COD 12800 120 mL
Only for *in vitro* use in the laboratory

D-GLUCOSE/D-FRUCTOSE

HEXOKINASE / PHOSPHOGLUCOSE ISOMERASE

INTENDED USE

Reagent for the measurement of D-glucose and D-fructose in several types of samples.

PRINCIPLE OF THE METHOD

D-fructose and D-glucose in the sample generate, by means of the reactions described below, NADPH that can be measured by spectrophotometry. The configuration of this reagent allows the determination of D-glucose/D-fructose if enzyme PGI is added or D-glucose if it is not added.

$$\text{D-fructose} + \text{ATP} \xrightarrow{\text{HK}} \text{Fructose-6-phosphate} + \text{ADP}$$

$$\text{D-glucose} + \text{ATP} \xrightarrow{\text{HK}} \text{Glucose-6-phosphate} + \text{ADP}$$

$$\text{Fructose-6-phosphate} \xrightarrow{\text{PGI}} \text{Glucose-6-phosphate}$$

$$\text{Glucose-6-phosphate} + \text{NADP}^+ \xrightarrow{\text{G6P-DH}} \text{Gluconate-6-phosphate} + \text{NADPH+H}^+$$

CONTENTS AND COMPOSITION

A1. Reagent. 2 x 40 mL. Buffer 70 mmol/L, Hexokinase >15 U/mL, NADP >1.5 mM, preservatives, pH 6.9.

A2. Reagent. 2 x 10 mL. Buffer 70 mmol/L, phosphoglucose isomerase >50 U/mL, preservatives, pH 6.9.

B. Reagent. 2 x 10 mL. Buffer 150 mmol/L, ATP >15 mmol/L, glucose-6-phosphate dehydrogenase >10 U/mL, preservatives, pH 8.9.

S. Multisugar standard: 1 x 5 mL. The assigned concentration values for components and their traceability are shown in the enclosed tables.
WARNING: H317: May cause an allergic skin reaction. P302+P352: IF ON SKIN: Wash with plenty of soap and water. P333+P313: If skin irritation or rash occurs: Get medical advice/attention.

STORAGE AND STABILITY

Store at 2-8 ºC.

Components are stable once opened until the expiry date marked in the label if they are stored well closed and care is taken to prevent contamination during their use.

Indications of deterioration: Absorbance of the blank over the limit indicated in "Test Parameters".

WARNING AND PRECAUTIONS

Exercise the normal precautions required for handling all laboratory reagents. Safety data sheet available for professional user on request. Disposal of all waste material should be in accordance with local guidelines. Any serious incident that might occur in relation to the device shall be reported to BioSystems S.A.

ADDITIONAL EQUIPMENT

Analyzer, spectrophotometer or photometer with cell holder thermostatable at 37ºC and able to read at 340 nm.

REAGENT PREPARATION

For D-glucose/D-fructose determination without diferentiation: prepare a Reagent A (RA) pouring the contents of the Reagent A2 into the Reagent A1 bottle. Mix gently. Other volumes can be prepared in the proportion: 4 mL Reagent A1 + 1 mL Reagent A2. Stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use. Reagent B provided ready to use.

For D-glucose determination: reagents are provided ready to use.

Standard (ST1): ready to use.

Standard (ST2): dilute ¼ with distilled water (1.00 g/L D-glucose + D-fructose / 0.75 g/L D-glucose).

PROCEDURE

Manual procedure (Notes 1, 2, 3 and 4)

1. Bring the Reagents and the photometer to 37ºC.
2. Pipette into a cuvette:

	D-glucose/D-fructose		D-glucose	
	Reagent Blank (RB)	Standard / Sample	Reagent Blank (RB)	Standard / Sample
Standard / Sample (ST1/ST2)	-	10 µL/32 µL	-	10 µL/32 µL
Distilled water (ST1/ST2)	10 µL/32 µL	-	10 µL/32 µL	-
Reagent A (A1+A2)	800 µL	800 µL	-	-
Reagent A1	-	-	800 µL	800 µL

3. Mix, incubate for 1 minute at room temperature (16-25ºC) or at 37°C, read absorbance (A1) at 340 nm.
4. Pipette into the cuvette:

Reagent B	160 µL	160 µL	200 µL	200 µL

5. Mix and incubate for 15 minutes at room temperature (16-25ºC) or for 10 minutes at 37ºC. Measure the absorbance (A2) of the Standard and Sample at 340 nm. The color is stable at least 30 minutes.
6. Calculate the concentration in the sample (C) using the following formula:

$$\frac{(A2 - 0.84 \times A1)_{Sample} - (A2 - 0.84 \times A1)_{RB}}{(A2 - 0.84 \times A1)_{Standard} - (A2 - 0.84 \times A1)_{RB}} \times C_{Standard\ (D\text{-}gluc/D\text{-}fruc)}\ [g/L] = C_{Sample\ (D\text{-}gluc/D\text{-}fruc)}\ [g/L]$$

$$\frac{(A2 - 0.80 \times A1)_{Sample} - (A2 - 0.80 \times A1)_{RB}}{(A2 - 0.80 \times A1)_{Standard} - (A2 - 0.80 \times A1)_{RB}} \times C_{Standard\ (D\text{-}glucose)}\ [g/L] = C_{Sample\ (D\text{-}glucose)}\ [g/L]$$

CALIBRATION

A reagent blank should be done every day and a calibration after reagent lot change or as required by quality control procedures.

QUALITY CONTROL

Each laboratory should establish its own internal quality control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

METROLOGICAL CHARACTERISTICS[1-2]

The metrological characteristics described below have been obtained using a Y15 analyzer. Details on evaluation data are available on request.

– Detection limit: D-glucose: 0.03 g/L; D-glucose/D-fructose: 0.02 g/L (ST1) / D-glucose: 0.003 g/L; D-glucose/D-fructose: 0.002 g/L (ST2).

– Linearity limit: D-glucose: 8.00 g/L; D-glucose/D-fructose: 8.00 g/L (ST1) / D-glucose: 2.40 g/L; D-glucose/D-fructose: 2.40 g/L (ST2).

– Precision:

Mean concentration D-glucose (ST1)	Repeatability (CV)	Within-laboratory (CV)
0.51 g/L	2.1 %	3.4 %
2.02 g/L	1.0 %	2.8 %

Mean concentration D-glucose/D-fructose (ST1)	Repeatability (CV)	Within-laboratory (CV)
1.04 g/L	1.4 %	2.4 %
4.10 g/L	0.7 %	2.8 %

Mean concentration D-glucose (ST2)	Repeatability (CV)	Within-laboratory (CV)
0.22 g/L	0.5 %	1.7 %
0.43 g/L	0.4 %	1.5 %

Mean concentration D-glucose/D-fructose (ST2)	Repeatability (CV)	Within-laboratory (CV)
0.23 g/L	0.4 %	0.6 %
0.45 g/L	0.4 %	0.7 %

– Trueness: Results obtained with this procedure did not show systematic differences when compared with a reference procedure. Details of the comparison experiments are available on request.

NOTES

1. Volumes proposed are to use a semi-micro cuvette. Other volumes can be used if the ratio between the reagents and sample is maintained.
2. Although calibration of each measurement series using the standard is recommended, the concentration in the sample (C) can also be calculated using the following factor:
$[(A2 - 0.84 \times A1)_{Sample} - (A2 - 0.84 \times A1)_{RB}] \times F = C_{Sample\ (D\text{-}gluc/D\text{-}fruc)}\ [g/L]$ (F = 2.77 (ST1) / 0.89 (ST2))
$[(A2 - 0.80 \times A1)_{Sample} - (A2 - 0.80 \times A1)_{RB}] \times F = C_{Sample\ (D\text{-}glucose)}\ [g/L]$ (F = 2.89 (ST1) / 0.92 (ST2))
3. The procedure and test parameters may vary depending on the sample type (Sample Type: ST).
4. When analyzing solid and semi-solid samples which are weighed out for sample preparation, the content (g/100 g) (CT) is calculated from the amount of sample weighed (W), the volume in which weighed sample is prepared (V), the concentration obtained in the sample(C) and the dilution factor (df) if necessary, as follows:

$$\frac{C_{Sample}\ (g/L) \times V\ (L)}{W_{Sample}\ (g)} \times 100 \times df = CT_{\ Sample}\ [g/100\ g]$$

BIBLIOGRAPHY

1. Association of Official Analytical Chemists (AOAC): Guidelines for standard method performance requirements, 2016.
2. International organization of vine and wine (OIV), Compendium of international methods of wine and must analysis Vol. 1 & 2, 2016.
3. International Fruit and Vegetable Juice Association. Methods of analysis (https://ifu-fruitjuice.com/).
4. Zoecklein BW, Fugelsang KC, Gump BH, Nury FS, Wine analysis and production. Van Nostrand Reinhold; 1 edition (December 31, 1990).

5. EBC Analysis Comitee. Analytica-EBC. Verlag Hans Carl; 7th edition (2010).
6. A.O.A.C 17th Ed., 2000 Official method 920.180 Honey (liquid, strained or comb), preparation of test sample.

SAMPLES

Preparation procedures

- Filter or centrifuge turbid solutions.
- Degas samples containing carbon dioxide in an ultrasonic bath or stir the sample in a beaker for approximately 1 minute.
- Decolorate strongly colored samples with polyvinylpolypyrrolidone (PVPP) (e.g. 1 g PVPP/100 mL sample), stir for 1 minute, and filter or centrifugue to eliminate PVPP.
- Dilute accordingly with distilled water samples with concentration over the specified linearity limit. Multiply obtained concentration by the dilution factor.
- Crush and/or homogenise solid samples.
- Clarify and/or deproteinise with Carrez reagent (BioSystems ref. 12837) by adding 5 mL Carrez-I and 5 mL Carrez-II to the sample liquid extracts in a 100 mL volumetric flask. Adjust to pH 7.5-8.5 with sodium hydroxide. Mix after each solution and fill the volumetric flask to the mark and filter.
- Extract samples containing fat with hot water at a temperature above the melting point of the fat. Let stand to room temperature and fill the volumetric flask to the mark with distilled water. Store on ice or in a refrigerator for 15-30 minutes and then filter. Use the clear or slightly opalescent supernatant for assay. Alternatively, clarify with Carrez Reagent.

Fruit juices and similar beverages[3] (ST1): Filter, clarify, decolorate and/or dilute juice if necessary as described in "Preparation procedures".

The recommended dilution of the sample is 1/10 for D-glucose and 1/20 for D-glucose/D-fructose, though this can be modified according to the needs of the user. Include the dilution factor (df) of the calculation formula if the manual procedure is used, or the field "predilution factor" if the automated procedure is used. Modification of predilution will affect the metrological characteristics.

Red and white wine[2,4] (ST1): Filter, clarify, decolorate and/or dilute wine if necessary as described in "Preparation procedures".

It is recommended to use the Wine Control (Red) (BioSystems ref. 12822) and Wine Control (White) (BioSystems ref. 12821) to verify the performance of the measurement procedure.

Beer[5] (ST1): Degas as described in "Preparation procedures".

Preserves and other vegetable and fruit products (ST1): Accurately weigh approximately 0.5 g of the homogeneous sample into a 100 mL volumetric flask, fill up to the mark with water, mix, and filter. Dilute sample if necessary.

Desserts and ice-cream (ST1): Accurately weigh approximately 3 g of sample into a 100 mL volumetric flask, add about 60 mL water and incubate for 15 min at approximately 70°C; shake from time to time. Let stand to room temperature, fill up to the mark with water, mix and filter. Clarify and/or dilute sample if necessary as described in "Preparation procedures".

Solid foodstuffs (ST1): Grind solid foodstuffs into a fine homogeneous compound. Weigh out a representative sample, extract with water (heated to 60°C, if necessary) and filter. Clarify and/or dilute sample if necessary as described in "Preparation procedures".

Honey[6] (ST1): For viscous or crystalline sample, transfer 5-10 g of honey to a beaker and heat for 5 min at approximately 60°C (there is no need to heat liquid honey), stirring with the spatula. Allow to cool. Weigh accurately 3 g of the liquid sample and dissolve with 60 mL of water in a 100 mL volumetric flask. Then fill up to the mark and mix. Prepare a 1/10 dilution of the 3% honey solution.

Fermentation samples and cell culture media (ST1): Centrifuge the sample at 5000 g for 5 min, filter the supernatant and use the filtrate, diluted if necessary, for the assay. It is recommended to stop enzymatic reactions by placing the sample in a water-bath at 80ºC for 15 minutes. Alternatively, deproteinise sample as described in "Preparation procedures".

Raw potatoes (ST2): Accurately weigh approximately 15 g of homogenized sample and mix with 30 mL of distilled water. Grind until the sample is suspended homogeneously. Filter and fill up to the mark in a 100 mL volumetric flask with distilled water.

Further applications: The method may also be used with other types of samples. Contact your supplier for more information.

TEST PARAMETERS (Notes 3 and 4)

These reagents may be used in several automatic analysers. Specific instructions for application in many of them are available on request.

BioSystems Y15

Reagent 1: Use Reagent A (A1+ A2) for D-glucose/D-fructose analysis or Reagent A1 for D-glucose analysis.
Reagent 2: Use Reagent B.

GENERAL		
Test name	**GLUCOSE-FRUCTOSE**	**GLUCOSE**
Analysis mode	differential bireagent.	differential bireagent
Sample type	ST1 / ST2	ST1 / ST2
Units	g/L	g/L
Reaction type	increasing	increasing
Decimals	2	2
PROCEDURE		
Reading	monochromatic	monochromatic
Sample	3 / 10	3 / 10
Reagent 1	250	240
Reagent 2	50	60
Washing	1.2	1.2
Predilution factor	*	*
Main filter	340	340
Reference filter	-	-
Reading 1	72 s	72 s
Reading 2	600 s	600 s
Reagent 2	96 s	96 s
CALIBRATION		
Calibration type	multiple / specific	multiple / specific
Calibration curve	-	-
OPTIONS		
Blank absorbance limit	0.300	0.300
Kinetic blank limit	-	-
Linearity limit	8.00 / 2.40	8.00 / 2.40

BioSystems Y25

Reagent 1: Use Reagent A (A1+ A2) for D-glucose/D-fructose analysis or Reagent A1 for D-glucose analysis.
Reagent 2: Use Reagent B.

GENERAL		
Test name	**GLUCOSE-FRUCTOSE**	**GLUCOSE**
Analysis mode	differential bireagent.	differential bireagent
Sample type	ST1 / ST2	ST1 / ST2
Units	g/L	g/L
Reaction type	increasing	increasing
Decimals	2	2
PROCEDURE		
Reading	monochromatic	monochromatic
Sample	3 / 10	3 / 10
Reagent 1	250	240
Reagent 2	50	60
Washing	1.2	1.2
Predilution factor	-	-
Main filter	340	340
Reference filter	-	-
Reading 1	75 s	75 s
Reading 2	600 s	600 s
Reagent 2	90 s	90 s
CALIBRATION		
Calibration type	multiple / specific	multiple / specific
Calibration curve	-	-
OPTIONS		
Blank absorbance limit	0.300	0.300
Kinetic blank limit	-	-
Linearity limit	8.00 / 2.40	8.00 / 2.40

BioSystems S.A. Costa Brava, 30. 08030 Barcelona (Spain)
www.biosystems.global