CO-F037 Glucose (GLU) Fluorimetric Assay Kit

Method: Fluorimetric method

Specification: 96T (Can detect 80 samples without duplication)

Measuring instrument: Fluorescence Microplate Reader

Sensitivity: 0.1 μmol/L

Detection range: 0.1-20 μmol/L

General information

▲Intended use

This kit can be used to measure glucose (Glu) content in serum, plasma, urine, saliva, milk and cell samples.

▲Background

Glucose is a monosaccharide with the molecular formula $C_6H_{12}O_6$. Glucose can be produced by breaking down glycogen or be synthesized in the liver and kidneys through gluconeogenesis. As a blood sugar, glucose circulates in the blood of animals. glucose is the main bioenergy substance in the body, which is used to produce energy. Blood sugar balance is achieved through a complex interaction between several organs and hormones.

▲Detection principle

Glucose oxidase can catalyze the oxidation of glucose into gluconic acid and produce hydrogen peroxide. In the presence of peroxidase, hydrogen peroxide reacts with the non-fluorescent substance to form fluorescent substance. The glucose content can be calculated indirectly by measuring the fluorescence intensity at the excitation wavelength of 535 nm and the emission wavelength of 590 nm.

Kit components & Storage

Item	Component	Specification	Storage
Reagent 1	Buffer Solution	50 mL × 2 vials	-20℃ , 6 months
Reagent 2	Enzyme Reagent	Powder × 1 vial	-20℃ , 6 months, shading light
Reagent 3	Chromogenic Agent	0.25 mL × 1 vial	-20℃ , 6 months, shading light
Reagent 4	5 mmol/L Standard	0.5 mL × 1 vial	-20℃ , 6 months
	Black Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other.

▲Materials prepared by users

Instruments

Fluorescence microplate reader (Ex/Em=535 nm/590 nm), Micropipettor, Incubator, Vortex mixer, Water bath, Centrifuge

Consumptive material

Tips (10 μL, 200 μL, 1000 μL), EP tubes (1.5 mL, 2 mL)

Reagents

Double distilled water

⚠Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

⚠Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

▲The key points of the assay

Avoid repeated freezing and thawing of reagent 2 working solution, it is recommended to aliquot the reagent 2 working solution into smaller quantities and store at -20℃ .

Pre-assay preparation

▲Reagent preparation

1. The preparation of reagent 2 working solution:
Dissolve a vial of reagent 2 with 250 μL of reagent 1 and mix fully. The unused solution can be stored at -20℃ for 1 month with shading light.
2. The preparation of chromogenic agent working solution:
Mix the reagent 1, reagent 2 working solution and reagent 3 at a ratio of 23:1:1. Prepare the fresh solution before use and stored with shading light.
3. The preparation of 50 μmol/L glucose standard:
Mix the reagent 4 and reagent 1 at a ratio of 1:99. Prepare the fresh solution before use. The unused solution can be stored at 2-8℃ for 7 days.

▲Sample preparation

1. Serum (Plasma):

Detect directly. Serum or plasma should be isolated from the sample tube as soon as possible without hemolysis. Serum or plasma can be stored at 2~8℃ for 24h.

2. Urine:

Collect the fresh urine and centrifuge the sample at 10000 g for 15 min at 4℃ . Take the supernatant for detection.

3. Milk sample:

Collect the milk sample, centrifuge at 10000 g for 10 min and collect middle layer liquid for measurement.

4. Saliva:

Collect the fresh saliva and centrifuge the sample at 10000 g for 15 min at 4℃ . Take the supernatant for detection.

5. Cells sample:

Collect the cells and wash the cells with PBS (0.01 M, pH 7.4) for 1~2 times. Centrifuge at 1000 g for 10 min and then discard the supernatant and keep the cell sediment. Add homogenization medium at a ratio of cell number (2×10^6): reagent 1 (μL) =1: 100. Sonicate or grind with hand-operated in ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant for detection. Meanwhile, determine the protein concentration of supernatant with BCA Assay kit.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (0.1-20 μmol/L).

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
Human plasma	300-600
Human serum	300-600
Chicken serum	600-1000
Human urine	1
Human milk	400-600
Saliva	3-5

Note: The diluent is reagent 1.

Assay protocol	
Ambient temperature	25-30℃
Optimum detection wavelength	Ex/Em=535 nm/590 nm

Instructions for the use of transferpettor:

(1) Equilibrate the pipette tip in that reagent before pipetting each reagent.

(2) Don't add the liquid outside the tips into the reaction system when pipetting each reagent.

Assay protocol

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	B	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	C	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	D	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	E	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	F	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	G	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	H	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

[Note]: A-H, standard wells; S1-S80, sample wells.

▲Operating steps

1. The preparation of standard curve
Dilute 50 μmo/L glucose standard with reagent 1 to a serial concentration. The recommended dilution gradient is as follows: 0, 2, 4, 6, 8, 10, 15, 20 μmol/L.
2. The measurement of samples
1) Standard well: add 50 μL of standards with different concentrations into the wells of 96 well microplate.
Sample well: add 50 μL of sample into the wells of 96 well microplate.
2) Add 50 μL of chromogenic agent working solution and mix fully.
3) Mix fully with microplate reader for 10 s and incubate at 37℃ for 15 min with shading light.
4) Measure the fluorescence intensity of each well at the excitation wavelength of 535 nm and the emission wavelength of 590 nm.

▲Operation table

	Standard well	Sample well
Standards with different concentrations (μL)	50	
Sample (μL)		50
Chromogenic agent working solution (μL)	50	50
Mix fully with microplate reader for 10 s and incubate at 37℃ for 15 min with shading light. Measure the fluorescence intensity of each well at the excitation wavelength of 535 nm and the emission wavelength of 590 nm.		

▲Calculation

Plot the standard curve by using fluorescence value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the fluorescence value of sample.

The standard curve is: $y= ax + b$.

For liquid samples:

$$\text{Glu content } (\mu mol/L)= (\Delta F- b) \div a \times f$$

For cell samples:

$$\text{Glu content } (\mu mol/gprot)= (\Delta F- b) \div a \times f \div C_{pr}$$

Note:

y: $F_{Standard} – F_{Blank}$, (F_{Blank} is the fluorescence value when the standard concentration is 0)

x: The concentration of standard;

a: The slope of standard curve;

b: The intercept of standard curve.

ΔF: Absolute fluorescence intensity of sample ($F_{Sample} – F_{Blank}$)

f: Dilution factor of sample before test.

C_{pr}: Concentration of protein in sample, gprot/L.

▲Notes

1. This kit is for research use only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The validity of kit is 6 months.
4. Do not use components from different batches of kit.

Appendix I Performance characteristics

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Detection range	0.1-20 μmol/L	Average intra-assay CV (%)	1.7
Sensitivity	0.1 μmol/L	Average inter-assay CV (%)	2.8
Average recovery rate (%)	99		

▲Inter-assay CV

Take three kits of different batches to detect the samples of high concentration, middle concentration and low concentration respectively. The concentrations should be within the detection range. The samples of each concentration were tested 3 times (n=3) parallelly with each kit. Then calculate the corresponding values according to the following formula. The average Inter-assay CV is 2.8%.

$$\bar{x}_T = \frac{\bar{x}_1 + \bar{x}_2 + \bar{x}_3}{3}$$

$$R = \frac{\bar{x}_{max} - \bar{x}_{min}}{\bar{x}_T} \times 100\%$$

$$\bar{R} = \frac{R_1 + R_2 + R_3}{3} \quad (n=3)$$

$\bar{x}_{max}$--- The max values of $\bar{x}_i$

$\bar{x}_{min}$--- The min values of $\bar{x}_i$

$\bar{x}_T$---The average values of $\bar{x}_i$

R_i---The value of each batch number kit

▲Intra-assay CV

Take one kit to detect the samples of high concentration, middle concentration and low concentration respectively. The concentrations should be within the detection range. The samples of each concentration were tested 6 times (n=6) parallelly, and the average Intra-assay CV is 1.7%, which was calculated according to the following formula.

$$CV = \frac{S}{\bar{x}} \times 100\%$$

S--- Standard deviation

▲Sensitivity

Fluorescence values of standard curve and 20 blank samples were measured according to the operation table. Plot the standard curve and calculate the standard deviation of blank, three standard deviations divided by the slope is the sensitivity (0.1 μmol/L) according to the formula of IUPAC.

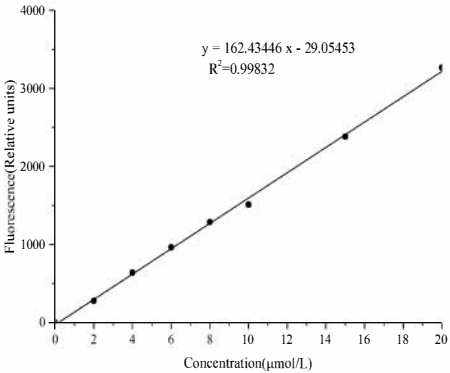
▲Recovery rate

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 99%.

▲Standard curve of total cholesterol

As the fluorescence value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only.

Concentration (μmol/L)	0	2	4	6	8	10	15	20
Average fluorescence	148	424	786	1110	1434	1658	2530	3414
Absoluted fluorescence	0	276	638	962	1286	1510	2382	3266



▲Example analysis

For human plasma, dilute for 300 times with reagent 1, take 50 μL of diluted sample according to the instructions and carry the assay according to the operation table.

The results are as follows:

standard curve: y = 162.43 x – 29.055, the average fluorescence value of the sample is 2741, the average fluorescence value of the blank is 80, and the calculation result is:

Glu content (μmo/L) = (2741 - 80 +29.055) ÷ 162.43 × 300=4968.40 μmol/L

Detect mouse serum (dilute for 200 times), rat serum (dilute for 600 times), rabbit serum (dilute for 200 times) and horse serum (dilute for 600 times) according to the protocol, the result is as follows: