GLUCOSE/FRUCTOSE (GLUC/FRU)

UV METHOD
MANUAL
RX ALTONA
FOOD AND WINE

INTENDED USE

For the quantitative *in vitro* determination of Glucose/Fructose in wine, fruit juice and honey.
This product is suitable for manual use and on the RX **altona** 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
	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

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

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

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

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

SAMPLE

WINE

Turbid samples should be filtered prior to assay. Strongly coloured samples should be decolourised with 0.2 g polyvinylpolypyrrolidone (PVPP) to approximately 10 ml of juice or wine. Shake vigorously for 5 minutes and filter. The clear filtrate can then be used in the assay undiluted. Carbonic acid should be removed by filtering or stirring with a glass rod for 30 seconds.

HONEY

Transfer 5g of viscous or crystalline honey into a beaker and heat for 5 minutes at +60°C, stir occasionally throughout. Allow to cool; then accurately weigh out 1g of liquid honey and dissolve in small amount of dIH2O, transfer into a 100ml volumetric flask and dilute to the mark and mix. Honeys greater than 75g/100g (7.5g/l) should be further diluted 1 in 10 with dIH2O.

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

STABILITY AND PREPARATION OF REAGENTS FOR RX ALTONA

R1. Buffer
Contents stable up to expiry when stored at +2 to +8°C.

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

Preparation of reagents for Glucose/Fructose
Prepare enough glucose R2 for the preparation of total sugars:

R2. Glucose Reagent (GF-G)
Add **795 µl** of enzyme reagent R2 to 15 ml of buffer R1. The working reagent is stable for 3 months at +2 to +8°C.

R2. Total sugars Reagent (GLFR)
Add **285 µl** of PGI R3 **to 7.5 ml** of Glucose reagent R2 (GF-G). Stable for 3 months at +2 to +8°C. Gently swirl/invert PGI R3 reagent before pipetting.

RX ALTONA PROCEDURE

GLUCOSE

Select GLFR GLU in the Test Screen. Then select Run Calibration or Run Sample and carry out a water blank as instructed.

Pipette into a cuvette:

	S0	S1	Sample
R1 Buffer	540 µl	540 µl	540 µl
dIH2O	5 µl	-	-
Standard	-	5 µl	-
Sample	-	-	5 µl

Mix, incubate for 1 minute at +37°C.
Insert the cuvette into the RX **altona** flowcell holder when prompted for Sample Blank and press Read. Then add

R2 (GF-G)	90 µl	90 µl	90 µl

Mix, incubate for a further 5 minutes at +37°C.
Insert the cuvette into the RX **altona** flowcell holder when prompted for Sample and press Read.

TOTAL SUGARS

Select GLFR Total in the Test Screen. Then select Run Calibration or Run Sample and carry out a water blank as instructed.

Pipette into a cuvette:

	S0	S1	Sample
R1 Buffer	540 µl	540 µl	540 µl
dIH2O	5 µl	-	-
Standard	-	5 µl	-
Sample	-	-	5 µl

Mix, incubate for 1 minute at +37°C.
Insert the cuvette into the RX **altona** flowcell holder when prompted for Sample Blank and press Read. Then add

R2 (GLFR)	90 µl	90 µl	90 µl

Mix, incubate for 15 minutes at +37°C.
Insert the cuvette into the RX **altona** flowcell holder when prompted for Sample and press Read.

CALIBRATION FOR RX ALTONA

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

CALCULATION

Fructose concentration is calculated as follows:
Fructose = Total Sugars content – glucose content of samples

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 or 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 to 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 to 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)

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

CALCULATION USING A STANDARD (Fructose)

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

SPECIFIC PERFORMANCE CHARACTERISTICS

The following performance characteristics were obtained using an RX **altona** analyser in cuvette mode at +37°C.

WINE

LINEARITY

The glucose, total sugars & fructose is linear to 7.5 g/l.

SENSITIVITY

The minimum detectable concentration of glucose, total sugars & fructose with an acceptable level of precision was determined as 0.3 g/l.

GLUCOSE PRECISION

Intra assay precision

	Level 1	Level 2	Level 3
Mean (g/l)	0.859	2.189	4.174
SD	0.031	0.065	0.148
CV (%)	3.58	2.99	3.53
n	20	20	20

Inter-Assay precision

	Level 1	Level 2	Level 3
Mean (g/l)	0.817	2.116	4.000
SD	0.028	0.113	0.260
CV (%)	3.45	5.34	6.51
n	20	20	20

TOTAL SUGARS PRECISION

Intra assay precision

	Level 1	Level 2	Level 3
Mean (g/l)	5.109	5.416	6.670
SD	0.130	0.137	0.117
CV (%)	2.54	2.53	1.75
n	20	20	20

Inter-Assay precision

	Level 1	Level 2	Level 3
Mean (g/l)	5.272	5.546	7.023
SD	0.222	0.273	0.213
CV (%)	4.22	4.92	3.03
n	20	20	20

HONEY

LINEARITY

The glucose, total sugars & fructose is linear to 7.5 g/l (75g/100g).

SENSITIVITY

The minimum detectable concentration of glucose, total sugars & fructose with an acceptable level of precision was determined as 0.3 g/l (3g/100g).

GLUCOSE PRECISION

Intra assay precision

	Level 1	Level 2	Level 3
Mean (g/100g)	20.265	26.060	29.305
SD	0.549	0.624	0.602
CV (%)	2.71	2.39	2.05
n	20	20	20

Inter-Assay precision

	Level 1	Level 2	Level 3
Mean (g/100g)	20.015	25.760	28.710
SD	1.206	0.657	0.636
CV (%)	6.03	2.55	2.21
n	20	20	20

TOTAL SUGARS PRECISION

Intra assay precision

	Level 1	Level 2	Level 3
Mean (g/100g)	45.300	59.420	65.099
SD	0.868	1.000	1.345
CV (%)	1.92	1.68	2.07
n	20	20	20

Inter-Assay precision

	Level 1	Level 2	Level 3
Mean (g/100g)	44.460	58.710	63.760
SD	1.673	1.668	2.072
CV (%)	3.76	2.84	3.25
n	20	20	20

REFERENCES

1. Stein, M.W, Methods of Enzymatic Analysis (Bergmeyer, H.K., ed.) Academic Prep, New York, 1974., 1196-1201.
2. Schmidt, F.H., (1961) Die enzymatische Bestimmung von Glucose und Fructose nebeneinander, Klin. Wschr **39:** 1244-1247.
3. Bergmeyer H.U., Bernt e., Schmidt, F. & Stork, H. (1974) in Methoden der Enzymatischen Analyse (Bergmeyer, H. U., Hrsg.) Bd. 2, S. 1241-1246: Verlag Chemie, Weinheim and (1974) in Methods of Enzymatic Analysis (Bergmeyer, H.U., ed.) 2nd ed., vol. 3, pp. 1196-1201, Verlag Chemie, Weinheim, Academic Press, Inc. New York and London.
4. Bernt, E & Bergmeyer, H.U. (1974) in Methoden der Enzymatischen Analyse (Bergmeyer, H.U., Hrsg.) Bd. 2, S. 1349-1352: Verlag Chemie, Weinheim, and (1974). in Methods of Enzymatic Analysis (Bergmeyer, H.U., ed.) 2nd ., vol 3, pp. 1304-1307, Verlag Chemie, Weinheim, Academic Press, Inc New York and London.

Revised 27 Sep 16 ml
Rev. 007