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

Catalog No：E-BC-F087

Specification：96T(40 samples)

Measuring instrument：Fluorescence Microplate Reader

(Ex/Em=535 nm/587 nm)

Detection range：1.06-35.00 μmol/L

Elabscience® Fructose-6-Phosphate(F-6-P)

Fluorometric Assay Kit

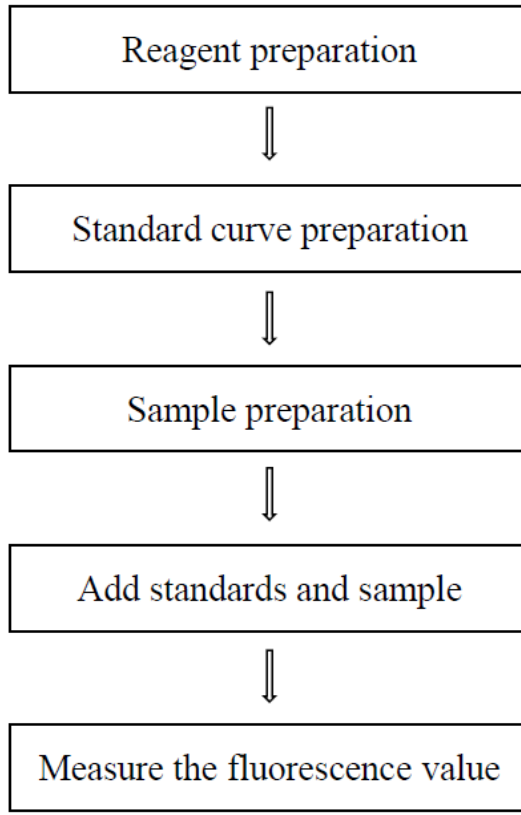
This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

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



Intended use

This kit can be used to measure fructose-6-phosphate (F-6-P) content in cell, animal and plant tissue samples.

Detection principle

Fructose-6-phosphate (F-6-P) is a six-carbon sugar formed by esterification of fructose and phosphate. It is an important intermediate in glycolysis and gluconeogenesis pathways. As the most basic metabolic substances in the metabolic process of living bodies and cells, it is of great significance to study them quantitatively.

Enzyme catalyze F-6-P to produce a specific product, which can react with chromogenic agent to produce the fluorescent substance. Detect the fluorescent intensity of fluorescent substance at the excitation wavelength of 535 nm and the emission wavelength of 587 nm.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution	30 mL × 1 vial	-20 ℃, 12 months shading light
Reagent 2	Substrate	Powder × 4 vials	-20 ℃, 12 months shading light
Reagent 3	Enzyme Reagent	Powder × 4 vials	-20 ℃, 12 months shading light
Reagent 4	1 mmol/L Standard Solution	1 mL × 1 vial	-20 ℃, 12 months shading light
	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. 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=535 nm/587 nm), Incubator, Vortex mixer

Reagents:

Normal saline (0.9% NaCl)

Consumptive material:

10kDa MWCO Spin Filter

Reagent preparation

① Equilibrate all reagents to 25℃ before use.

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

③ The preparation of enzyme working solution：
Dissolve one vial of enzyme reagent with 3 mL of buffer solution, mix well to dissolve. Store at 2-8 ℃ for 3 days protected from light.

④ The preparation of 35 μmol/L standard solution：
Before testing, please prepare sufficient 35 μmol/L standard solution. For example, prepare 1000 μL of 35 μmol/L standard solution (mix well 35 μL of 1 mmol/L standard solution and 965 μL of double distilled water). Store at 2-8 ℃ for a week protected from light.

⑤ The preparation of standard curve：
Always prepare a fresh set of standards. Discard working standard dilutions after use.
Dilute 35 μmol/L standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 7, 14, 17.5,

21, 24.5, 28, 35 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**7**	**14**	**17.5**	**21**	**24.5**	**28**	**35**
35 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

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 100 mg).

② Wash tissue in cold normal saline (0.9% NaCl).

③ Homogenize 100 mg tissue in 900 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.

⑤ Collect supernatant and add it to 10kDa MWCO Spin Filter. Centrifuge at 12000×g for 15 min at 4℃.

⑥ Collect the filtrate and preserve it on ice for detection.

Cell (adherent or suspension) samples:

① Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells).

② Homogenize 1×10^6 cells in 200 μL 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.

④ Collect supernatant and add it to 10kDa MWCO Spin Filter. Centrifuge at 12000×g for 15 min at 4℃.

⑤ Collect the filtrate and preserve it on ice for detection.

② Dilution of sample

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

Sample type	Dilution factor
10% Mouse liver tissue homogenate	2-4
10% Mouse heart tissue homogenate	3-6
10% Mouse kidney tissue homogenate	2-4
10% Mouse lung tissue homogenate	3-6
10% Orange tissue homogenate	2-5
10% Orange peel tissue homogenate	1
10% Epipremnum aureum tissue homogenate	2-5
1×10^6 Jurkat cells	1
1×10^6 293T cells	1
1×10^6 Hela cells	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 200 μL of substrate working solution into standard and sample wells.

③ Add 200 μL of enzyme working solution into control wells.

④ Mix fully with fluorescence microplate reader for 5s. Incubate at 37 ℃ for 5 min and measure the fluorescence intensity at the excitation wavelength of 535 nm and the emission wavelength of 587 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. Tissue sample:

$$\text{F-6-P content (μmol/kg wet weight)} = \frac{\Delta F - b}{a} \div \frac{m}{V} \times f$$

2. Cell sample:

$$\text{F-6-P content (nmol/10^6)} = \frac{\Delta F - b}{a} \div \frac{n}{V} \times f$$

[Note]

ΔF:$\Delta F = F_{sample} - F_{control}$.

m: The weight of tissue, g.

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

n: The number of cell sample, 10^6.

f: Dilution factor of sample before tested.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three mouse liver tissue 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)	28.00	18.00	23.00
%CV	4.6	4.7	5.0

Inter-assay Precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	28.00	18.00	23.00
%CV	5.7	6.8	9.8

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 93.3%.

	Standard 1	Standard 2	Standard 3
Expected Conc.(μmol/L)	28	18	23
Observed Conc.(μmol/L)	25.5	17.3	21.4
Recovery rate (%)	91	96	93

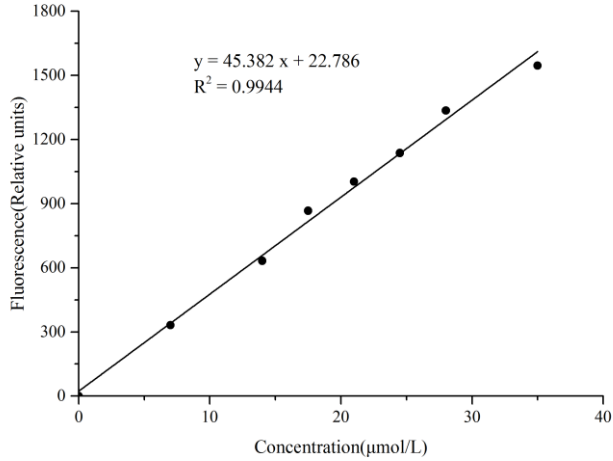
Sensitivity

The analytical sensitivity of the assay is 1.06 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	7	14	17.5	21	24.5	28	35
Fluorescence value	940	1292	1592	1783	1944	2065	2269	2439
	950	1262	1564	1841	1952	2099	2293	2543
Average fluorescence value	945	1277	1578	1812	1948	2082	2281	2491
Absoluted fluorescence value	0	332	633	867	1003	1137	1336	1546



Appendix II Example Analysis

Example analysis:

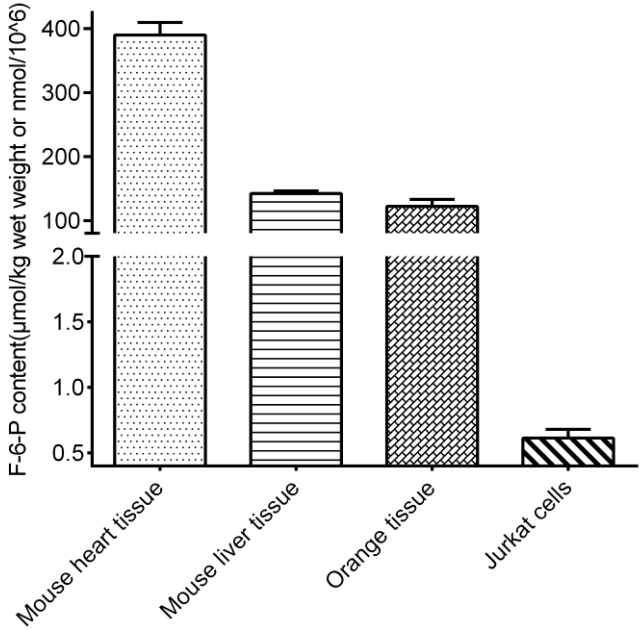
Take 20 μL of 10% mouse heart tissue filtrate which dilute for 4 times and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 45.382x + 22.786, the average fluorescence value of the sample well is 1706, the average fluorescence value of the control well is 1191, and the calculation result is:

F-6-P content (μmol/kg wet weight) = (1706 - 1191 - 22.786) ÷45.382 ×0.9 ÷0.1 ×4

= 390.46 μmol/kg wet weight

Detect 10% mouse heart tissue homogenate (dilute for 4 times), 10% mouse liver tissue homogenate (dilute for 4 times), 10% orange tissue homogenate(dilute for 5 times) and 1×10^6 Jurkat cells 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.