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

Catalog No：E-BC-F047

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

Measuring instrument：Fluorescence Microplate Reader

(Ex/Em=535 nm/587 nm)

Detection range：0.60-100 μmol/L

Elabscience® α-Ketoglutarate (α-KG) 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
Tel: 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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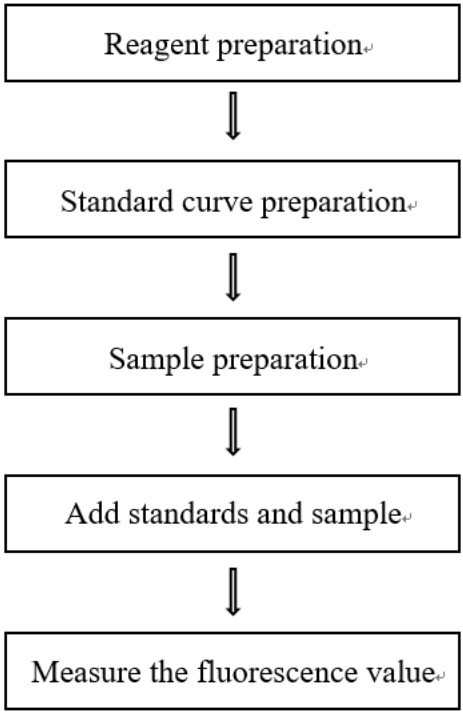
Assay summary



Intended use

This kit can be used for determination of α-Ketoglutarate (α-KG) content in serum, plasma, urine, animal tissue and cell samples.

Detection principle

α-Ketoglutarate (α-KG) is an important intermediate metabolite in the tricarboxylic acid cycle and a key node connecting the metabolism of carbon and nitrogen in cells. As a short chain carboxylic acid molecule, α-ketoglutaric acid is the precursor of many important amino acids such as glutamine and glutamic acid. It not only directly participates in energy supply, but also participates in various chemical reactions in cells, and has a variety of physiological effects.

α-Ketoglutaric acid and alanine can be combined with fluorescent probe products under the action of a series of enzymes. The content of α-ketoglutaric acid in samples can be determined by measuring the fluorescence value.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	13 mL ×1 vial	26 mL ×1 vial	-20℃, 12 months
Reagent 2	Substrate	Powder ×1 vial	Powder ×2 vials	-20℃, 12 months
Reagent 3	Enzyme Reagent	Powder ×1 vial	Powder ×2 vials	-20℃, 12 months shading light
Reagent 4	Probe	1.2 mL ×1 vial	2.4 mL ×1 vial	-20℃, 12 months shading light
Reagent 5	Standard	Powder ×2 vials	Powder ×2 vials	-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:

Incubator, Centrifuge, Fluorescence microplate reader (Ex/Em=535 nm/587 nm), 50 KD ultrafiltration tube

Reagents:

Normal saline (0.9% NaCl)

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② The preparation of substrate working solution:
Dissolve one vial of substrate with 12 mL of buffer solution, mix well to dissolve. Store at -20 ℃ for 3 days. Avoid repeated freeze/thaw cycles is advised.

③ The preparation of enzyme working solution:
Dissolve one vial of enzyme reagent with 1.2 mL of double distilled water, mix well and keep it on ice during use. The enzyme working solution should be prepared on spot.

④ The preparation of 50 mmol/L standard solution:
Dissolve one vial of standard with 1 mL of double distilled water, mix well. Store at -20 ℃ for 3 days protected from light.

⑤ The preparation of 100 μmol/L standard solution :
Dilute 2 μL of 50 mmol/L standard solution with 998 μL of double distilled water and mix well. Store at -20 ℃ for 3 days protected from light.

⑥ The preparation of standard curve：

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

Dilute 100 μmol/L pyruvic acid standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 10, 20, 30, 50, 60, 80, 100 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**10**	**20**	**30**	**50**	**60**	**80**	**100**
100 μmol/L standard (μL)	0	20	40	60	100	120	160	200
Double distilled water (μL)	200	180	160	140	100	80	40	0

Sample preparation

① Sample preparation

Serum and plasma: The samples were centrifuged with a 50 KD ultrafiltration tube at 4 ℃ 12000×g for 15 min, and filtrate was collected to be measured.

Tissue sample:

① Harvest 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 12000×g at 4℃ for 15 min to remove insoluble material. Collect supernatant and keep it with a 50 KD ultrafiltration tube at 4 ℃ 12000×g for 15 min, and filtrate was collected to be measured.

Cell (adherent or suspension) samples:

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

② Wash cells with PBS (0.01 M, pH 7.4).

③ Homogenize 2×10^6 cells in 200 μL normal saline (0.9% NaCl) with a ultrasonic cell disruptor at 4℃.

④ Centrifuge at 12000 × g for 15 minutes to remove insoluble material. Collect supernatant and keep it with a 50 KD ultrafiltration tube at 4℃ 12000×g for 15 min, and filtrate was collected to be measured.

② Dilution of sample

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

Sample type	Dilution factor
10% Rat kidney tissue homogenate	1
10% Rat lung tissue homogenate	1
10% Porcine heart tissue homogenate	1
10% Rat brain tissue homogenate	1
10% Mouse liver tissue homogenate	1
10^6 Jurkat cell	1
Human serum	1
Rat serum	2-3

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 wells.

② Add 140 μL of substrate working solution into each well.

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

④ Add 20 μL of probe into each well.

⑤ Mix fully with microplate for 5 s and 37 ℃ for 20 min protected from light. Measure the fluorescence value of each well at Ex/Em=535/587 nm. The fluorescence values of the control and sample.

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) samples:

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

2. Tissue samples:

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

3. Cells samples:

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

[Note]

ΔF: Absoluted fluorescence intensity of sample ($F_{Sample} - F_{Blank}$).

m: The weight of tissue sample, g.

V: The volume of homogenate, mL.

n: The number of cells, 10^6.

f: Dilution factor of sample before test.

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)	1.00	25.00	50.00
%CV	2.3	1.9	1.8

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)	1.00	25.00	50.00
%CV	4.5	4.1	3.4

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%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (μmol/L)	15	45	70
Observed Conc. (μmol/L)	15.2	44.6	67.9
Recovery rate (%)	101	99	97

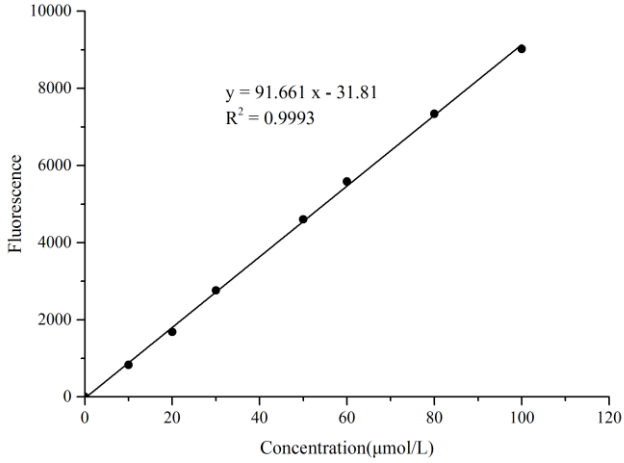
Sensitivity

The analytical sensitivity of the assay is 0.60 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**	**10**	**20**	**30**	**50**	**60**	**80**	**100**
Fluorescence value	547	1406	2078	3267	5050	6140	7629	9415
	568	1369	2406	3370	5271	6145	8164	9746
Average fluorescence value	557	1388	2242	3318	5161	6143	7897	9581
Absoluted fluorescence value	0	830	1685	2761	4603	5585	7339	9023



Appendix II Example Analysis

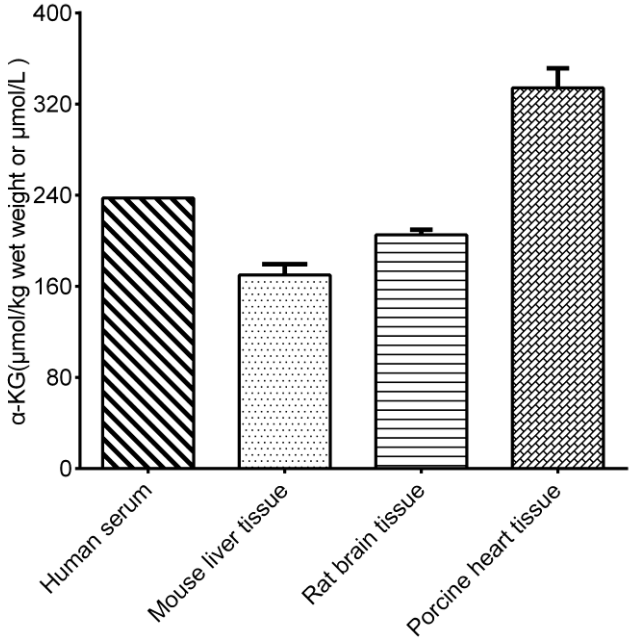
Example analysis：

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

standard curve: y = 91.661 x – 31.81, The average fluorescence value of blank well is 557.45. The average fluorescence value of the sample well is 2256.69, and the calculation result is:

$$\text{α-KG content (μmol/kg wet weight)} = \frac{2256.69 - 557.45 + 31.81}{91.661} \div \frac{0.1}{0.9} = 169.97 \text{ μmol/kg wet weight}$$

Detect human serum, 10% mouse liver tissue homogenate, 10% rat brain tissue homogenate, and 10% porcine heart 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.