TECHNICAL MANUAL

alpha-Ketoglutarate Dehydrogenase (alpha-KGDH)Activity Assay Kit

- **SKU CODE:** MAES0236
- **SIZE:** 96 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Sample preparation
- Add standard and sample
- Add working solution
- Add chromogenic agent
- Incubate
- Add clarificant
- Measure the absorbance

2. Intended use

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

3. Detection principle

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

4. Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Extraction Solution	55 mL × 1 vial	-20°C, 12 months
Reagent 2	Buffer Solution	28 mL × 2 vials	-20°C, 12 months
Reagent 3	Substrate	Powder × 2 vials	-20°C, 12 months, protect from light
Reagent 4	Chromogenic Agent	3 mL × 1 vial	-20°C, 12 months, protect from light
Reagent 5	Clarificant	3 mL × 1 vial	-20°C, 12 months

Item	Component	Size (96 T)	Storage
Reagent 6	Standard	Powder × 1 vial	-20°C, 12 months
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

5. Materials prepared by users

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

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **The preparation of working solution:** Dissolve one vial of substrate with one vial of buffer solution, mix well to dissolve. Store at 2-8°C for 6 hours protected from light.
3. **The preparation of 0.5 mmol/L standard solution:** Dissolve one vial of standard with 2 mL of double distilled water, mix well to dissolve. Store at 2-8°C for 6 hours protected from light.
4. **The preparation of standard curve:** Always prepare a fresh set of standards. Discard working standard dilutions after use. 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.

7. Sample preparation

Serum and plasma: Detect directly.

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 extraction solution with a dounce homogenizer at 4°C.

4. Centrifuge at 10,000×g for 10 minutes at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection.

5. Meanwhile, determine the protein concentration of supernatant (MAES0177).

Dilution of sample:

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

- 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 extraction solution. For the dilution of other sample types, please perform a pretest to confirm the dilution factor.

8. The key points of the assay

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

9. 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. 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 chromogenic agent to each well.
4. Mix fully with microplate reader for 3 seconds, incubate at 37°C for 10 minutes protected from light.
5. Add 20 µL of clarificant to each well.
6. Mix fully with microplate reader for 3 seconds, and measure the OD value of each well at 450 nm with microplate reader.

10. 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 absolute OD value.

3. Plot the standard curve by using absolute 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:

1. Serum (plasma) sample:

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

α-KGDH activity (U/L) = (ΔA_450 - b) ÷ a ÷ T × 1000 × f

2. Tissue sample:

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

α-KGDH activity (U/gprot) = (ΔA_450 - b) ÷ a ÷ T × 1000 ÷ C_{pr} × f

[Note]

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 test.

1000: 1 mmol = 1000 μmol.

11. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

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

Intra-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	12.50	27.60	33.80
%CV	1.7	1.4	1.4

Inter-assay Precision

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

Inter-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	12.50	27.60	33.80
%CV	8.2	8.5	8.8

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 100%.

Recovery

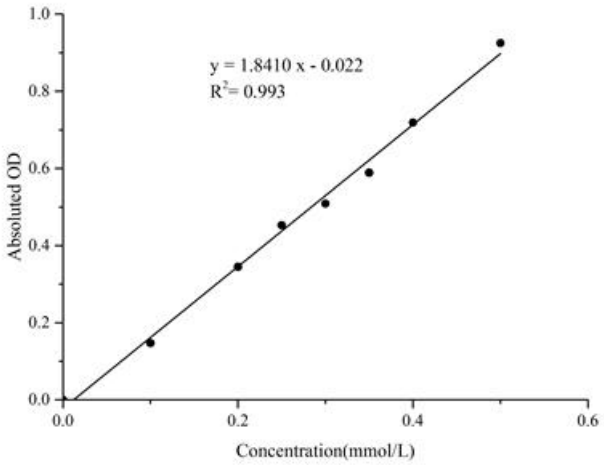
	Standard 1	Standard 2	Standard 3
Expected Conc. (mmol/L)	0.15	0.28	0.37
Observed Conc. (mmol/L)	0.2	0.3	0.4
Recovery rate (%)	101	98	101

Sensitivity

The analytical sensitivity of the assay is 8.3 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.

Standard curve

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), the standard curve and data are provided below for reference only:



Standard curve

Concentration (mmol/L)	Average OD	Absolute OD
0	0.091	0
0.1	0.238	0.147
0.2	0.436	0.345
0.25	0.545	0.453
0.3	0.601	0.509
0.35	0.681	0.589
0.4	0.811	0.719
0.5	1.017	0.925

12. Appendix II Example Analysis

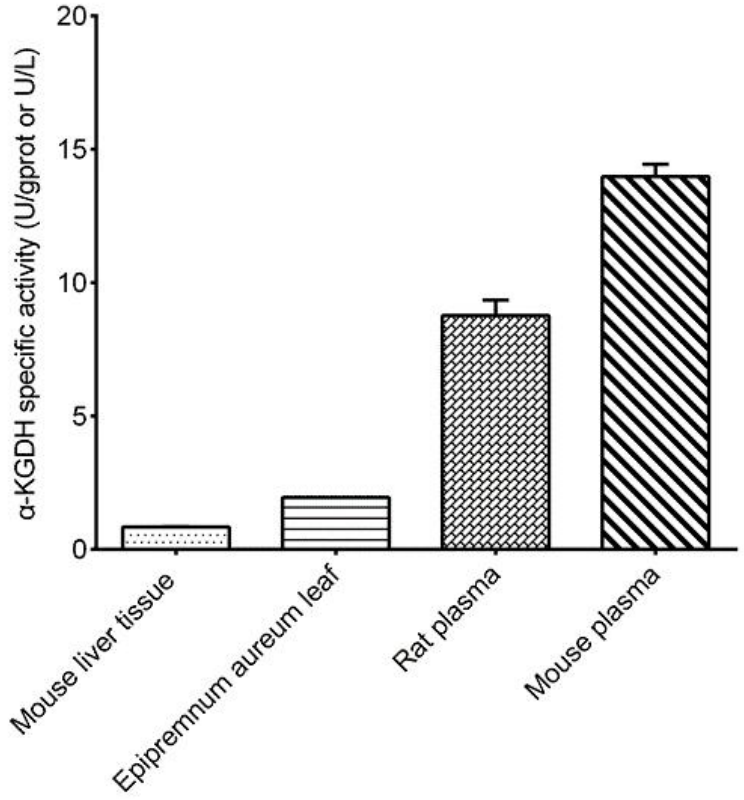
Example analysis:

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

Standard curve: y = 1.7013x – 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:

α-KGDH activity (U/gprot) = (0.397 – 0.113 + 0.0006) ÷ 1.7013 ÷ 10 × 1000 ÷ 13.89 = 1.20 U/gprot

Detect 10% mouse liver tissue homogenate (the concentration of protein is 13.89 gprot/L), 10% epipremnum aureum tissue homogenate (the concentration of protein is 3.63 gprot/L), rat plasma and mouse plasma according to the protocol, the result is as follows:



13. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.
2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.
3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.
4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.

5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.
6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.