

GLUCOSE - FRUCTOSE

UV DETERMINATION IN BIOLOGICAL FLUIDS

Kit: 5 x 20 ml
Code GF8815

SUMMARY

Fructose and Glucose are monosaccharides. When happens a deficiency or an absence of enzyme(s) that participates to carbohydrate metabolism, may have an accumulation of these sugars, who overflow into the urine.

PRINCIPLE

In the presence of ATP, NADP and G6PDH (glucose-6P-dehydrogenase) and HK (hexokinase) **the glucose** produces NADPH. The intensity of the UV-colour at this wavelength is proportional to the conc. of **GLUCOSE** in the sample. The HK is able to phosphorylate also Fructose.

Adding PGI (phosphogluc. isomerase) new NADPH is produced from the changes of fructose-6P in glucose-6P. The new increase of the UV-colour at this wavelength is proport. to the conc. of **FRUCTOSE** in the sample.

REAGENTS

Components of the kit:	Code GF8815
*REAGENT 1 (buffer, liquid, ready to use)	3 x 70 ml
*REAGENT 2 (lyo)	5 x 20 ml
*REAGENT 3 (starter 3, suspension, ready to use)	1 x 2.5 ml
*REAGENT 4 (starter 4, suspension, ready to use)	1 x 2.5 ml
Good buffer	> 20 mmol/L
NADP	> 0.2 mmol/L
ATP	> 2 mmol/L
HK	> 10 U/L
G6PDH	> 5 U/L
PGI	> 50 U/L

STABILITY: the reagents, at 2-8°C, are stable up to the expiry date shown on the package **if not contaminated during handling.**

AUXILIARY REAGENTS FOR QUALITY CONTROL (Not supplied with this kit)

To assure proper test performance, we suggest following kits:
 - Elevated Control SUBSTRATE (lyo) 6 x 2 mL Cod. SCE3005
that contains a few substrates in different ranges (see the inserts).

PREPARATION OF THE WORKING REAGENT

Dissolve a vial of *Reagent 2 with 20 ml of *Reagent 1 and mix gently till dissolution. Please avoid foaming. A suggestion could be to aliquote in vials the quantity for each stage of analysis; to put the need for the day at 2-8°C for use, to freeze the remaining vials for next stages.

Let the reagents reach the working temperature before use.
Close immediately after handling. Incompetent handling will release us from any responsibility.

STABILITY: the diluted *Reagent 2 is stable 8 days at 2-8°C.
 Till 40 days freezed at -20°C. FREEZE only ONE TIME.
DO NOT REPEAT FREEZING.

SAMPLE

- Please use limpid sample, at a NEUTRAL pH.
- If necessary, filter the sample.
- We strong suggest to neutralize the Acidic Samples at a neutral pH (max pH ≤ 8,0).

PROCEDURE

- Wavelength: 340 nm (334-365 nm)
- Pathlength: 1 cm
- Reading: against air or distilled water
- Temperature: 37°C
- Method: end-point
- Reaction: 8 - 18 minutes
- Linearity: 30 - 1000 mg/L at 37°C as (Glucose + Fructose)
- Sample/reagents: 1/40/1/1

Let reagents reach the working temperature before using.

Pipette in a test tube or cuvette so labelled:

R/B: Reagent Blank, S: Sample:

	R/B	S
*Working reagent	1000 µl	1000 µl
Distilled water	25 µl	---
Sample	---	25 µl

Mix and incubate for about 3 minutes at 37°C. Measure the absorbance AS0 and AR/B0. For the GLUCOSE then add:

*Reagent 3 suspension	25 µl	25 µl
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Mix and wait the end of the react. (5-10 min.). Read AS1 and AR/B1. For the FRUCTOSE start the reactions with:

*Reagent 4 suspension	25 µl	25 µl
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Mix carefully and wait the end of the reaction (5-15 min.).
 Read AS2 and AR/B2.

Calculate for the Sample Glucose

$$ASG = (AS1 - AS0);$$

calculate for the Reagent/Blank Glucose

$$AR/BG = (AR/B1 - AR/B0).$$

Calc. the difference for GLUCOSE $\Delta AG = ASG - AR/BG$.

Calculate for the Sample Fructose

$$ASF = (AS2 - AS1);$$

calculate for the Reagent/Blank Fructose

$$AR/BF = (AR/B2 - AR/B1).$$

Calc. the difference for FRUCTOSE $\Delta AF = ASF - AR/BF$.

CALCULATION FOR GLUCOSE

Use this general formula to calculate the concentration:

$$\text{Glucose conc. (g/L)} = V/v \times 1/\epsilon d \times MW/1000 \times \Delta AG$$

V = total test volume = 1.050 ml

v = sample volume = 0.025 ml

d = pathlength = 1 cm

ϵ = molar coeff. NADH = 6.3 L / mmol x cm

MW = glucose MW = 180.16

so it becomes:

$$\text{Glucose conc. (g/L)} = 1.201 \times \Delta AG$$

CALCULATION FOR FRUCTOSE

Use this general formula to calculate the concentration:

$$\text{Fructose conc. (g/L)} = V/v \times 1/\epsilon d \times MW/1000 \times \Delta AF$$

V = total test volume = 1.075 ml

v = sample volume = 0.025 ml

d = pathlength = 1 cm

ϵ = molar coeff. NADH = 6.3 L / mmol x cm

MW = fructose MW = 180.16

so it becomes:

$$\text{Fructose conc. (g/L)} = 1.230 \times \Delta AF$$

REFERENCE VALUES

Fructose

Serum	10 - 60 mg/L
Urine	<30 - 65 mg/24h
Seminal fluid	> 1500 mg/L

Glucose

Serum (adult)	740 - 1060 mg/L
Urine	< 500 mg/24h
CSF (cerebro spinal fluid) (adult)	400 - 700 mg/L

It is suitable that every laboratory determine its reference values.

PERFORMANCE CHARACTERISTICS

These performance characteristics was determined using a spectrophotometer or analyzers typically found in clinical laboratories, under the stated assay conditions.

Linearity: The Glucose- Fructose Reagent is linear up to 1000 mg/L (as 500 Fructose+500 Glucose or 1000 Fructose alone).
For concentrations ≥ 1000 mg/L, dilute the sample 1:4 with distilled water., repeat the determ. and multiply the result $\times 4$.

Sensitivity: The minimum detectable is 10 mg/L.

FRUCTOSE

Within-run Precision:

	Mean (mg/L) \pm 2s	CV %
Serum 1	44 \pm 0,36	1,8
Serum 2	472 \pm 3,3	0,8

Run-to-run (Day-to-day) Precision:

	Mean (mg/L) \pm 2s	CV %
Serum 1	45 \pm 0,76	2,3
Serum 2	472 \pm 3,3	0,9

GLUCOSE

Within-run Precision:

	Mean (mg/L) \pm 2s	CV %
Serum 1	44 \pm 0,4	2,1
Serum 2	425 \pm 3,6	0,6

Run-to-run (Day-to-day) Precision:

	Mean (mg/L) \pm 2s	CV %
Serum 1	46 \pm 0,6	2,5
Serum 2	426 \pm 4,2	0,8

Interferences: See References point 2.

FRUCTOSE

Correlation: A group of 20 samples from 20 to 450 mg/L was assayed by this procedure and using a similar commercially available Fructose Reag. Comparison of the data gave following results:

Linear regression equation $y = 1,004x - 0,3$
Correlation coefficient $r = 0,9958$

GLUCOSE

Correlation: A group of 20 samples from 20 to 500 mg/L was assayed by this procedure and using a similar commercially available Glucose Reag. Comparison of the data gave following results:

Linear regression equation $y = 1,0024x + 0,4$
Correlation coefficient $r = 0,9978$

NOTE

- 1.A proportional. variation of the reaction volumes does not change the result.
2. We suggest do not mix Reagents from different Production lots.
3. For concentrations higher than 1000 mg/L (500 glucose + 500 fructose) dilute the sample 1:4 with distilled water, repeat the determination and multiply the result by 4.
4. Very deep attention must be given to interfering substances: certain drugs and other substances are able to influence levels of Fructose and Glucose (see References 2.).
5. PAY ATTENTION!
Applications on routine Analyzers may be totally different from what we developed as manual determination, and also from themselves.
6. The reagent must be used only for the intended destinations. by expert people and in the due lab. conditions.
7. The clinical diagnosis cannot be done using the result of only one test, but have to be done integrating different lab. and clinical data.
8. For proteic samples may be required deproteinization (spermatic liquid for instance):
 - add perchloric acid (0,33N) 2,000 mL to the sample 0,200 mL
 - mix and centrifuge 5 min \times 5000 rpm
 - keep 1 mL of the supernatant
 - neutralize with 1 mL sodium hydroxide (NaOH 0,3 N)
 - use directly this solution as sample.
- REM that the Dilution Factor will be $F = 22$; keep in mind for the Calculation
- if it will be need further dilutions, please return to Note 3).
9. The *Reagent 1 is supplied in surplus.

REFERENCES

1. Textbook of Clinical Chemistry, Ed. by N.W. Tietz, W.B. Saunders Co., Philadelphia (1999).
2. Young D.S. et al., Clin. Chem. 21, 302D (1975).

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