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

Catalog No：E-BC-K134-M

Specification：96T(80 samples)

Measuring instrument：Microplate reader（265-305 nm）

Detection range：0.2-22.2 mmol/L

Elabscience® Fructose Colorimetric 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.

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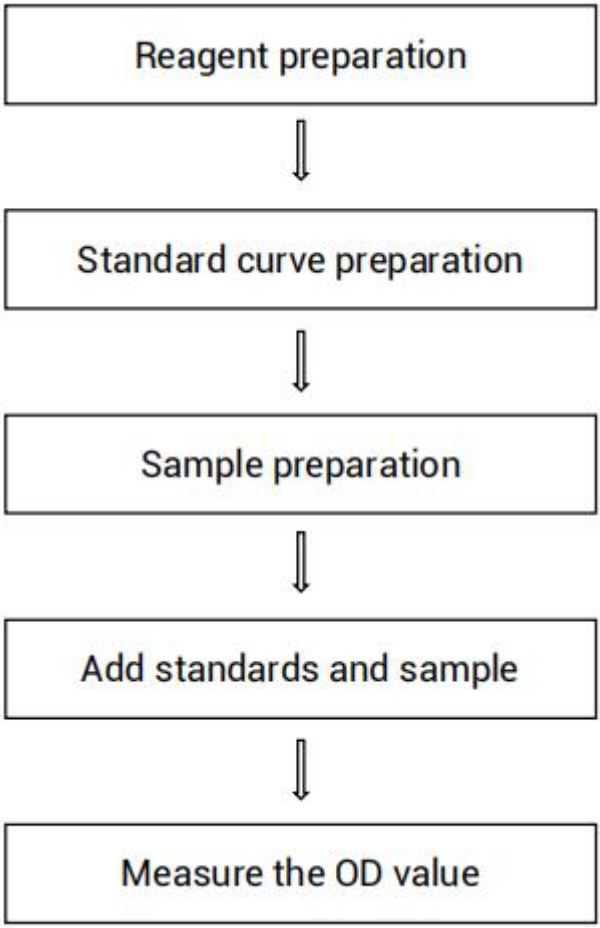
Assay summary



Intended use

This kit can measure fructose content in serum, plasma and plant tissue samples.

Detection principle

Fructose is one of the most common hexaketose sugars, which is abundant as a monosaccharide in free form 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 interact with the matrix solution to form a specific product, which has a maximum absorption peak at 285 nm.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Matrix Solution	75 mL × 2 vials	2-8°C, 12 months
Reagent 2	Standard	Powder × 3 vials	2-8°C, 12 months
	UV 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:

Microplate reader (265-305 nm, optimum wavelength: 285 nm), Incubator, Water bath, Vortex mixer

Reagents:

Normal saline (0.9% NaCl)

Reagent preparation

① Equilibrate all reagents to 25℃ before use.

② The preparation of 22.2 mmol/L standard solution:
Dissolve one vial of standard with 2.5 mL of double distilled water, mix well to dissolve. Store at 2-8°C for a week protected from light.

③ The preparation of standard curve:
Always prepare a fresh set of standards. Discard working standard dilutions after use.
Dilute 22.2 mmol/L standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 4.44, 8.88, 11.1, 13.32, 15.75, 17.76, 22.2 mmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**4.44**	**8.88**	**11.1**	**13.32**	**15.75**	**17.76**	**22.2**
22.2 mmol/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 (plasma): detect directly.

Tissue sample:

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

② Homogenize 20 mg plant tissue in 180 μL of normal saline (0.9% NaCl) with a dounce homogenizer at 4℃.

③ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

④ Meanwhile, determine the protein concentration of supernatant (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 pulp tissue homogenization	2-3
10% Mango pulp tissue homogenization	2-3
10% Cantaloupe pulp tissue homogenization	2-3
10% Grape pulp tissue homogenization	4-5
10% Orange pulp tissue homogenization	2-3
10% Pineapple pulp tissue homogenization	2-3
10% broad bean tissue homogenization	2-3
10% jackfruit pulp tissue homogenization	10-15
10% carrot tissue homogenization	2-3
10% banana pulp tissue homogenization	3-5
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 tube: Add 0.025 mL of standard solution with different concentrations into 2 mL EP tube.
Sample tube: Add 0.025 mL of sample into 2 mL EP tube.

② Add 1.5 mL of matrix solution into each tube.

③ Vortex mixing and boiling water bath for 8 min, and cool the tubes to room temperature with running water. Centrifuge at 10000×g for 10 min and collect supernatant.

④ Take 200 μL of supernatant of each tube to the microplate. Measure the OD values of each well at 285 nm with microplate reader.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

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

3. Plot the standard curve by using absoluted OD 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) samples:

$$\text{fructose content (mmol/L)} = \frac{\Delta A - b}{a} \times f$$

2. Tissue sample (measured in tissue weight):

$$\text{fructose content (mmol/kg wet weight)} = \frac{\Delta A - b}{a} \div \frac{m}{V} \times f$$

3. Tissue sample (measured in protein concentration):

$$\text{fructose conten (mmol/gprot)} = \frac{\Delta A - b}{a} \div C_{pr} \times f$$

[Note]

ΔA: $\Delta A = OD_{Sample} - OD_{blank}$.

m： The wet weight of sample, g.

V: The volume of normal saline (0.9% NaCl) in the sample preparation, mL.

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

f： Dilution factor of sample before test.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three mouse 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 (mmol/L)	8.88	13.32	17.76
%CV	3.1	3.0	2.7

Inter-assay Precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	8.88	13.32	17.76
%CV	5.3	4.0	3.2

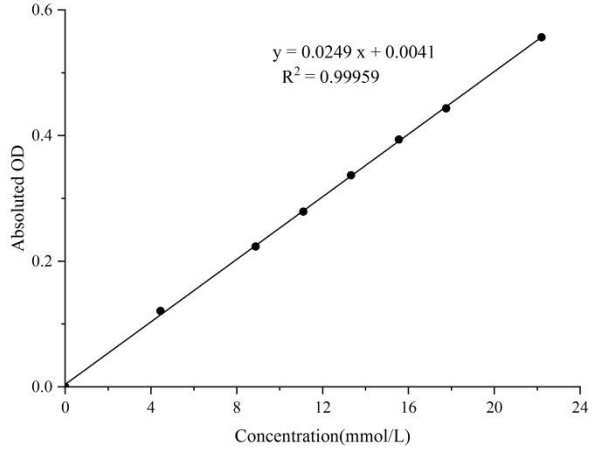
Sensitivity

The analytical sensitivity of the assay is 0.2 mmol/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 OD 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 (mmol/L)	0	4.44	8.88	11.1	13.32	15.75	17.76	22.2
OD value	0.306	0.431	0.529	0.584	0.643	0.71	0.757	0.871
	0.312	0.429	0.536	0.592	0.649	0.696	0.748	0.86
Average OD	0.309	0.430	0.532	0.588	0.646	0.703	0.752	0.866
Absoluted OD	0	0.121	0.224	0.279	0.337	0.394	0.444	0.557



Appendix Π Example Analysis

Example analysis：

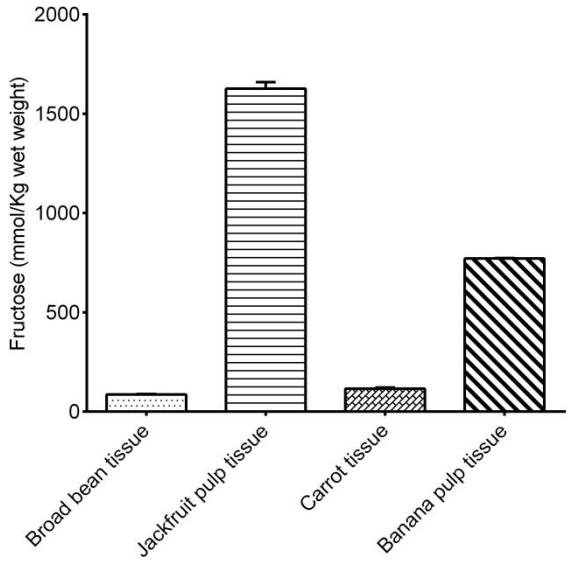
Take 0.025 mL of 10% banana pulp tissue homogenization supernatant which dilute for 5 times and carry the assay according to the operation steps. The results are as follows:

standard curve: y = 0.0249 x + 0.0041. The average OD of sample is 0.741, the average OD of blank is 0.309, and the calculation result is:

fructose content (mmol/kg wet weight) = (0.741 – 0.309 – 0.0041) ÷ 0.0249 ÷ 0.1 × 0.9 × 5

= 773.31 mmol/kg wet weight

Detect 10% broad bean tissue homogenization (dilute for 3 times), 10% jackfruit pulp tissue homogenization (dilute for 10 times), 10% carrot tissue homogenization (dilute for 3 times) and 10% banana pulp tissue homogenization (dilute for 5 times), 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.