（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-F080

Specification：96T(40 samples)

Measuring instrument：Fluorescence Microplate Reader

(Ex/Em=530 nm/590 nm)

Detection range：0.72-500 μmol/L

Elabscience® Fructose Fluorometric Assay Kit

This manual must be read attentively and completely before using this product.

If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623

Tell: 1-832-243-6086

Fax: 1-832-243-6017

Email: techsupport@elabscience.com

Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Table of contents

Assay summary 3

Intended use 4

Detection principle 4

Kit components & storage 5

Reagent preparation 6

Sample preparation 8

Operating steps 9

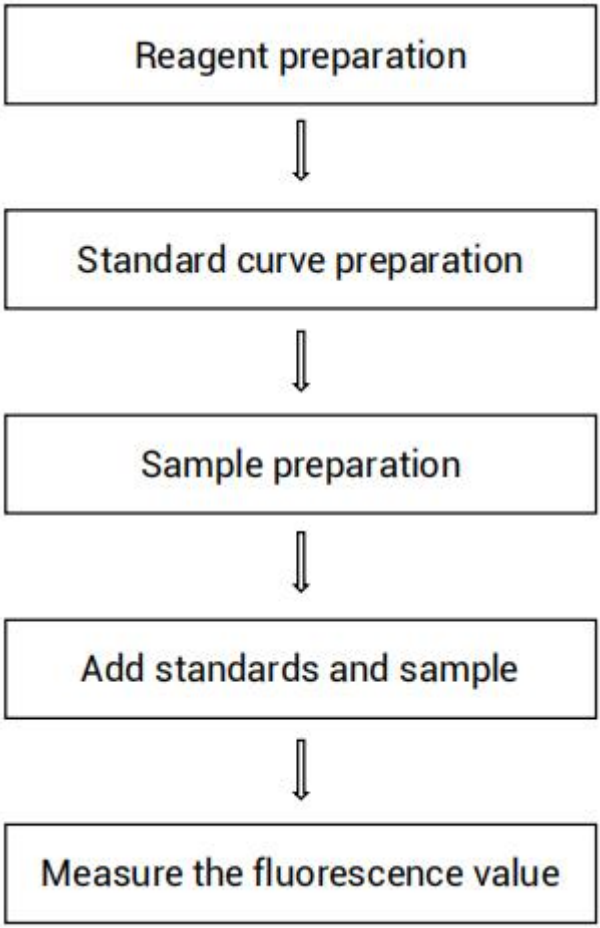
Calculation 10

Appendix I Performance Characteristics 11

Appendix П Example Analysis 13

Statement 14

Assay summary



Intended use

This kit can be used to measure fructose content in serum, plasma and tissue samples.

Detection principle

Fructose is the most common hexketoose, which is often present in large amounts as a free monosaccharide in fruits and honey. A large number of epidemiological data and experimental studies have shown that excessive fructose intake may be an important factor in the increased incidence of metabolic diseases.

Fructose can produce a specific product under the action of enzymes, which reacts with the chromogenic agent to produce a fluorescent substance (Ex/Em: 530 nm/590 nm).

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution	50 mL × 1 vial	-20°C, 12 months
Reagent 2	Matrix Solution	50 mL × 1 vial	-20°C, 12 months shading light
Reagent 3	Enzyme Reagent	Powder × 8 vials	-20°C, 12 months shading light
Reagent 4	Chromogenic Agent	Powder × 8 vials	-20°C, 12 months shading light
Reagent 5	Substrate	Powder × 8 vials	-20°C, 12 months shading light
Reagent 6	Accelerant	1 mL × 1 vial	-20°C, 12 months shading light
Reagent 7	5 mmol/L Standard Solution	1.6 mL × 1 vial	-20°C, 12 months
	Black Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
	Sample Layout Sheet	1 piece	

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. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Fluorescence microplate reader (Ex/Em=530 nm/590 nm), Incubator (37℃), Vortex mixer

Reagents:

Normal saline (0.9% NaCl)

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② The preparation of enzyme working solution：
Dissolve one vial of enzyme reagent with 1.125 mL of buffer solution, mix well to dissolve and add 2 μL of accelerant to mix well. Store at 2-8°C for 1 week protected from light.

③ The preparation of chromogenic working solution：
Dissolve one vial of chromogenic agent with 1.5 mL of matrix solution, mix well to dissolve. Store at 2-8°C for 3 days protected from light.

④ The preparation of substrate working solution:
Dissolve one vial of substrate with 1.5 mL of matrix solution, mix well to dissolve. Store at 2-8°C for 3 days protected from light.

⑤ The preparation of 500 μmol/L standard solution:
Before testing, please prepare sufficient 500 μmol/L standard solution according to the test wells. For example, prepare 1000 μL of 500 μmol/L standard solution (mix well 100 μL of 5 mmol/L standard solution and 900 μL of double distilled water). Store at 2-8°C for a week protected from light.

⑥ The preparation of standard curve：
Always prepare a fresh set of standards. Discard working standard dilutions after use.
Dilute 500 μmol/L standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 100, 200, 250, 300, 350, 400, 500 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**100**	**200**	**250**	**300**	**350**	**400**	**500**
500 μmol/L standard (μL)	0	40	80	100	120	140	160	200
Double distilled water (μL)	200	160	120	100	80	60	40	0

Sample preparation

① Sample preparation

Serum and plasma: detect directly. If not detected on the same day, the serum or plasma can be stored at -80℃ for a month.

Tissue sample:

① arvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 180 μL normal saline (0.9% NaCl) with a dounce homogenizer at 4℃.

④ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M; E-BC-K168-M).

② Dilution of sample

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

Sample type	Dilution factor
10% Watermelon tissue homogenate	40-90
10% Cantaloupe tissue homogenate	30-90
10% Orange tissue homogenate	20-80
10% Mango tissue homogenate	30-90
10% Grape tissue homogenate	60-200
10% Pineapple tissue homogenate	15-40
10% Mouse liver tissue homogenate	1
Mouse serum	1
Human serum	1
Rabbit serum	1

Note: The diluent is normal saline (0.9% NaCl). For the dilution of other sample types, please do pretest to confirm the dilution factor.

Operating steps

① Standard well: add 20 μL of standard with different concentrations into the well.
Sample well: add 20 μL of sample into the well.
Control well: add 20 μL of sample into the well.

② Add 60 μL of enzyme working solution into each well.

③ Mix fully with microplate reader for 3 s. Incubate at 37°C for 90 min protected from light.

④ Add 100 μL of chromogenic working solution into the standard well and sample well.

⑤ Add 100 μL of substrate working solution into the control well.

⑥ Mix fully with microplate reader for 5 s. Incubate at 37°C for 30 min. Measure the fluorescence intensity at the excitation wavelength of 530 nm and the emission wavelength of 590 nm.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean fluorescence value of the blank (Standard #①) from all standard readings. This is the absoluted fluorescence value.

3. Plot the standard curve by using absoluted fluorescence value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

1. Serum (plasma) sample:

$$\text{Fructose content (μmol/L)} = \frac{\Delta F - b}{a} \times f$$

2. Tissue sample:

$$\text{Fructose content (mmol/gprot)} = \frac{\Delta F - b}{a} \div C_{pr} \div 1000 \times f$$

[Note]

ΔF: The absolute fluorescence value of sample, $F_{sample} - F_{control}$.

1000: 1 mmol=1000 μmol.

f: Dilution factor of sample before tested.

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

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	50.00	150.00	350.00
%CV	1.7	2.3	3.1

Inter -assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	50.00	150.00	350.00
%CV	3.2	5.2	7.3

Recovery

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 101%.

	Standard 1	Standard 2	Standard 3
Expected Conc.(μmol/L)	50	150	350
Observed Conc.(μmol/L)	48.5	142.5	388.5
Recovery rate (%)	97	95	111

Sensitivity

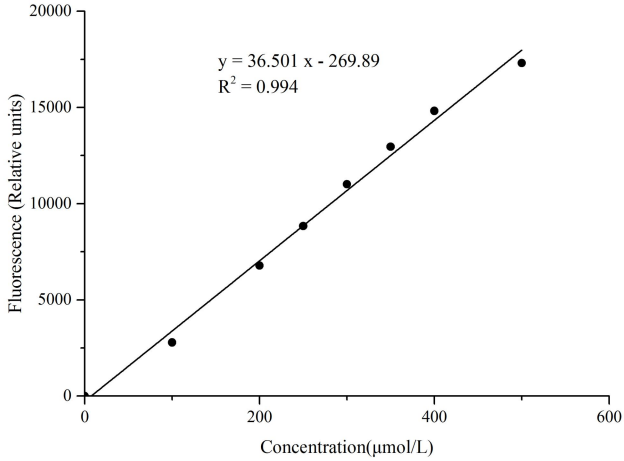
The analytical sensitivity of the assay is 0.72 μmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the

corresponding concentration.

2. Standard curve:

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	100	200	250	300	350	400	500
Fluorescence value	490	3163	7128	8990	11105	13218	14882	17205
	497	3401	7417	9664	11908	13678	15740	18396
Average fluorescence value	493	3282	7273	9327	11506	13448	15311	17800
Absoluted fluorescence value	0	2789	6779	8834	11013	12955	14818	17307



Appendix Π Example Analysis

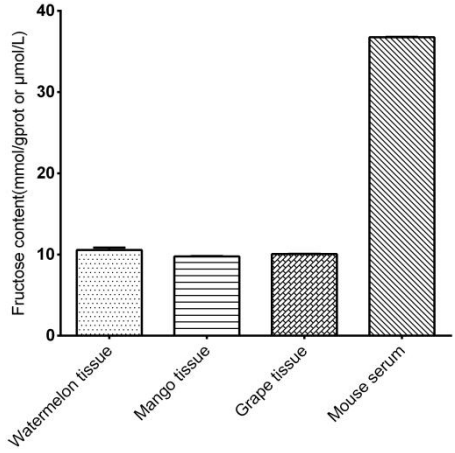
Example analysis：

Dilute 10% grape tissue homogenate, dilute for 30 times, take 20 μL of diluted sample and carry the assay according to the operation steps. The results are as follows:

standard curve: y = 36.501x - 269.89, the fluorescence value of the sample is 17288, the fluorescence value of the control is 686, the concentration of protein in sample is 1.42 gprot/L, and the calculation result is:

Fructose content (mmol/gprot) = (17288 – 686 + 269.89) ÷ 36.501 ÷ 1.42 ÷ 1000 ×30 = 9.77 mmol/gprot

Detect 10% watermelon tissue homogenate (the concentration of protein is 1.97 gprot/L, dilute for 40 times), 10% mango tissue homogenate (the concentration of protein is 1.42 gprot/L, dilute for 30 times), 10% grape tissue homogenate (the concentration of protein is 3.27 gprot/L, dilute for 60 times) and mouse serum according to the protocol, the result is as follows:



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.