Glucose

CYPRESS DIAGNOSTICS

CE

IVD

Enzymatic. Colorimetric
GOD-POD
Liquid

Store at 2 - 8 °C

Configuration

REF	HBL04	HBL04A	HBL04M
VOL	2 x 125 mL	8 x 125 mL	8 x 30 mL
Reagent	2 x 125 mL	8 x 125 mL	8 x 30 mL
Standard	1 x 5 mL	4 x 5 mL	-

Intended use

The Cypress Diagnostics kit Glucose is an *in vitro* diagnostic medical device intended to be used for the quantitative measurement of glucose in human fluoride plasma, free of hemolysis and turbidity. The device is not automated. The measurement of glucose is intended to be used for screening for and diagnosis of (pre)diabetes, the diagnosis of hypoglycemia and the aid in diagnosis of non-diabetic hyperglycemia. This kit is intended to be used by healthcare professionals in a laboratory-based testing environment.
For *in vitro* diagnostic use only. For professional use only.

Clinical significance

Glucose is the major carbohydrate present in the peripheral blood. The oxidation of glucose is the major source of cellular energy in the body. Glucose determinations are run primarily to aid in the diagnosis and treatment of diabetes mellitus. Elevated glucose levels may be associated with pancreatitis, pituitary or thyroid dysfunction, renal failure and liver disease, whereas low glucose levels may be associated with insulinoma, hypopituitarism, neoplasms, or insulin-induced-hypoglycemia.
Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

Principle

In the presence of glucose oxidase (GOD), β-D-glucose is oxidized to gluconic acid and hydrogen peroxide (H_2O_2). As glucose exists in both α- and β-forms in solution, a complete conversion of glucose therefore requires mutarotation of α-D-glucose to β-D-glucose. The latter reaction is accelerated in the presence of the enzyme mutarotase (MRO). After glucose oxidation, the formed hydrogen peroxide is measured through the oxidative coupling of 4-aminoantipyrine (4-AAP) to 4-chlorophenol (4-CP) in the presence of peroxidase (POD), yielding a red quinoneimine dye.

$$\alpha\text{-D-Glucose} \xrightarrow{MRO} \beta\text{-D-Glucose}$$

$$\beta\text{-D-Glucose} + O_2 + H_2O \xrightarrow{GOD} \text{Gluconic acid} + H_2O_2$$

$$H_2O_2 + \text{4-CP} + \text{4-AAP} \xrightarrow{POD} \text{Quinoneimine} + HCl + 2H_2O$$

The intensity of the color formed is proportional to the glucose concentration in the sample.

Reagent composition

Reagent	Phosphate buffer pH 7,4 (14 mmol/L) 4-Chlorophenol (4-CP) (7,3 mmol/L) 4-Aminoantipyrine (4-AAP) (0,3 mmol/L) Mutarotase (MRO) (25 U/L) Glucose oxidase (GOD) (11500 U/L) Peroxidase (POD) (750 U/L) *BSA (2 g/L)
Standard	Glucose aqueous (see value on label) *BSA (25 g/L)

**This is a material of animal origin, but the risk of this material was assessed as non-hazardous and non-critical in the Risk Analysis*

Precautions

- All body fluid samples should be considered potentially infectious materials and the appropriate precautions should be taken. Wear personal protective equipment such as gloves, safety glasses, lab coats or aprons when working with possible biohazard contaminants.
- Use Good Laboratory Practices (GLP) when handling this product.
- Please refer to the MSDS, available on our website, for further information.

Traceability

Traceable to certified reference material LNE CRM Bio 101a.
Reference method: ID-GC-MS.

Preparation

Reagent and standard are ready to use.

Storage, stability and disposal

All the components of the kit are stable up to the date of expiration as specified on the label, when stored tightly closed, protected from light and contaminations prevented during their use. Storage temperature for this kit is 2 - 8 °C.

Handle standards very carefully to prevent contamination. The reagent should be a clear solution. If turbidity or precipitation has occurred or if blank absorbance at 510 nm ≥ 0,32, the reagent should be discarded.
Do not use the product if deterioration or contamination is suspected or beyond the expiration date or open container stability period. Dispose unused or deteriorated product and waste in compliance with local regulations.

Additional material required but not provided

- Spectrophotometer or colorimeter. Minimum analyzer specifications:
  Measuring at 510 nm (490-550)
  Linear measuring range: 0 - 2 AU
- Cuvettes, matching the analyzer used (1,0 cm light path)
- General laboratory equipment

Samples

Sample type: human fluoride plasma, free of hemolysis and turbidity
Plasma should be isolated in blood tubes containing sodium fluoride (NaF) to inhibit glycolysis. In fluoride plasma, the glucose concentration is stable for up to 3 days at room temperature. For fasting glucose determination, fasting for at least 12 hours is recommended before sample collection.

Procedure

Make sure the reagents and samples are at room temperature.
1. Wavelength 510 nm (490-550); Temperature 37 °C/15-25 °C; Cuvette (1 cm light path).
2. Adjust the instrument to zero with distilled water.
3. Pipette into a cuvette:

For Blank	1,00 mL Reagent
For Standard [Note 4]	10 µL Standard + 1,00 mL Reagent
For Sample	10 µL Sample + 1,00 mL Reagent
Mix, incubate 10 min at 37 °C or 15 min at room temperature (15 - 25 °C). Read the absorbance (A) of sample and standard against blank. The color is stable for at least 40 min.	

Calculation

$$\text{Glucose (mg/dL)} = \frac{A_{Sample} - A_{Blank}}{A_{Standard} - A_{Blank}} \times stand.conc.\,(mg/dL)$$

Conversion Factor: mmol/L = 0,0555 x mg/dL

Quality control

Control sera are recommended for the qualification of the reagents and to monitor the performance of assay procedures. Next to legal requirements, we recommend to perform Quality Control on a daily basis and after each calibration.
Use Biochemistry Normal and Pathological Controls (HBC01, HBC02). If other controls (not manufactured by Cypress) are used, they have to be validated by the user as they can vary. Prepare and measure these controls the same as samples. Measure at least one replicate per control.
If control values are found outside the defined range, check the instrument, reagents and calibrator for problems. Do not continue testing as the results will be invalid. Each laboratory should establish its own QC scheme and corrective actions if controls do not meet the acceptable tolerances.

Reference values[6]

Fasting Plasma - Child	60 - 100 mg/dL (3,3 - 5,6 mmol/L)
Fasting Plasma - Adult (< 60y)	74 - 100 mg/dL (4,1 - 5,6 mmol/L)
Decision Limit - Diabetes	≥ 126 mg/dL (≥ 7,0 mmol/L)
Newborn 1 day	40 - 60 mg/dL (2,2 - 3,3 mmol/L)
Newborn > 1 day	50 - 80 mg/dL (2,8 - 4,4 mmol/L)

These values are for orientation purpose. Each laboratory should establish its own reference range.

Performance characteristics

Measuring range: from 14,1 mg/dL (limit of quantitation) to 420 mg/dL (linearity limit). If the obtained results are greater than 420 mg/dL, dilute the sample 1:2 with saline solution, repeat the determination, and multiply the result by factor 2.

Precision:
Precision studies were performed on 5 plasma samples with specified concentrations ranging from 40,8 to 204 mg/dL. The table below shows the results at glucose concentrations of 60,8, 130 and 204 mg/dL.

	Repeatability (n=80)			Within-laboratory (n=80)		
Mean (mg/dL)	60,8	130	204	60,8	130	204
SD	0,75	1,68	6,37	2,30	4,62	10,4
CV (%)	1,24	1,29	3,12	3,79	3,54	5,08

Sensitivity: 1 mg/dL = 0,0054 AU

Accuracy: Results obtained using CYPRESS DIAGNOSTICS reagents did not show systematic differences when compared with other commercial reagents.

Cypress Diagnostics: Nijverheidsstraat 8 • 2235 Hulshout • Belgium
www.diagnostics.be • Tel: ++ 32 15 67 67 68 • e-mail: cypress@diagnostics.be

The results of the performance characteristics depend on the analyzer used.

Interferences

Endogenous interferences: **No interference up to: 711 mg/dL hemoglobin, 20 mg/dL bilirubin and 70 mg/dL lipids.**
All results of the analytical specificity study met the acceptance criteria, except for lipid interference at a glucose level of 40 mg/dL. The latter interference effect is most likely caused by turbidity of the sample. Therefore, a note to avoid lipemic/turbid samples was added to the limitations of the test.
The following endogenous substances may potentially interfere: creatinine, uric acid (source: clinfx website AACC/Wiley).[3]

Exogenous interferences: **The following exogenous substances may potentially interfere: acetaminophen, acetylsalicylic acid, amidotrizoic acid, amikacin, aminopyrine, ascorbic acid, chlorpropamide, cysteine, cystine, dextran, diclofenac, gentisic acid, guanoclor, hydralazine, iproniazid, iron dextran, iron sorbitex, isocarboxazid, isoniazid, lactose, levodopa, methyldopa, nitrazepam, novaminsulfon, oxyphenbutazone, p-aminophenol, phenazopyridine, phenformin, tetracycline, tolazamide, tolbutamide, or trypan blue (source: clinfx website AACC/Wiley).[3]**

Limitations

- **Turbid samples can give rise to elevated absorbance values and should be avoided.**

Notes

1. **For best use of this kit on a Cypress Diagnostics analyzer, we kindly advise to follow the application sheets of the respective analyzer. Please log in to our website (www.diagnostics.be) as a registered user to download the latest application sheets, which are located under the section of the corresponding analyzer. Compatible Cypress analyzers: CYANSmart, CYANStart, CYANExpert 130, CYANVision.**
2. **In case other instruments (not manufactured by Cypress Diagnostics) are used, the laboratory is responsible to validate the reagents in this kit on those analyzers before testing patient samples.**
3. **For this kit, application sheets for the following Mindray analyzers are available (see website): BS-120, BS-200, BS-200E.**
4. **Calibration with the aqueous standard may cause a systematic error in automatic procedures. In this case, it is recommended to use a serum based Biochemistry calibrator (HBC03).**

Bibliography

1. **Kaplan L.A. Glucose. Kaplan A et al. Clin Chem The C.V. Mosby CO. St.Louis. Toronto. Princeton. 1984; 1032-1036**
2. **Trinder P. Ann. Clin.Biochem. 1969, 6: 24-33.**
3. **https://clinfx.wiley.com/aaccweb/aacc/ (accessed February 14, 2022)**
4. **Burtis A et al. Tietz Textbook of Clinical Chemistry, 3rd ed. AACC 1999**
5. **Tietz N W et al. Clinical Guide to Laboratory tests, 3rd ed. AACC 1995**
6. **Rifal N et al. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 6th ed AACC 2018**
7. **Larson D Clinical Chemistry: Fundamentals and Laboratory Techniques, Elsevier 2017**

Notice: Any serious incident that has occurred in relation to the device shall be reported to Cypress Diagnostics and the competent authority of the Member State in which the user and/or patient is established.

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Cypress Diagnostics: Nijverheidsstraat 8 • 2235 Hulshout • Belgium
www.diagnostics.be • Tel: ++ 32 15 67 67 68 • e-mail: cypress@diagnostics.be