized to gluconate-6-phosphate, by the enzyme glucose-6-phosphate dehydrogenase. Absorbance increase at 340 nm is proportional to the amount of D-glucose.

D-Fructose determination:

D-fructose in the presence of phosphoglucose isomerase (PGI), is converted into one of the products of the reaction, thus taking part in the enzymatic sequence described above.

KIT COMPONENTS

The components of the kit are stable until expiration date on the label.
Keep away from direct light sources.

GL+FR R1: 5 x 20 ml (liquid) blue cap
Composition: TEA pH 7.0, MgCl₂ 4 mM, dinucleotide > 4 mM, hexokinase > 2 kU/l, glucose-6-phosphate dehydrogenase > 2 KU/l, preservatives.

GL+FR R2: 1 x 2.5 ml (liquid) white cap
Composition: TEA pH 7.8, ATP > 50 mM, preservatives.

GL+FR R3: 1 x 2.5 ml (liquid) blue cap
Composition: Good buffer pH 8.0, PGI > 30 kU/l, preservatives.

GL+FR BL: 2 x 50 ml (liquid) white cap
Composition: TEA pH 7.8, preservatives.

Store all components at 2-8°C.

In vitro use only.

MATERIALS REQUIRED BUT NOT SUPPLIED

Current laboratory instrumentation. Spectrophotometer UV/VIS with thermostatic cuvette holder. Automatic micropipettes. Glass or high quality polystyrene cuvettes. Standard solution. Standard solution of glucose+fructose+sucrose 20 g/l (code SQPE063151), containing 5 g/l of **D-Glucose**, 5 g/l of **D-Fructose** and 10 g/l of **Sucrose**, is available on request. Please contact customer service for further information.

REAGENT PREPARATION

Procedure 1:

Use separate reagents.

Stability: until expiration date on the label at 2-8°C.

Procedure 2:

D-Glucose determination: mix 40 parts of reagent R1 with 1 part of reagent R2 (*Working reagent G*)

D-Glucose and D-Fructose determination: mix 40 parts of reagent R1 with 1 part of reagent R2 and 1 part of reagent R3 (*Working reagent F*)

It is suggested to prepare strictly the amount needed for the analysis, and any residue has to be stored at 2-8°C away from direct light sources.
Analytical performances of mixed reagent begin to fall off 48 hours after its preparation.

Preparation of standard glucose and fructose 1 g/l:

if you have available the standard glucose + fructose + sucrose, SQPE063151, containing 5 g/l of glucose and 5 g/l of fructose should be diluted 1: 5 (1 part of standard and 4 parts of distilled water) thus obtaining a standard of concentration 1 g/l of glucose and 1 g/l of fructose.

PRECAUTIONS

Reagent may contain some non-reactive and preservative components. It is suggested to handle carefully it, avoiding contact with skin and swallow.
Perform the test according to the general "Good Laboratory Practice" (GLP) guidelines.

SPECIMEN

Wine or any foodstuff once its utilization has been tested. Red wine samples can be analyzed without decolorization.

PROCEDURE 1

Wavelength:	340 nm		
Lightpath:	1 cm		
Temperature:	37°C		
dispense:	blank	standard	sample
reagent R1	2 ml	2 ml	2 ml
water	50 µl	-	-
standard	-	50 µl	-
sample	-	-	50 µl

Mix, incubate at 37°C for 2 minutes.
Read absorbances of standard (As₁) and sample (Ac₁) against reagent blank.

dispense:	blank	standard	sample
reagent R2	50 µl	50 µl	50 µl

Mix, incubate at 37°C for 5 minutes.
Read absorbances of standard (As₂) and sample (Ac₂) against reagent blank.

For Fructose determination

dispense:	blank	standard	sample
reagent R3	50 µl	50 µl	50 µl

Mix, incubate at 37°C for 5 minutes.
Read absorbances of standard (As₃) and sample (Ac₃) against reagent blank.

RESULTS CALCULATION

$$\text{glucose g/l} = \frac{Ac_2 - Ac_1}{As_2 - As_1} \times 1 \text{ (standard value)}$$

$$\text{fructose g/l} = \frac{Ac_3 - Ac_2}{As_3 - As_2} \times 1 \text{ (standard value)}$$

$$\text{glucose-fructose g/l} = \frac{Ac_3 - Ac_1}{As_3 - As_1} \times 2 \text{ (standard value)}$$

PROCEDURE 2 (cell flow instruments)

Wavelength:	340 nm			
Lightpath:	1 cm			
Temperature:	37°C			
dispense:	reagent blank	standard	sample blank	sample
reagent	2 ml	2 ml	-	2 ml
water	50 µl	-	-	-
standard	-	50 µl	-	-
sample	-	-	50 µl	50 µl
blank	-	-	2 ml	-

Mix, incubate at 37°C for 10 minutes.
Read absorbances of standard (As₁), sample (Ac₁), reagent blank (Ar₁) and sample blank (Ac₂).

RESULTS CALCULATION

Working reagent G:

$$\text{Glucose g/l} = \frac{(Ac_1 - Ar_1) - Ac_2}{As_1 - Ar_1} \times 1 \text{ (standard value)}$$

Working reagent F:

$$\text{Glucose+Fructose g/l} = \frac{(Ac_1 - Ar_1) - Ac_2}{As_1 - Ar_1} \times 2 \text{ (standard value)}$$

TEST PERFORMANCE

Specificity

The method is specific for glucose and fructose.

Linearity

The method is linear up to 2 g/l of glucose and fructose.

If the limit value is exceeded, it is suggested to dilute the sample 1+4 with distilled water and to repeat the test, multiplying the result by 5.

Precision Glucose-Fructose

White wine dil 1:2

intra-assay (n=10)	mean (g/l)	SD (g/l)	CV%
sample	1.461	0.011	0.75

inter-assay (n=20)	mean (g/l)	SD (g/l)	CV%
sample	1.464	0.040	2.74

Red wine dil 1:2

intra-assay (n=10)	mean (g/l)	SD (g/l)	CV%
sample	1.658	0.015	0.93

inter-assay (n=20)	mean (g/l)	SD (g/l)	CV%
sample	1.659	0.018	1.06

Rose wine dil 1:2

intra-assay (n=10)	mean (g/l)	SD (g/l)	CV%
sample	1.617	0.014	0.88

inter-assay (n=20)	mean (g/l)	SD (g/l)	CV%
sample	1.612	0.043	2.67

WASTE DISPOSAL

This product is made to be used in professional laboratories.

P501: Dispose of contents according to national/international regulations.

REFERENCES

H.U.Bergmeyer ed. 3, "Methods of enzymatic analysis" vol. VI pp. 163-171.

H.U.Bergmeyer ed. 3, "Methods of enzymatic analysis" vol. VI pp. 321-327..

Tietz Textbook of Clinical Chemistry, Second Edition, Bur-tis-Ashwood (1994).

MANUFACTURER

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SYMBOLS

	lot of manufacturing
	code number
	storage at temperature interval
	expiration date (year/month)
	warning, read enclosed documents
	read the directions