

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

Catalog No: E-BC-F172

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

Measuring instrument: Fluorescence Microplate Reader (Ex/Em=535 nm/587 nm)

Detection range: 0.0004 - 3.33 U/L

Elabscience® α -Ketoglutarate Dehydrogenase (α -KGDH) Activity 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

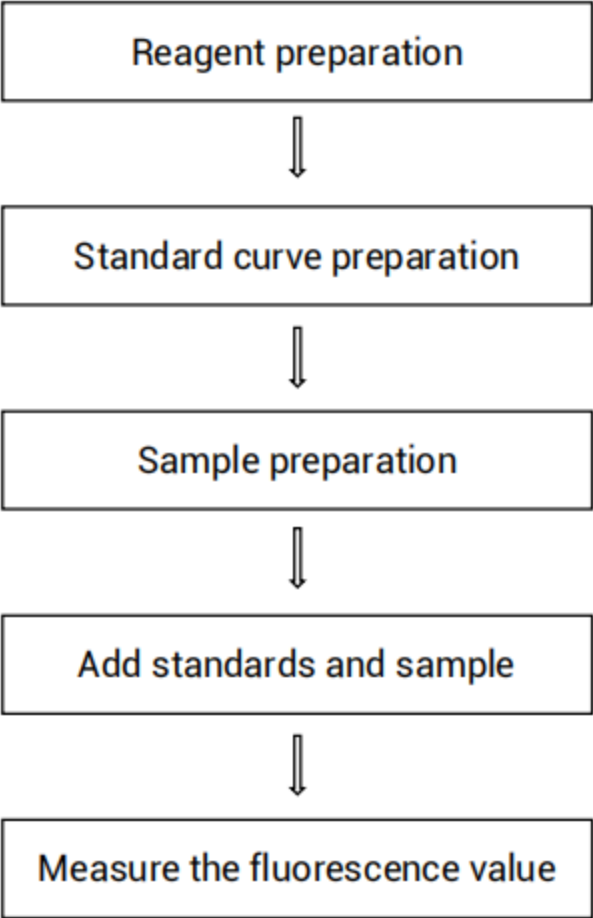
Website: www.elabscience.com

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

The kit is suitable for detecting the activity of α -Ketoglutarate Dehydrogenase (α -KGDH) in animal tissues and cells.

Detection principle

α -Ketoglutarate Dehydrogenase (α -KGDH) is a highly regulated mitochondrial enzyme that catalyzes the conversion of α -ketoglutarate to succinyl-CoA in the tricarboxylic acid (TCA) cycle.

α -KGDH catalyzes the decarboxylation of α -ketoglutarate, reducing NAD^+ to NADH; NADH reduces resazurin to the strongly fluorescent resorufin under the catalysis of diaphorase, and α -KGDH activity is calculated by measuring the fluorescence value at the excitation wavelength of 535 nm and the emission wavelength of 587 nm.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Extraction Solution	40 mL $\times$ 1 vial	40 mL $\times$ 2 vials	-20°C, 12 months, shading light
Reagent 2	Buffer Solution	11 mL $\times$ 1 vial	22 mL $\times$ 1 vial	-20°C, 12 months, shading light
Reagent 3	Substrate	Powder $\times$ 3 vials	Powder $\times$ 6 vials	-20°C, 12 months, shading light
Reagent 4	Chromogenic Agent	0.5 mL $\times$ 1 vial	1 mL $\times$ 1 vial	-20°C, 12 months, shading light
Reagent 5	0.5 mmol/L Standard	0.9 mL $\times$ 1 vial	1.8 mL $\times$ 1 vial	-20°C, 12 months, shading light
	Black Microplate	96 wells		No requirement
	Plate Sealer	2 pieces		
	Sample Layout Sheet	1 piece		

Note: All the reagents should be stored according to the table. The reagents from different kits can not be mixed or used interchangeably. For liquid reagents with small volumes or powders, centrifuge them before use to prevent loss.

Instruments

Fluorescence Microplate Reader (Ex/Em=535 nm/587 nm), Incubator (37°C)

Materials required but not provided

Double distilled water

Reagent preparation

- ① Equilibrate all the reagents to 25°C before use.
- ② The preparation of substrate working solution:
Dissolve one vial of substrate with 0.2 mL of buffer solution. The prepared substrate working solution can be aliquoted and stored at -20°C protected from light for 2 days.
- ③ The preparation of working solution:
For each well, prepare 200 µL of working solution (mix well 185 µL of buffer solution, 10 µL of substrate working solution and 5 µL of chromogenic agent). The working solution should be prepared on spot protected from light and used up within 0.5 h at room temperature.

④ The preparation of 12.5 $\mu\text{mol/L}$ standard solution :

Before testing, please prepare sufficient 12.5 $\mu\text{mol/L}$ standard solution according to the test wells. For example, prepare 1000 μL of 12.5 $\mu\text{mol/L}$ standard solution (mix well 975 μL of buffer solution and 25 μL of 0.5 mmol/L standard solution). The 12.5 $\mu\text{mol/L}$ standard solution should be freshly prepared.

⑤ Preparation of standard solutions with different concentrations :

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

Dilute 12.5 $\mu\text{mol/L}$ standard solution with Buffer Solution as the diluent to a serial concentration. The recommended dilution gradient is as follows: 0, 2.50, 3.75, 5.00, 6.25, 7.50, 8.75, 12.50 $\mu\text{mol/L}$.

Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration ($\mu\text{mol/L}$)	0	2.50	3.75	5.00	6.25	7.50	8.75	12.50
12.5 $\mu\text{mol/L}$ Standard (μL)	0	40	60	80	100	120	140	200
Buffer Solution (μL)	200	160	140	120	100	80	60	0

Sample preparation

Tissue sample:

- ① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).
- ② Wash tissue in cold extraction solution.
- ③ Homogenize 20 mg tissue in 180 μ L extraction solution with a dounce homogenizer at 4°C.
- ④ Centrifuge at 10000 $\times$ g for 10 min to remove insoluble material. Collect supernatant and keep it on ice for detection.
- ⑤ Meanwhile, determine the protein concentration of supernatant. (E-BC-K318-M).

Cell sample

- ① Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells).
- ② Wash cells with cold extraction solution.
- ③ Homogenize 1×10^6 cells in 0.2 mL of extraction solution with an ultrasonic cell disruptor at 4°C.
- ④ Centrifuge at 10000 $\times$ g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection.
- ⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

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 homogenization	5-10
10% Mouse kidney tissue homogenization	5-10
10% Mouse lung tissue homogenization	5-10

10% Mouse spleen tissue homogenization	5-10
1×10^6 HepG2 cells	1
1×10^6 Jurkat cells	1
1×10^6 293T cells	1
1×10^6 Hela cells	1

Note: The diluent is extraction solution. For the dilution of other sample types, please do pretest to confirm the dilution factor.

Operating steps

- ① Standard well: add 20 μ L of standard with different concentrations into the corresponding well.
Sample well: add 20 μ L of sample into the corresponding well.
Sample blank well: add 20 μ L of extraction solution into the corresponding well.
- ② Add 180 μ L of working solution to the sample wells, and add 180 μ L of buffer solution to the standard wells.
- ③ Mix fully for 5 s. Measure the fluorescence value of each well at the excitation wavelength of 535 nm and the emission wavelength of 587 nm with a fluorescence microplate reader, recorded as F1.
- ④ Incubate at 37°C for 30 min (protected from light).
- ⑤ Mix fully for 5 s. Measure the fluorescence value of each well at the excitation wavelength of 535 nm and the emission wavelength of 587 nm with a fluorescence microplate reader, recorded as F2. The standard curve is fitted to the F1 value of the standard wells.

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 Corrected fluorescence value.
3. Plot the standard curve by using Corrected 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:

Tissue or cell sample:

Definition: One unit of enzyme is defined as the amount of enzyme in 1 g protein that catalyzes the production of 1 μmol of NADH per minute at 37°C.

$$\alpha\text{-KGDH activity (U/gprot)} = (\Delta F_{\text{sample}} - \Delta F_{\text{control}} - b) \div a \times f \div C_{\text{pr}} \div T$$

[Note]

ΔF_{sample} : The fluorescence value of the sample well, $F_2 - F_1$.

$\Delta F_{\text{control}}$: The fluorescence value of the control well, $F_2 - F_1$.

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

f: Dilution factor of sample before test.

T: Reaction time, 30 min.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three mouse liver tissue samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Three human serum samples were assayed in replicates of 20 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	0.02	0.10	2.60
%CV	3.2	4.5	4.7

Inter-assay Precision

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

Three human serum samples were assayed 17 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	0.02	0.10	2.60
%CV	8.7	8.5	8.3

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 99.3%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/L)	0.02	0.10	2.60
Observed Conc. (U/L)	0.02	0.10	2.55
Recovery rate (%)	100	100	98

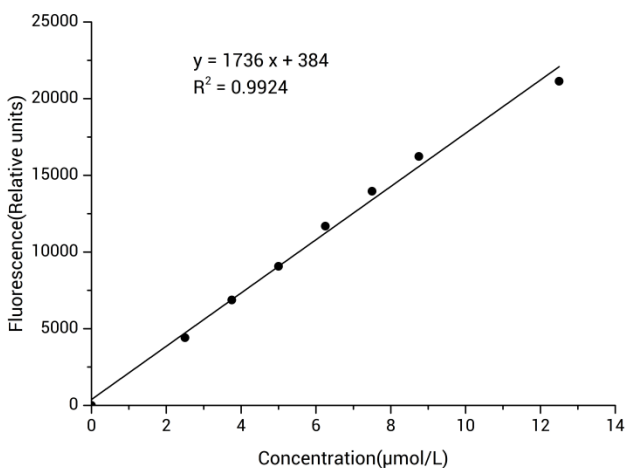
Sensitivity

The analytical sensitivity of the assay is 0.0004 U/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 ($\mu\text{mol/L}$)	0	2.50	3.75	5	6.25	7.50	8.75	12.50
Fluorescence value	10	4459	6844	9353	11771	14174	15720	22059
	14	4394	6919	8810	11628	13781	16764	20233
Average fluorescence value	12	4427	6882	9082	11700	13978	16242	21146
Absoluted fluorescence value	0	4415	6870	9070	11688	13966	16230	21134



Appendix II Example Analysis

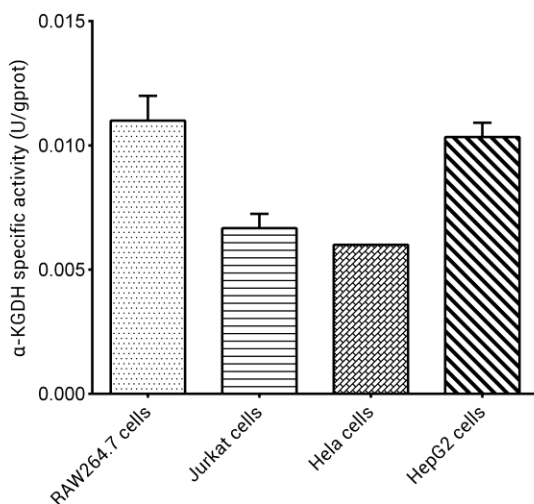
Example analysis:

Take 20 μL of 10% RAW264.7 cells homogenate supernatant to the well of microplate. Proceed according to the operating steps. The results are as follows:

standard curve: $y = 1736x + 384$. The F_1 of sample is 3499, the F_2 of sample is 6095, $\Delta F_{\text{sample}} = F_2 - F_1 = 6095 - 3499 = 2596$, The F_1 of control is 3245, the F_2 of control is 3606, $\Delta F_{\text{control}} = F_2 - F_1 = 3606 - 3245 = 361$, the concentration of protein is 3.10 gprot/L, and the calculation result is:

$$\begin{aligned}\alpha\text{-KGDH activity (U/gprot)} &= (2596 - 362 - 384) \div 1736 \div 3.1 \div 30 \\ &= 0.011 \text{ U/gprot}\end{aligned}$$

Detect 1×10^6 RAW264.7 cells (the concentration of protein is 3.10 gprot/L), 1×10^6 Jurkat cells (the concentration of protein is 3.39 gprot/L), 1×10^6 Hela cells (the concentration of protein is 3.01 gprot/L) and 1×10^6 HepG2 cells (the concentration of protein is 3.78 gprot/L), 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.

