PRODUCT INFORMATION & MANUAL

alpha-Ketoglutarate Dehydrogenase Activity Assay Kit (Colorimetric)

NBP3-25918

For research use only.
Not for diagnostic or therapeutic procedures.

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αlphα-Ketoglutαrαte Dehydrogenαse Activity Assαy Kit (Colorimetric)

Catalog No: NBP3-25918

Method: Colorimetric method

Specification: 96T (Can detect 40 samples without duplication)

Instrument: Microplate reader

Sensitivity: 8.3 U/L

Detection range: 8.3-42.3 U/L

Average intra-assay CV (%): 1.5

Average inter-assay CV (%): 8.5

Average recovery rate (%): 100

▲ This kit is for research use only.
▲ Instructions should be followed strictly, changes of operation may result in unreliable results.
▲ Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

This kit can measure α-ketoglutarate dehydrogenase activity in serum, plasma, animal and plant tissue samples.

▲Detection principle

α-ketoglutarate dehydrogenase (α-KGDH) is a key enzyme in the tricarboxylic acid (TCA) cycle. While the substrate being catalyzed by α-KGDH, NAD^+ is reduced to NADH, and electrons are transfered to WST-8 under the action of hydrogen transmitter to produce yellow product. The activity of α-KGDH can be calculated by measuring the change of absorbance value at 450 nm.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Extraction Solution	55 mL × 1 vial	-20℃ , 12 months
Reagent 2	Buffer Solution	28 mL × 1 vial	-20℃ , 12 months
Reagent 3	Substrate	powder × 1 vial	-20℃ , 12 months, shading light
Reagent 4	Chromogenic Agent	3 mL×1 vial	-20℃ , 12 months, shading light
Reagent 5	Clarificant	3 mL×1 vial	-20℃ , 12 months
Reagent 6	Standard	powder × 1 vial	-20℃ , 12 months
	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.			

▲Materials prepared by users

Instruments

Incubator, Centrifuge, Microplate reader (440-460 nm, optimum wavelength: 450 nm)

⚠ Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

⚠ Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

▲ The key points of the assay

The powder must be completely dissolved while preparing the working solution.

Pre-assay preparation

▲ Reagent preparation

1. Bring all reagents to room temperature before use.
2. Preparation of working solution:

   Dissolve a vial of reagent 3 with a vial of reagent 2 and mix fully. The prepared solution can be stored at 2-8℃ for 6 h with shading light.
3. Preparation of 0.5 mmol/L standard solution:

   Dissolve a vial of reagent 6 with 2 mL double distilled water and mix fully. The prepared solution can be stored at 2-8℃ for 6 h with shading light.

▲ Sample preparation

1. Serum and plasma samples:

   Detect the sample directly.
2. Tissue sample:

   Accurately weigh the tissue, add reagent 1 at a ratio of Weight (g): Volume (mL) =1:9 and homogenize the sample in ice water bath. Then centrifuge at 10000 g for 15 min, then take the supernatant for detection. Meanwhile, determine the protein concentration of supernatant. If the supernatant is turbidity after centrifugation, repeated centrifuge until clear before use.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (8.3-42.3 U/L).

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

Sample type	Dilution factor
10% Rat liver tissue homogenate	1
10% Rat lung tissue homogenate	1
10% Mouse liver tissue homogenate	1
10% Epipremnum aureum tissue homogenate	1
10% Mouse spleen tissue homogenate	1
Rat plasma	1

Note: The diluent is reagent 1.

Assay protocol

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S1'	S9	S9'	S17	S17'	S25	S25'	S33	S33'
B	B	B	S2	S2'	S10	S10'	S18	S18'	S26	S26'	S34	S34'
C	C	C	S3	S3'	S11	S11'	S19	S19'	S27	S27'	S35	S35'
D	D	D	S4	S4'	S12	S12'	S20	S20'	S28	S28'	S36	S36'
E	E	E	S5	S5'	S13	S13'	S21	S21'	S29	S29'	S37	S37'
F	F	F	S6	S6'	S14	S14'	S22	S22'	S30	S30'	S38	S38'
G	G	G	S7	S7'	S15	S15'	S23	S23'	S31	S31'	S39	S39'
H	H	H	S8	S8'	S16	S16'	S24	S24'	S32	S32'	S40	S40'

Note:A-H, standard wells; S1-S40, sample wells; S1'-S40', control wells.

▲Detailed operation steps

1. The preparation of standard curve

Dilute 0.5 mmol/L standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 0.1, 0.2, 0.25, 0.3, 0.35, 0.4, 0.5 mmol/L. Reference is as follows:

Number	Standard concentrations (mmol/L)	0.5 mmol/L standard solution (μL)	Double distilled water (μL)
A	0	0	200
B	0.1	40	160
C	0.2	80	120
D	0.25	100	100
E	0.3	120	80
F	0.35	140	60
G	0.4	160	40
H	0.5	200	0

2. The measurement of samples

(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.

Control well: Add 20 μL of sample to the corresponding wells..

(2) Add 200 μL of working solution to standard well and sample well.

Add 200 μL of double distilled water to control well.

(3) Add 20 μL of regent 4 to each well.

(4) Mix fully with microplate reader for 3 s, incubate at 37°C for 10 min with shading light.

(5) Add 20 μL of regent 5 to each well.

(6) Mix fully with microplate reader for 3 s, and measure the OD value of each well at 450 nm with microplate reader.

▲Summary operation table

	Standard well	Sample well	Control well
Standard solution with different concentrations (μL)	20		
Sample (μL)		20	20
Working solution (μL)	200	200	
Double distilled water (μL)			200
Regent 4 (μL)	20	20	20
Mix fully, incubate at 37°C for 10 min with shading light.			
Regent 5 (μL)	20	20	20
Mix fully, and measure the OD value of each well.			

▲Calculation

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample. The standard curve is: y= ax + b.

1. Tissue sample:

Definition: The amount of α-KGDH in 1 g tissue protein per 1 minute that hydrolyze the substrate to produce 1 μmol NADH at 37 °C is defined as 1 unit.

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

2. Serum/plasma sample:

Definition: The amount of α-KGDH in 1 L serum (plasma) per 1 minute that hydrolyze the substrate to produce 1 μmol NADH at 37°C is defined as 1 unit.

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

Note:

y: $OD_{Standard}$ – OD_{Blank} (OD_{Blank} is the OD value when the standard concentration is 0).

x: The concentration of Standard.

a: The slope of standard curve.

b: The intercept of standard curve.

ΔA_{450}: OD_{Sample} – $OD_{Control.}$

T: The time of reaction, 10 min.

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

f: Dilution factor of sample before tested.

1000: 1 mmol =1000 μmol.

Appendix I Data

▲Example analysis

For mouse liver tissue, take 20 μL of 10% mouse liver tissue homogenate, and carry the assay according to the operation table.

The results are as follows:

standard curve: y = 1.7013 x – 0.0006, the average OD value of the control is 0.113, the average OD value of the sample is 0.397, the concentration of protein in sample is 13.89 gprot/L, and the calculation result is:

$$\text{α-KGDH activity (U/gprot)} = (0.397 - 0.113 + 0.0006) \div 1.7013 \div 10 \times 1000 \div 13.89$$
$$= 1.20 \text{ U/gprot}$$