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

Catalog No：E-BC-F151

Specification：96T(40 samples)

Measuring instrument：Fluorescence Microplate Reader

(Ex/Em=535 nm/587 nm)

Detection range：0.63-100 μmol/L

Elabscience® Dihydroxyacetone Phosphate (DHAP) Fluorometric Assay Kit

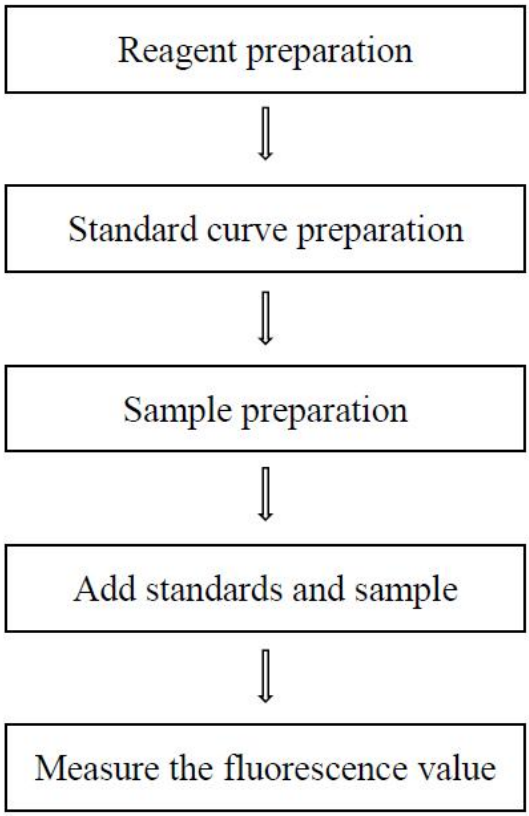
This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

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 dihydroxyacetone phosphate (DHAP) content in serum, plasma and animal tissue samples.

Detection principle

Dihydroxyacetone phosphate (DHAP) is an intermediate product present in the glycolysis and gluconeogenesis processes of living organisms. Most animals, due to the absence of the glyoxylate cycle, cannot convert fatty acids into glucose like plants. They can only convert the glycerol in fats into glucose through DHAP or enter the glycolysis pathway.

Detection principle: The products formed after a series of enzymatic reactions of DHAP can cause the fluorescent probe to emit fluorescence. The fluorescence value can be detected using a fluorescence microplate reader at the maximum excitation wavelength of 535 nm and the maximum emission wavelength of 587 nm to calculate the content of DHAP.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution	20 mL × 1 vial	-20°C, 12 months, shading light
Reagent 2	Enzyme Reagent	0.12 mL × 1 vial	-20°C, 12 months, shading light
Reagent 3	Substrate	Powder × 2 vials	-20°C, 12 months, shading light
Reagent 4	Accelerant	0.9 mL × 1 vial	-20°C, 12 months, shading light
Reagent 5	Chromogenic Agent	6 mL × 1 vial	-20°C, 12 months, shading light
Reagent 6	1 mmol/L Standard Solution	0.2 mL × 1 vial	-20°C, 12 months, shading light
	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=535 nm/587 nm), Incubator

Reagents:

PBS (0.01 M, pH 7.4), 1 mol/L Perchloric acid, 3 mol/L $KHCO_3$

Consumptive material:

pH test paper

Reagent preparation

① Equilibrate all reagents to 25°C before use.

② The preparation of enzyme working solution：

Before testing, please prepare sufficient enzyme working solution. For example, prepare 350 μL of enzyme working solution (mix well 10 μL of enzyme reagent and 340 μL of buffer solution). Store at -20°C protected from light and used up within 3 days.

③ The preparation of substrate working solution：

Dissolve one vial of substrate with 1.4 mL of buffer solution, mix well. Store at -20°C protected from light and used up within 7 days.

④ The preparation of chromogenic working solution：

Before testing, please prepare sufficient chromogenic working solution. For example, prepare 320 μL of chromogenic working solution (mix well 90 μL of buffer solution, 30 μL of accelerant and 200 μL of chromogenic agent). Store at -20°C protected from light and used up within 3 days.

⑤ The preparation of 100 μmol/L standard solution：

Before testing, please prepare sufficient 100 μmol/L standard solution. For example, prepare 1000 μL of 100 μmol/L standard solution (mix well 100 μL of 1 mmol/L standard solution and 900 μL of buffer solution). The 100 μmol/L standard solution should be prepared on spot. Store at -20°C protected from light and used up within 3 days.

⑥ The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 100 μmol/L standard solution with buffer solution to a serial concentration. The recommended dilution gradient is as follows: 0, 5, 10, 15, 20, 50, 80, 100 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**5**	**10**	**15**	**20**	**50**	**80**	**100**
100 μmol/L Standard (μL)	0	10	20	30	40	100	160	200
Buffer Solution (μL)	200	190	180	170	160	100	40	0

Sample preparation

① Sample preparation

Serum (plasma) samples:

① Add 40 μL of plasma (serum) and 10 μL of 1 mmol/L perchloric acid and vortex-mix for at least 1 min. Add 3 mol/L $KHCO_3$ to adjust the pH to 7-8 (the recommended initial volume is 60 μL. Adding $KHCO_3$ to adjust the pH causes vigorous gas production. Please add slowly and carefully).

② Centrifuge at 10000×g for 10 min at 4℃. Collect supernatant and keep it on ice for detection. (The dilution factor of the sample treatment process using acids and bases needs to be calculated based on the specific volume of $KHCO_3$ added. For example, if 400 μL of sample is mixed with 100 μL of 1 mmol/L perchloric acid and then add 60 μL of 3 mol/L $KHCO_3$ to adjust the pH to 7-8, then the dilution factor f_1 is (total volume of sample, perchloric acid and $KHCO_3$)/(the volume of sample) = 560/400 = 1.4).

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 60 mg).

② Homogenize 60 mg tissue in 540 μL PBS(0.01 M，pH 7.4) with a dounce homogenizer at 4°C.

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

④ Add 40 μL of supernatant and 10 μL of 1 mmol/L perchloric acid and vortex-mix for at least 1 min. Add 3 mol/L $KHCO_3$ to adjust the pH to 7-8 (the recommended initial volume is 60 μL. Adding $KHCO_3$ to adjust the pH causes vigorous gas production. Please add slowly and carefully).

⑤ Centrifuge at 10000×g for 10 min at 4℃. Collect supernatant and keep it on ice for detection. (The dilution factor of the sample treatment

process using acids and bases needs to be calculated based on the specific amount of $KHCO_3$ added. For example, if 400 μL of sample is mixed with 100 μL of 1 mmol/L perchloric acid and then 60 μL of 3 mol/L $KHCO_3$ is added to adjust the pH to 7-8, then the dilution factor f_1 is(total volume of sample, perchloric acid and $KHCO_3$)/(the volume of sample) = 560/400 = 1.4).

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	1
10% Mouse kidney tissue homogenate	1
10% Mouse brain tissue homogenate	1
10% Mouse lung tissue homogenate	1
10% Mouse heart tissue homogenate	1
10% Mouse spleen tissue homogenate	1
Mouse serum	1
Mouse plasma	1

Note: The diluent is PBS(0.01 M，pH 7.4). 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 70 μL of enzyme working solution into standard and sample wells. Add 70 μL of buffer solution into control wells.

③ Add 20 μL of substrate working solution into each well.

④ Add 80 μL of chromogenic working solution into each well.

⑤ Mix fully with fluorescence microplate reader for 5 s. Incubate at 37°C for 30 min protected from light. 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. Serum and plasma samples:

$$\text{DHAP content (μmol/L)} = (\Delta F - b) \div a \times f_1 \times f$$

2. Tissue samples:

$$\text{DHAP content (μmol/kg wet weight)} = (\Delta F - b) \div a \div m \times V \times f_1 \times f$$

[Note]

ΔF: The absoluted fluorescence of sample, $\Delta F = F_{sample} - F_{control}$.

f_1: Dilution factor of sample preparation, calculated according to the formula in the sample preparation section

f: Dilution factor of sample before tested.

m: The weight of sample, kg

V: The volume of PBS(0.01 M，pH = 7.4) in the sample preparation step, L

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three mouse serum 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)	30.00	60.00	90.00
%CV	1.1	1.6	3.3

Inter-assay Precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	30.00	60.00	90.00
%CV	1.9	2.2	4.7

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 95.3%.

	Standard 1	Standard 2	Standard 3
Expected Conc.(μmol/L)	30.00	60.00	90.00
Observed Conc.(μmol/L)	28.2	57.6	86.4
Recovery rate (%)	94.0	96.0	96.0

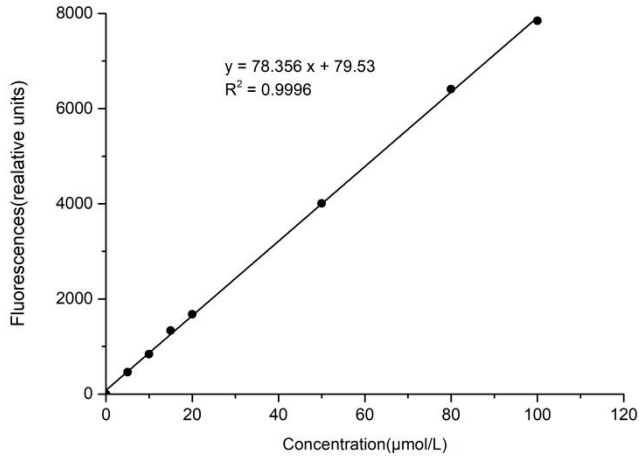
Sensitivity

The analytical sensitivity of the assay is 0.63 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	5	10	15	20	50	80	100
Fluorescence value	910	1356	1731	2256	2567	4876	7161	8607
	906	1381	1767	2231	2599	4959	7473	8899
Average fluorescence value	908	1368	1749	2244	2583	4917	7317	8753
Absoluted fluorescence value	0	460	841	1336	1675	4010	6409	7845



Appendix Π Example Analysis

Example analysis：

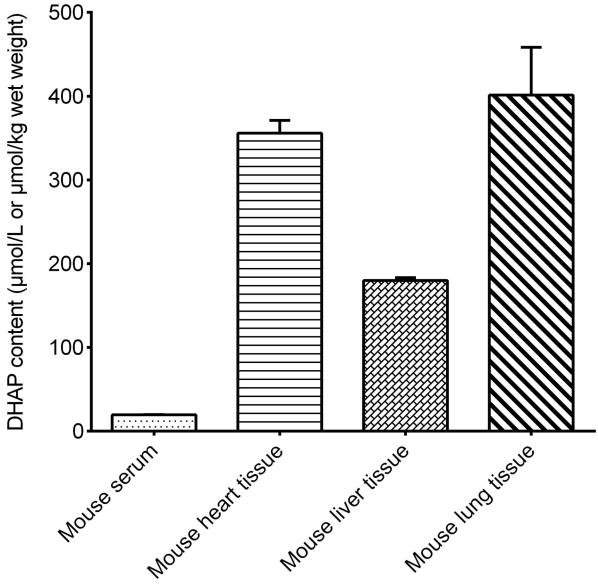
Take 20 μL of 10% mouse liver tissue supernatant and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 78.356 x + 79.53, the average fluorescence value of the sample well is 2945, the average fluorescence value of the control well is 1946, $\triangle F = F_{sample} - F_{control}$ = 2945 –1946 = 1199, and the calculation result is:

$$\text{DHAP content (μmol/kg wet weight)} = (1199 - 79.53) \div 78.356 \div 0.0001 \times 0.0009 \times 1.4$$

$$= 180 \text{ μmol/kg wet weight}$$

Detect mouse serum, 10% mouse heart tissue homogenate, 10% mouse liver tissue homogenate, 10% mouse lung tissue homogenate 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.