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

Catalog No：E-BC-K912-M

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

Measuring instrument：Microplate reader (440-460 nm)

Detection range：0.054-40 U/L

Elabscience® α-Ketoglutarate Dehydrogenase (α-KGDH) Activity Colorimetric 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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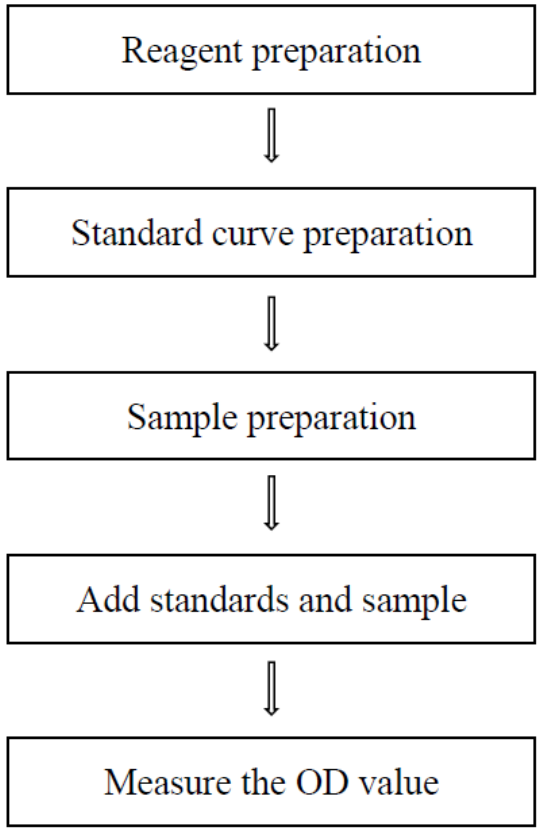
Assay summary



Intended use

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

Detection principle

α-Ketoglutarate Dehydrogenase (α-KGDH) is a key enzyme in the tricarboxylic acid (TCA) cycle. α-KGDH catalyzes the conversion of α-ketoglutarate and NAD^+ to NADH and succinyl-CoA. Concurrently, NADH transfers hydrogen to convert WST-8 into the chromogenic formazan, and the activity of α-KGDH is determined by measuring the absorbance at 450 nm.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Extraction Solution	53 mL ×1 vial	53 mL × 2 vials	-20°C, 12 months
Reagent 2	Buffer Solution	13 mL ×1 vial	26 mL ×1 vial	-20°C, 12 months
Reagent 3	Substrate	powder × 4 vials	powder × 8 vials	-20°C, 12 months, shading light
Reagent 4	Chromogenic Agent	1.3 mL × 1 vial	2.6 mL × 1 vial	-20°C, 12 months, shading light
Reagent 5	Stop Solution	1.3 mL × 1 vial	2.6 mL × 1 vial	-20°C, 12 months
Reagent 6	Standard	powder × 1 vial	powder × 2 vials	-20°C,12 months, shading light
	Microplate	48 wells	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

Microplate reader (440-460 nm, optimum wavelength: 450 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 Preparation of reaction working solution:
Dissolve one vial of substrate with 3.35 mL of buffer solution. The reaction working solution should be freshly prepared before use. Stable for 8 h when stored at -20°C protected from light.

③ Preparation of 1.2 mmol/L Standard Solution:
Dissolve one vial of substrate with 1.67 mL of double distilled water. Aliquot and store unused standard solution at -20°C protected from light for up to 7 days.

④ Standard curve preparation：

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

Dilute 1.2 mmol/L standard solution with Buffer solution diluent to a serial concentration. The recommended dilution gradient is as follows: 0, 0.24, 0.36, 0.48, 0.6, 0.72, 0.84, 1.2 mmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**0.24**	**0.36**	**0.48**	**0.6**	**0.72**	**0.84**	**1.2**
1.2 mmol/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 μL extraction solution with a dounce homogenizer at 4℃.

④ Centrifuge at 10000×g for 10 min at 4℃ 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 extraction solution.

③ Homogenize 1×10^6 cells in 200 μL extraction solution with a ultrasonic cell disruptor at 4℃.

④ Centrifuge at 10000×g for 10 min at 4℃ 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).

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	1
10% Mouse kidney tissue homogenization	1
10% Mouse heart tissue homogenization	1
10% Mouse lung tissue homogenization	1
1×10^6 RAW264.7 cells	1
1×10^6 Jurkat 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 μL of standard solution with different concentrations to the corresponding wells.
Sample well: Add 20 μL of sample to the corresponding wells.

② Add 200 μL of reaction working solution to each well.

③ Add 20 μL of chromogenic Agent to each well.

④ Mix fully with microplate reader for 3 s. Measure the initial OD value (A_1) of each well at 450 nm with microplate reader.

⑤ Incubate at 37°C protected from light for 30 min.

⑥ Add 20 μL of stop solution to each well. Mix fully with microplate reader for 3 s. Measure the initial OD value (A_2) of each well at 450 nm with microplate reader.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard #①) from all standard readings. This is the Corrected OD value.

3. Plot the standard curve by using Corrected OD 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 enzyme in 1 g protein will catalyze substrate to produce 1 μmol product per 1 min at 37 °C.

$$\underset{(U/L)}{\text{α-KGDH activity}} = (\Delta A_{450} - b) \div a \div T \times f \times 1000$$

Serum and plasma sample

Definition: One unit of enzyme is defined as the amount enzyme in 1 L plasma or serum will catalyze substrate to produce 1 μmol product per 1 min at 37 °C.

$$\underset{(U/gprot)}{\text{α-KGDH activity}} = (\Delta A_{450} - b) \div a \div T \times f \div C_{pr} \times 1000$$

[Note]

ΔA_{450}: The OD value of the sample well changes., A_2 - A_1.

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

f: Dilution factor of sample before test.

T: Reaction time, 30 min.

1000: 1 mmol/L= 1000 μmol/L.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three mouse liver 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)	4	16	40
%CV	3.1	3.9	4.5

Inter-assay Precision

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

Three mouse liver 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)	4	16	40
%CV	5.2	6.4	7.3

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%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/L)	4	16	40
Observed Conc. (U/L)	3.92	15.98	39.99
Recovery rate (%)	98	100	100

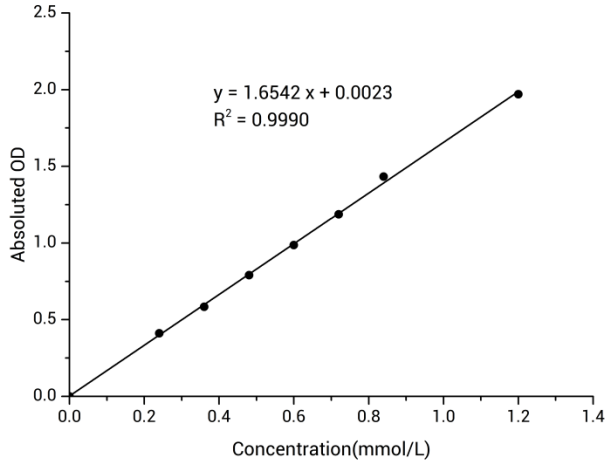
Sensitivity

The analytical sensitivity of the assay is 0.054 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 OD 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 (mmol/L)	0	0.24	0.36	0.48	0.60	0.72	0.84	1.2
OD	0.048	0.480	0.639	0.854	1.062	1.254	1.525	2.018
	0.048	0.439	0.625	0.822	1.007	1.217	1.438	2.018
Average OD	0.048	0.460	0.632	0.838	1.035	1.236	1.482	2.018
Absoluted OD	0.000	0.412	0.584	0.790	0.987	1.188	1.434	1.970



Appendix Ⅱ Example Analysis

Example analysis：

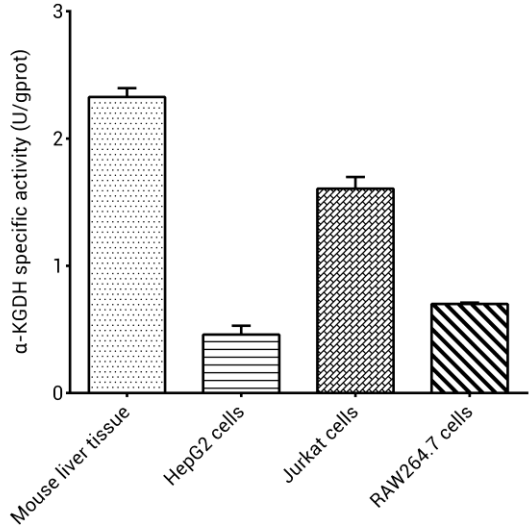
Take 20 μL of 10% mouse liver tissue homogenate supernatant to the well of microplate. Proceed according to the operating steps. The results are as follows:

standard curve: y = 1.393 x + 0.0023. The A_1 of sample is 0.254, the A_2 of sample is 1.393, $\Delta A_{405} = A_2 - A_1 = 1.393 - 0.254 = 1.139$, the concentration of protein is 9.86 gprot/L, and the calculation result is:

$$\text{α-KGDH activity (U/gprot)} = (1.139 - 0.0023) \div 1.6542 \div 30 \div 9.86 \times 1000$$

$$= 2.32 \text{ U/gprot}$$

Detect 10% mouse liver tissue homogenization (the concentration of protein is 13.22 gprot/L), 1.0×10^6 HepG2 cells (the concentration of protein is 3.78 gprot/L), 1.0×10^6 RAW264.7 cells (the concentration of protein is 3.10 gprot/L) and 1.0×10^6 Jurkat cells (the concentration of protein is 3.39 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.