RANDOX

GLUCOSE/FRUCTOSE (GLUC/FRU)

UV METHOD
MANUAL
RX MONZA

INTENDED USE

For the quantitative *in vitro* determination of Glucose/Fructose in wine, beer and fruit juices. This product is suitable for manual use and on the RX **monza** analyser. **Applications for a variety of additional analysers are available from www.randoxfooddiagnostics.com.**

FOR THE ANALYSIS OF FOOD AND WINE. Not for use in diagnostic procedures.

Cat. No.			
GF 2635	R1.	Buffer	1 x 100 ml
1 x 100 ml	R2.	Enzyme Reagent	1 x 1.1 ml
	R3.	PGI	1 x 0.6 ml
	CAL	Glucose Standard	1 x 5.5 ml

UV METHOD[1-4]

Glucose is measured enzymatically utilising both hexokinase and glucose - 6- phosphate dehydrogenase.
The total sugar content (glucose + fructose) is determined by converting the fructose - 6- phosphate
(F-6-P) to glucose-6-phosphate (G-6-P) by phosphoglucose isomerase (PGI). The G-6-P is then converted to gluconate-6-phosphate and the NADH formed is stoichiometric with the amount of total sugars.

Fructose content = Total sugar - glucose content.

REACTION PRINCIPLE

D - Glucose + ATP $\xrightarrow{\text{hexokinase}}$ G-6-P + ADP

D -Fructose + ATP $\xrightarrow{\text{hexokinase}}$ F-6-P + ADP

F-6-P $\xrightarrow{\text{PGI}}$ G-6-P

G-6-P+ NAD^+ $\xrightarrow{\text{G-6-PDH}}$ D-gluconate-6-Phosphate + NADH + H^+

SAMPLE[5]

Red wine, white wine, beer or fruit juices. Decolourisation of red wine is not necessary. Fruit juices can also be assayed but must be filtered first if turbid. Strongly coloured juices with suspected low values should be decolourised with 0.1 g polyamide powder, gelatine or polyvinylpolypyrrolidone (PVPP) to approximately 10 ml of juice. Stir for 1 minute and filter. The clear filtrate can then be used in the assay undiluted. Carbonic acid should be removed from beer by filtering or stirring with a glass rod for 30 seconds.

REAGENT COMPOSITION

Contents		Concentrations in the Test
R1.	**Buffer**	
	Pipes	100 mmol/l, pH 7.6
	ATP	4 mmol/l
	NAD^+	3 mmol/l
	Magnesium ions	15 mmol/l
R2.	**Enzyme Reagent**	
	Hexokinase	≥ 0.5 U/ml
	G-6-PDH	≥ 1.5 U/ml
R3.	**PGI**	
	Phosphoglucose Isomerase	≥ 6.8 U/ml
CAL	**Standard**	
	Glucose	See lot specific insert

SAFETY PRECAUTIONS AND WARNINGS

For *in vitro* use only. Do not pipette by mouth. Exercise the normal precautions required for handling laboratory reagents.

Solution R1 contains Sodium Azide. Avoid ingestion or contact with skin or mucous membranes. In case of skin contact, flush affected area with copious amounts of water. In case of contact with eyes or if ingested, seek immediate medical attention.

Sodium Azide reacts with lead and copper plumbing, to form potentially explosive azides. When disposing of such reagents flush with large volumes of water to prevent azide build up. Exposed metal surfaces should be cleaned with 10% sodium hydroxide.

Health and Safety data sheets are available on request.

Please dispose of all biological and chemical materials according to local guidelines.

The reagents must be used only for the purpose intended by suitably qualified laboratory personnel, under appropriate laboratory conditions.

MATERIALS PROVIDED

Buffer
Enzyme Reagent
PGI
Glucose Standard

RX MONZA PROCEDURE

STABILITY AND PREPARATION OF REAGENTS FOR RX MONZA

R1. Glucose Reagent (GF-G)
Add 50 µl of enzyme reagent R2 to 10 ml of buffer R1. The working reagent is stable for 3 months at +2 to +8°C or 2 weeks at +15 to +25°C protected from light.

R1. Fructose Reagent (Total sugars) (GLFR)
Contents stable to expiry date as supplied when stored at +2 to +8°C.
Add **55 µl** of PGI R3 to **10 ml** of Glucose reagent R1 (GF-G). Stable for 3 months at +2 to +8°C.

CAL. Glucose Standard
The glucose standard is ready to use.

GLUCOSE

Select GF-G (programme 64) in the Run Test screen and carry out a water blank as instructed.

Pipette into a cuvette:

	S0 Blank	SO*	S1 Blank	S1	Sample Blank	Sample
Redist. Water	5 µl	5 µl	-	-	-	-
Standard	-	-	5 µl	5 µl	-	-
Sample	-	-	-	-	5 µl	5 µl
Buffer	500 µl	-	500 µl	-	500 µl	-
GF-G R1	-	500 µl	-	500 µl	-	500 µl

Mix, incubate for 10 - 15 minutes at +20 to +25°C.
Insert the cuvette into the RX **monza** flowcell holder and press Read.

*Reagent Blank

FRUCTOSE (TOTAL SUGARS)

Select GLFR (programme S32) in the Run Test screen and carry out a water blank as instructed.

Pipette into a cuvette:

	S0 Blank	SO*	S1 Blank	S1	Sample Blank	Sample
Redist. Water	5 µl	5 µl	-	-	-	-
Standard	-	-	5 µl	5 µl	-	-
Sample	-	-	-	-	5 µl	5 µl
Buffer	500 µl	-	500 µl	-	500 µl	-
GLFR R1	-	500 µl	-	500 µl	-	500 µl

Mix, incubate for 10 - 15 minutes at +20 to +25°C.
Insert the cuvette into the RX **monza** flowcell holder and press Read.

*Reagent Blank

CALIBRATION FOR RX MONZA

Calibration is recommended with change of reagent lot, or as indicated by QC procedures. Use CAL Standard provided in the kit.

CALCULATION

Fructose content = Total sugar - Glucose content.

MANUAL PROCEDURE

STABILITY AND PREPARATION OF REAGENTS FOR MANUAL USE

Buffer Solution, Enzyme reagent and Standard are ready for use as supplied. PGI should be inverted before use. All components are stable to the expiry date when stored at +2 to +8°C.

INSTRUCTIONS FOR USE

If measuring samples containing sugars in the range 0.6 - 4.0 g/l follow procedure A. If measuring samples less than 0.6 g/l sugars then follow procedure B and dilute the standard 1 in 10. If using 3 - 4 ml cuvettes follow macro procedures. If using 1 ml cuvettes follow semi-micro procedure.

(A)

Samples 0.6 g/l - 4 g/l

Wavelength:	340 nm (Hg 334 nm or Hg 365 nm)
Cuvette:	1 cm path length
Temperature:	+20 to+25°C
Measurements:	against water (increasing absorbance)

Pipette into 1 ml Cuvettes

	Blank	Standard	Sample
Buffer R1	1000 µl	1000 µl	1000 µl
Sample	-	-	10 µl
Standard (optional)	-	10 µl	-
Distilled Water	10 µl	-	-

Mix and measure absorbance A_1

Enzyme Reagent R2	10 µl	10 µl	10 µl

Mix well and incubate for 10 - 15 minutes at +20 to +25°C.
Measure absorbance A_2

PGI R3	5 µl	5 µl	5 µl

Mix well and incubate for 10 - 15 minutes at +20 to +25°C.
Measure absorbance A_3.

CALCULATION USING A FACTOR

A. Glucose $(A_2 - A_1)_{sample} - (A_2 - A_1)_{Blank} = \Delta A_{glucose.}$

B. Fructose $(A_3 - A_2)_{sample} - (A_3 - A_2)_{Blank} = \Delta A_{fructose.}$

Wavelength	Conc Glucose (g/l)	Conc Fructose (g/l)
Hg 365 nm	5.250 x $\Delta A_{glucose}$	5.276 x $\Delta A_{fructose}$
Hg 334 nm	2.974 x $\Delta A_{glucose}$	2.99 x $\Delta A_{fructose}$
Hg 340 nm	2.917 x $\Delta A_{glucose}$	2.93 x $\Delta A_{fructose}$

CALCULATION USING A STANDARD (Glucose)

$$\text{Concentration of glucose} = \text{standard Conc.} \times \frac{\Delta A_{sample\ (glucose)}}{\Delta A_{standard\ (glucose)}}\ (g/l)$$

CALCULATION USING A STANDARD (Fructose)

$$\text{Concentration of Fructose} = \text{standard conc.} \times \frac{\Delta A_{sample\ (fructose)}}{\Delta A_{standard\ (glucose)}}\ (g/l)$$

(B)
Samples less than 0.6 g/l

Wavelength:	340 nm (Hg 334 nm or Hg 365 nm)
Cuvette:	1 cm path length
Temperature:	+20 to+25°C
Measurements:	against water (increasing absorbance)

Pipette into 1 ml Cuvettes

	Blank	Standard	Sample
Buffer Solution R1	1000 µl	1000 µl	1000 µl
Sample	-	-	50 µl
Standard (diluted 1 + 9)	-	50 µl	-
Distilled Water	50 µl	-	-
Mix and measure absorbance A_1			
Enzyme Reagent R2	10 µl	10 µl	10 µl
Mix well and incubate 10 – 15 minutes at +20 to +25°C. Measure absorbance A_2			
PGI R3	5 µl	5 µl	5 µl
Mix well and incubate for 10 - 15 minutes at +20 to +25°C. Measure absorbance A_3.			

CALCULATION USING A FACTOR

A. Glucose $(A_2 - A_1)_{sample} - (A_2 - A_1)_{Blank} = \Delta A_{glucose}$.

B. Fructose $(A_3 - A_2)_{sample} - (A_3 - A_2)_{Blank} = \Delta A_{fructose}$.

Wavelength	Conc Glucose (g/l)	Conc Fructose (g/l)
Hg 365 nm	1.091 x $\Delta A_{glucose}$	1.096 x $\Delta A_{fructose}$
Hg 334 nm	0.618 x $\Delta A_{glucose}$	0.621 x $\Delta A_{fructose}$
Hg 340 nm	0.606 x $\Delta A_{glucose}$	0.609 x $\Delta A_{fructose}$

CALCULATION USING A STANDARD (Glucose)

$$\text{Concentration of glucose} = \frac{\text{standard conc./10}}{} \times \frac{\Delta A_{sample\ (glucose)}}{\Delta A_{standard\ (glucose)}} \text{ (g/l)}$$

CALCULATION USING A STANDARD (Fructose)

$$\text{Concentration of fructose} = \frac{\text{standard conc./10}}{} \times \frac{\Delta A_{sample\ (fructose)}}{\Delta A_{standard\ (glucose)}} \text{ (g/l)}$$

SPECIFIC PERFORMANCE CHARACTERISTICS

The following performance characteristics were obtained using an RX **monza** analyzer in cuvette mode at +25°C.

LINEARITY

The glucose assay is linear to 5.10 g/l. The total sugars assay is linear to 7.11 g/l.

SENSITIVITY

The minimum detectable concentration of glucose with an acceptable level of precision was determined as 0.53 g/l. The total sugars assay is sensitive to 0.275 g/l.

GLUCOSE PRECISION

Within run precision

	Level 1	Level 2
Mean (g/l)	2.55	4.29
SD	0.009	0.099
CV (%)	0.37	0.23
n	20	20

Inter-Assay

	Level 1	Level 2
Mean (g/l)	2.55	4.29
SD	0.021	0.050
CV (%)	1.10	1.17
n	20	20

CORRELATION

The RX **monza** analyzer (Y) was compared to the RX **daytona** (X) and the following linear regression equation obtained:

$$Y = 1.0427\ X - 0.1423$$

and a correlation coefficient r = 0.9911

43 unknown samples were analysed spanning the range 0.55 to 4.99 g/l.

TOTAL SUGARS PRECISION

Within run precision

	Level 1	Level 2
Mean (g/l)	1.82	3.52
SD	0.033	0.043
CV (%)	1.81	1.23
n	20	20

Total Precision

	Level 1	Level 2
Mean (g/l)	1.82	3.52
SD	0.054	0.103
CV (%)	2.95	2.94
n	20	20

CORRELATION

This method (Y) was compared to another commercially available method and the following linear regression equation obtained:

$$Y = 1.0059\ X - 0.0$$

and a correlation coefficient r = 0.9992

46 unknown samples were analysed spanning the range 0.4 to 5.9 g/l.

REFERENCES

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