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

Catalog No：E-BC-F058

Specification：48T(32 samples)96T(80 samples)

Measuring instrument：Fluorescence Microplate Reader

(Ex/Em=535 nm/587 nm)

Detection range：1.00-100 μmol/L

Elabscience® Pyruvate 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.

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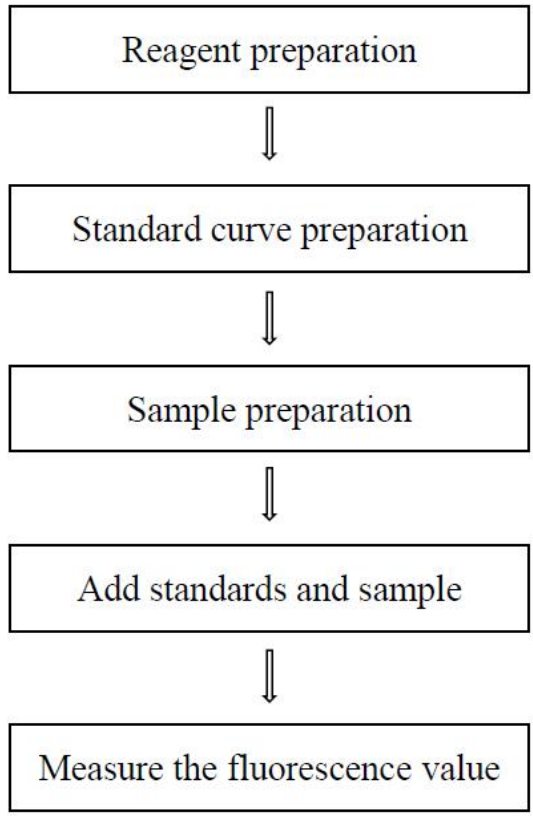
Assay summary



Intended use

This kit can be used to measure pyruvate content in serum (plasma), urine, animal tissue and cell samples.

Detection principle

Pyruvate is an important component of the organism. It mainly participates in the metabolism of sugar and fat, and is also one of the intermediate products of carbohydrate metabolism. The detection principle of this kit is that pyruvate undergoes a series of reactions under the action of enzyme reagents, and the resulting substance can react with the color developer. The content of pyruvate in the sample is calculated by measuring the fluorescence value of excitation wavelength 535 nm and emission wavelength 587 nm.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	14 mL × 1 vial	28 mL × 1 vial	-20°C, 12 months shading light
Reagent 2	Coenzyme I	Powder × 1 vial	Powder × 2 vials	-20°C, 12 months shading light
Reagent 3	Substrate	Powder × 2 vials	Powder × 3 vials	-20°C, 12 months shading light
Reagent 4	Coenzyme II	Powder × 1 vial	Powder × 2 vials	-20°C, 12 months shading light
Reagent 5	Enzyme Solution	0.9 mL × 1 vial	1.8 mL × 1 vial	-20°C, 12 months shading light
Reagent 6	Chromogenic Agent	0.1 mL × 1 vial	0.2 mL × 1 vial	-20°C, 12 months shading light
Reagent 7	1 mmol/L Standard	0.8 mL × 1 vial	1.6 mL × 1 vial	-20°C, 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

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 coenzyme I working solution:
Dissolve one vial of coenzyme I with 2 mL of buffer solution, mix well to dissolve. Store at -20°C for 1 month protected from light.

③ The preparation of substrate working solution:
Dissolve one vial of substrate with 2 mL of buffer solution, mix well to dissolve. Store at -20°C for 7 days protected from light.

④ The preparation of coenzyme II working solution:
Dissolve one vial of coenzyme II with 0.2 mL of buffer solution, mix well to dissolve. Store at -20°C for 1 month protected from light.

⑤ The preparation of enzyme working solution:
Before testing, please prepare sufficient enzyme working solution according to the test wells. For example, prepare 400 μL of enzyme working solution (mix well 100 μL of enzyme solution and 300 μL of buffer solution). The enzyme working solution should be prepared on spot and keep it on ice.

⑥ The preparation of reaction working solution:

For each well, prepare 100 μL of reaction working solution (mix well 20 μL of coenzyme I working solution, 20 μL of substrate working solution and 60 μL of enzyme working solution). The reaction working solution should be prepared on spot and keep it on ice.

⑦ The preparation of chromogenic working solution:

Before testing, please prepare sufficient chromogenic working solution according to the test wells. For example, prepare 495 μL of chromogenic working solution (mix well 15 μL of coenzyme II working solution, 5 μL of chromogenic agent and 475 μL of buffer solution). The chromogenic working solution should be prepared on spot and keep it on ice.

⑧ 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 and 900 μL of double distilled water). Store at -20°C for 1 month protected from light.

⑨ 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 double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 20, 30, 40, 50, 60, 70, 100 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**20**	**30**	**40**	**50**	**60**	**70**	**100**
100 μmol/L Standard (μL)	0	40	60	80	100	120	140	200
Double distilled water (μL)	200	160	140	120	100	80	60	0

Sample preparation

① Sample preparation

Serum, plasma and urine samples: Add 200-400μL of serum (plasma) into 10 kDa MWCO Spin Filter and centrifuge at 12000×g for 10 min. Collect the filtrate and preserve it on ice for detection. Detect the prepared sample within 1 day.

Tissue sample:

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

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

③ Homogenize 100 mg tissue in 900 μL normal saline (0.9% NaCl) with a dounce homogenizer at 4°C.

④ Centrifuge at 12000×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 10 min at 4℃.

⑥ Collect the filtrate and preserve it on ice for detection. Detect the prepared sample within 1 day.

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°C.

③ Centrifuge at 12000×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 10 min at 4℃.

⑤ Collect the filtrate and preserve it on ice for detection. Detect the prepared sample within 1 day.

② **Dilution of sample**

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

Sample type	Dilution factor
Guinea pig serum	1
Rabbit serum	1-2
Human serum	1
Rat serum	1-2
10% Mouse kidney tissue homogenate	1
10% Mouse liver tissue homogenate	1
1×10^6 239T cells	1
1×10^6 CHO cells	1
1×10^6 Hela cells	1
1×10^6 Jurkat 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.

② Add 100 μL of reaction working solution into each well.

③ Add 100 μL of chromogenic working solution into each well.

④ Mix fully with fluorescence microplate for 3s. Incubate at 37°C for 20 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 (plasma) and urine samples:

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

2. Tissue sample:

$$\text{pyruvate content (μmol/kg wet weight)} = (\Delta F - b) \div a \div \left(\frac{m}{n}\right) \times f$$

3. Cell sample:

$$\text{pyruvate content (μmol/10^9)} = (\Delta F - b) \div a \div \left(\frac{n}{v}\right) \times f$$

[Note]

ΔF: $\Delta F = F_{sample} - F_{blank}$. ($F_{blank}$ is the fluorescence value when the standard concentration is 0).

m: The weight of tissue, g.

v: The volume of normal saline in the preparation of sample, L.

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 rat 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)	15.00	55.00	85.00
%CV	1.0	2.0	1.5

Inter-assay Precision

Three rat 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)	15.00	55.00	85.00
%CV	2.0	4.0	3.6

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 99.2%.

	Sample 1	Sample 2	Sample 3
Expected Conc (μmol/L)	15	55	85
Observed Conc (μmol/L)	14.7	55.0	84.6
Recovery rate (%)	98	100	99.5

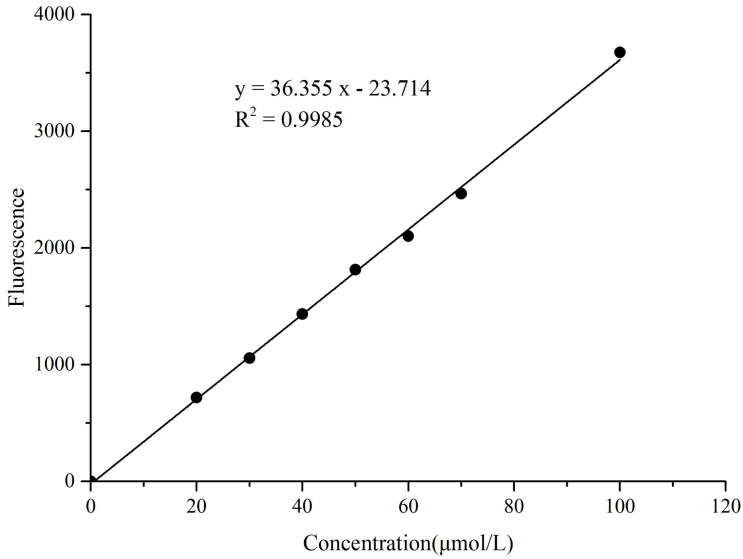
Sensitivity

The analytical sensitivity of the assay is 1.00 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	20	30	40	50	60	70	100
Fluorescence value	482	1208	1546	1913	2316	2570	2961	4162
	502	1212	1551	1938	2295	2613	2955	4171
Average fluorescence value	492	1210	1548	1926	2306	2592	2958	4166
Absoluted fluorescence value	0	718	1056	1434	1814	2100	2466	3674



Appendix II Example Analysis

Example analysis:

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

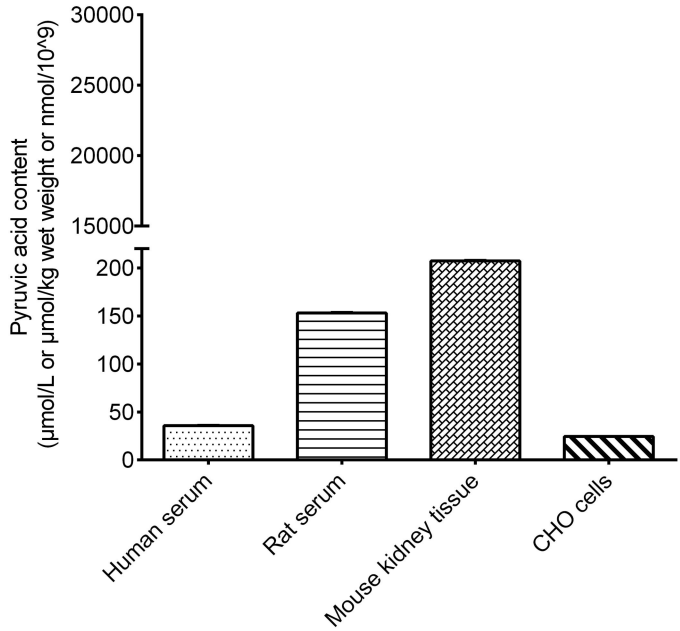
Standard curve: y = 36.355 x - 23.714, the average fluorescence value of the sample well is 1278, the average fluorescence value of the blank well is 462,

ΔF = 1278 - 462 = 816, and the calculation result is:

pyruvate content (μmol/kg wet weight) = (816 + 23.714) ÷ 36.355 ÷ (0.1 ÷ 0.9)

= 207.88 μmol/kg wet weight

Detect human serum, rat serum, 10% mouse kidney tissue homogenate and 1×10^6 CHO 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.