Biorex food diagnostics

GLUCOSE FRUCTOSE

PRODUCT CODE: BXEFC02

QUALITY MANAGEMENT SYSTEM
ISO 13485 CERTIFIED COMPANY

BXEFC02A/B/C
R1 1x100ml / R2 1x20ml / R3 1x0.75ml **R1 2x100ml / R2 2x20ml / R3 2x0.75ml** **R1 1x50ml / R2 1x10ml / R3 1x0.38ml**
STORE AT 2-8°C
INSTRUCTIONS FOR USE
FOR USE IN THE ANALYSIS OF FOOD ONLY Not for use in diagnostic procedures

LOT Lot Number
REF Catalogue Number
Storage Temperature
Expiry Date (Year / Month)
Warning, Read Enclosed Documents
Instructions For Use
Manufactured By

GLUCOSE FRUCTOSE in honey

(LIQUID STABLE)

Kit Contents:	BXEFC02A 100 TEST	BXEFC02B 200 TEST	BXEFC02C 50 TEST
R1 Buffer (FC02-01)	1 x 100ml	2 x 100ml	1 x 50ml
R2 Enzyme Reagent (FC02-02)	1 x 20ml	2 x 20ml	1 x 10ml
R3 PGI (FC02-03)	1 x 0.75ml	2 x 0.75ml	1 x 0.38ml
R4 Standard (FC02-04)	1 x 1ml	2 x 1ml	1 x 0.5ml

Intended Use:
For quantitative determination of Glucose and Fructose in honey samples.

Summary:
While sugar is 100% sucrose, honey is composed primarily of the simple sugars Glucose and Fructose (monosaccharides) and a further 17-20% of water.

Fructose is slightly sweeter than glucose and is the predominant sugar in most honeys, making the honey taste slightly sweeter than sugar. These proportions may vary depending on the source of the nectar and differences in climatic conditions, which can affect the various properties of honey. These 2 carbohydrates are responsible for some of the key functional properties in honey, to include the ability to hold moisture, extend shelf-life, its microwave reactivity and its ability to promote colour and flavour.

In good quality honey the fructose content should generally exceed that of glucose.

Test Principle:
Glucose content in honey samples is measured enzymatically using both hexokinase and glucose - 6- phosphate dehydrogenase.

Total sugar content (glucose + fructose) is established by converting the fructose - 6- phosphate (F-6-P) to glucose-6-phosphate (G-6-P) using phosphoglucose isomerase (PGI). The G-6-P is then converted to gluconate-6-phosphate and the NADH produced is directly proportional to the amount of total sugars present.

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

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

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

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

Fructose content = Total sugars - glucose content

Reagent Concentration:

R1 Buffer	Triethanolamine Buffer pH 8.0	50 mmol/l
	$MgCl_2$	3.8 mmol/l
R2 Enzyme Reagent	NADP	1.2 mmol/l
	ATP	1.2 mmol/l
	Hexokinase	>1200 U/l
	G-6-P-DH	>2160 U/l
R3 PGI	Phosphoglucose Isomerase	approx. 490 units
R4 Standard	Glucose	500 mg/dl

Reagent Handling and Preparation:
All components are liquid stable, ready-to-use. Unopened kit components are stable at 2-8°C up to expiry. Once opened all components are stable for 28 days when stored at 2-8°C.

Sample:
Honey.
Glucose/Fructose is stable in diluted honey samples for 4 weeks 2-8 °C.

Sample Preparation:

- Take 1g of honey and add 10ml DDH_2O
- Vortex for 2 minutes to dissolve
- Take 100µl of the dissolved honey solution and add 900µl DDH_2O
- Vortex for 5 seconds
- Apply sample to Analyst 2010 (GLUC/FRUC program) following the Procedure outlined in the table below
- Dilution Factor = 100

Analyst 2010/Manual Procedure:

Wavelength	Temperature	Cuvette	Measurement
340 nm	37°C	1 cm light path	Against reagent blank

Pipette into test tubes as follows:		
	Blank	**Standard/Sample**
Buffer (R1)	1000 µl	1000 µl
DDH_2O	10 µl	---
Standard/Sample	---	10 µl
Enzyme Reagent (R2)	200 µl	200 µl
Vortex and incubate for 5 minutes at 37 °C. Determine the absorbance of sample against the reagent blank for Glucose. Then add:		
PGI	7.5 µl	7.5 µl
Vortex and incubate for 7 minutes at 37 °C. Determine the absorbance of sample for Total Sugars.		

Calculation with Standard:

$$\text{Glucose/Total Sugars conc.} = \frac{\text{Sample abs}}{\text{Standard abs}} \times \text{Standard conc.}$$

Fructose content = Total sugars - Glucose content

Calculation with Factor:
340 nm = Sample Abs x 700 = Glucose/Total sugars in mg/dl
Fructose content = Total sugars - Glucose content

Linearity:
The test is linear up to:
Glucose
93.8g/100g (938mg/dl)
Total Sugars
163.8g/100g (1638mg/dl)

Samples above this concentration should be diluted 1:3 with 0.9% saline, reassayed and the result multiplied by 3.

Sensitivity:
This method will accurately measure levels down to:
Glucose
2.3g/100g (23mg/dl)
Total Sugars
2.8g/100g (28 mg/dl)

Precision:

	Intra Assay – Within run	
Glucose conc (mg/dl)	**n**	**CV%**
100	20	3.21 %
300	20	2.34 %
500	20	1.63 %

	Intra Assay – Within run	
Total Sugars conc (mg/dl)	**n**	**CV%**
240	20	1.01 %
700	20	0.96 %
1100	20	1.01 %

	Inter Assay – Between Run	
Glucose conc (mg/dl)	**n**	**CV%**
100	20	4.35 %
300	20	2.79 %
500	20	2.46 %

	Inter Assay – Between Run	
Total Sugars conc (mg/dl)	**n**	**CV%**
240	20	4.36 %
700	20	1.68 %
1100	20	1.76 %

These characteristics were established using an Analyst 2010 analyser. Results may vary depending on the system in use.

Quality Control:
It is recommended that a laboratory uses normal and abnormal reference controls to verify the performance of the procedure, for both performance of the reagent and any instrumentation employed in the determination. Results obtained should fall within the specified ranges.

Biorex Glucose/Fructose Control Level 1 and Level 2 BXEFC04A

If results fall outside the acceptable range appropriate action as determined by the laboratory's internal quality procedures should be taken.

Some common reasons for incorrect results can be:
1. Wavelength used for the determination
2. Light source
3. Temperature
4. Cleanliness, e.g of cuvettes used in measurements
5. Bacterial contamination of reagent
6. Reagent expiry
7. Calibration frequency

Health & Safety:
This kit is designed for use by suitably qualified laboratory personnel only. Exercise the normal precautions required for the handling of laboratory reagents. Do not ingest the material. Dispose of material according to local guidelines.

References:

1. Barham D & Trinder P Analyst 1972:97:142
2. Teuscher, A & Richterich, P. Schweiz Med Wschr. 1971; 101:345 & 390
3. Tietz NW, Clinical guide to Laboratory Tests, 3rd Edition
4. Beutler, H.O (1984) in Methods of Enzymatic Analysis (Bergmeyer, H.U. ed) 3rd rd, vol. VI, pp 321-327
5. Official Methods of Analysis of the Association of Official Analytical Chemists (1990), 15th ed., vol 2, pp 741-742