PRODUCT INFORMATION & MANUAL

alpha-Ketoglutarate Assay Kit (Fluorometric)

NBP3-25913

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

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alpha-Ketoglutarate Assay Kit (Fluorometric)

Catalog No: NBP3-25913

Method: Fluorimetric method

Specification: 96T (Can detect 80 samples without duplication)

Measuring instrument: Fluorescence Microplate Reader

Sensitivity: 0.60 µmol/L

Detection range: 0.60-100 µmol/L

Average intra-assay CV (%): 2.0

Average inter-assay CV (%): 4.0

Average recovery rate (%): 99

- ▲ 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 be used for the 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.

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

▲Kit components & Storage

Item	Component	Specification	Storage
Reagent 1	Buffer Solution	26 mL × 1 vial	-20℃ , 12 months
Reagent 2	Substrate	Powder × 2 vials	-20℃ , 12 months
Reagent 3	Enzyme Reagent	Powder ×2 vials	-20℃ , 12 months, shading light
Reagent 4	Probe	2.4 mL ×1 vial	-20℃ , 12 months, shading light
Reagent 5	Standard	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.			

▲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)

⚠ 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.

Pre-assay preparation

▲Reagent preparation

1. Bring all reagents to room temperature before use.
2. Preparation of reagent 2 working solution:
   Dissolve a vial of reagent 2 with 12 mL of reagent 1. Mix fully for use and the prepared solution can be stored at -20℃ for 3 days, and avoid repeated freeze-thaw.
3. The preparation of reagent 3 working solution:
   Dissolve a vial of reagent 3 with 1.2 mL of double distilled water. Mix fully and preserve it on the ice box with shading light for use. Prepare the fresh needed amount before use.
4. The preparation of 50 mmol/L standard solution:
   Dissolve a vial of reagent 5 with 1 mL of double distilled water. Mix fully for use and the prepared solution can be stored at -20℃ with shading light for 3 days.
5. The preparation of 100 μmol/L standard solution:

   Mix the 50 mmol/L standard and double distilled water at a ratio of 1:499. The prepared solution can be stored at -20℃ with shading light for 3 days.

▲Reagent preparation

1. Serum and plasma and urine samples:

The samples were centrifuged with a 50 KD ultrafiltration tube at 4°C 12000 g for 15 min, and filtrate was collected to be measured.

2. Tissue sample:

Accurately weigh the tissue, add normal saline (0.9% NaCl) at a ratio of Weight (g): Volume (mL) =1:9 and homogenize the sample. Centrifuged with 12000 g at 4°C for 15 min, and the supernatant was centrifuged with a 50 KD ultrafiltration tube with 12000 g at 4°C for 15 min. The filtrate was collected for measurement.

3. Cell sample:

Collect the cells and wash the cells with PBS (0.01 M, pH 7.4) for 1~2 times. Centrifuge at 1000 g for 10 min, discard the supernatant and keep the cell sediment. Add homogenization medium at a ratio of cell number (10^6): normal saline (0.9% NaCl) (μL) =1: 200. Sonicate or mechanical homogenate in ice water bath. Then centrifuge at 12000 g for 15 min at 4°C. The supernatant was centrifuged at 12000 g at 4°C for 15 min with a 50 KD ultrafiltration tube, and the filtrate was collected for measurement.

▲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 (0.60-100 μmol/L).

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
Rat serum	2-3
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

Note: The diluent is normal saline (0.9% NaCl).

Assay protocol

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	B	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	C	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	D	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	E	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	F	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	G	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	H	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

Note:A-H, standard wells; S1-S80, sample wells.

▲ Detailed operation steps

1. The preparation of standard curve:

Dilute 100 μmol/L 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:

Number	Standard concentrations (μmol/L)	100 μmol/L standard solution (μL)	Double distilled water (μL)
A	0	0	200
B	10	20	180
C	20	40	160
D	30	60	140
E	50	100	100
F	60	120	80
G	80	160	40
H	100	200	0

2. The measurement of samples:

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 reagent 2 working solution into each well.

3) Add 20 μL of reagent 3 working solution into each well.

4) Add 20 μL of reagent 4 into each well.

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

▲Summary operation table

	Standard well	Sample well
Standard with different concentrations (μL)	20	
Sample (μL)		20
Reagent 2 working solution (μL)	140	140
Reagent 3 working solution (μL)	20	20
Reagent 4 (μL)	20	20
Mix fully with microplate reader for 5 s and incubate at 37℃ for 20 min with shading light. Measure the fluorescence value of each well at Ex/Em=535/587 nm. The fluorescence values of the control and sample.		

▲Calculation

Plot the standard curve by using fluorescence value (F) 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 F value of sample.

The standard curve is: y= ax + b.

1. For serum (plasma):

α-KG content (μmol/L) = (ΔF - b) ÷ a × f

2. For tissue:

α-KG content (μmol/kg wet weight) = (ΔF - b) ÷ a ÷ (m ÷ V) × f

3. For cells:

α-KG content (mmol/10^6) = (ΔF - b) ÷ a ÷ (n ÷ V) × f

Note:

y: $F_{Standard} - F_{Blank}$ (F_{Blank} is the F value when the standard concentration is 0).

x: The concentration of standards.

a: The slope of standard curve.

b: The intercept of standard curve.

ΔF: Absolute 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 cell sample, 10^6.

f: Dilution factor of sample before test.

Appendix I Data

▲Example analysis

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 = 91.661 x - 31.81, The average fluorescence value of blank well was 557.45, The average fluorescence value of the ample was 2256.69. Then the calculation result is:

α-KG content (μmol/kg wet weight) = ((2256.69 - 557.45 + 31.81) ÷ 91.661) ÷ (0.1 ÷ 0.9) = 169.97 μmol/kg wet weight