TECHNICAL MANUAL

Alpha-Ketoglutarate Fluorometric Assay Kit

- **SKU CODE:** MAES0258
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Add standards and samples
- Measure fluorescence values

2. Intended use

This kit can be used for determination of α-Ketoglutarate (α-KG) content in serum, plasma, urine, animal tissue and cell samples.

3. 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.

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

4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	13 mL × 1 vial	26 mL × 1 vial	-20°C, 12 months
Reagent 2	Substrate	Powder × 1 vial	Powder × 2 vials	-20°C, 12 months
Reagent 3	Enzyme Reagent	Powder × 1 vial	Powder × 2 vials	-20°C, 12 months, shading light
Reagent 4	Probe	1.2 mL × 1 vial	2.4 mL × 1 vial	-20°C, 12 months, shading light

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 5	Standard	Powder × 2 vials	Powder × 2 vials	-20°C, 12 months, shading light
	Black Microplate	96 wells	96 wells	No requirement
	Plate Sealer	2 pieces	2 pieces	

5. 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)

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of substrate working solution:** Dissolve one vial of substrate with 12 mL of buffer solution, mix well to dissolve. Store at -20°C for 3 days. Avoid repeated freeze/thaw cycles.
3. **Preparation of enzyme working solution:** Dissolve one vial of enzyme reagent with 1.2 mL of double distilled water, mix well and keep on ice during use. The enzyme working solution should be prepared fresh.
4. **Preparation of 50 mmol/L standard solution:** Dissolve one vial of standard with 1 mL of double distilled water, mix well. Store at -20°C for 3 days protected from light.
5. **Preparation of 100 μmol/L standard solution:** Dilute 2 μL of 50 mmol/L standard solution with 998 μL of double distilled water and mix well. Store at -20°C for 3 days protected from light.
6. **Preparation of standard curve:** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 100 μmol/L pyruvic acid 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.

7. Sample preparation

Sample preparation:

Serum and plasma: The samples were centrifuged with a 50 KD ultrafiltration tube at 4°C, 12000×g for 15 min, and filtrate was collected for measurement.

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 normal saline (0.9% NaCl) with a dounce homogenizer at 4°C.

4. Centrifuge at 12000×g at 4°C for 15 min to remove insoluble material. Collect supernatant and keep it with a 50 KD ultrafiltration tube at 4°C, 12000×g for 15 min, and filtrate was collected for measurement.

Cell (adherent or suspension) samples:

1. Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells).

2. Wash cells with PBS (0.01 M, pH 7.4).

3. Homogenize 2×10^6 cells in 200 µL normal saline (0.9% NaCl) with an ultrasonic cell disruptor at 4°C.

4. Centrifuge at 12000×g for 15 minutes to remove insoluble material. Collect supernatant and keep it with a 50 KD ultrafiltration tube at 4°C, 12000×g for 15 min, and filtrate was collected for measurement.

Dilution of sample:

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

10% Rat kidney tissue homogenate: 1

10% Rat lung tissue homogenate: 1

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

Rat serum: 2-3

Note: The diluent is normal saline (0.9% NaCl). For the dilution of other sample types, please perform a pretest to confirm the dilution factor.

8. Operating steps

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 substrate working solution into each well.
3. Add 20 µL of enzyme working solution into each well.
4. Add 20 µL of probe into each well.
5. Mix fully with microplate for 5 s and incubate at 37°C for 20 min protected from light. Measure the fluorescence value of each well at Ex/Em=535/587 nm.

9. 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 absolute fluorescence value.

3. Plot the standard curve by using absolute fluorescence value of standard and corresponding 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) samples:

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

2. Tissue samples:

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

3. Cell samples:

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

[Note]

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 cells, 10^6.

f: Dilution factor of sample before test.

10. 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)

Inter-assay Precision

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

Recovery

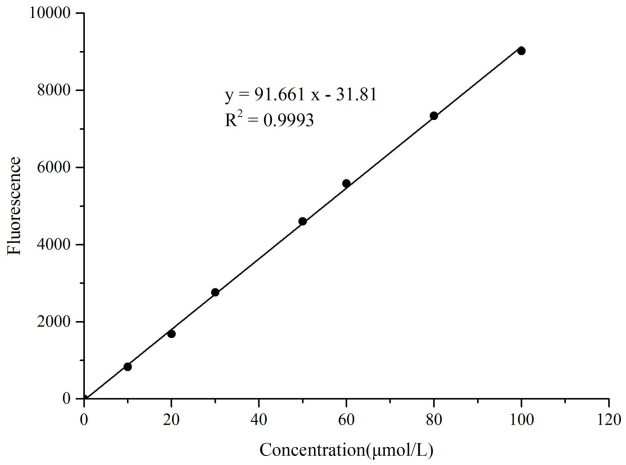
Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times in parallel to get the average recovery rate of 99%.

Sensitivity

The analytical sensitivity of the assay is 0.60 µmol/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 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), the standard curve and data are provided below for reference only:



11. Appendix II Example Analysis

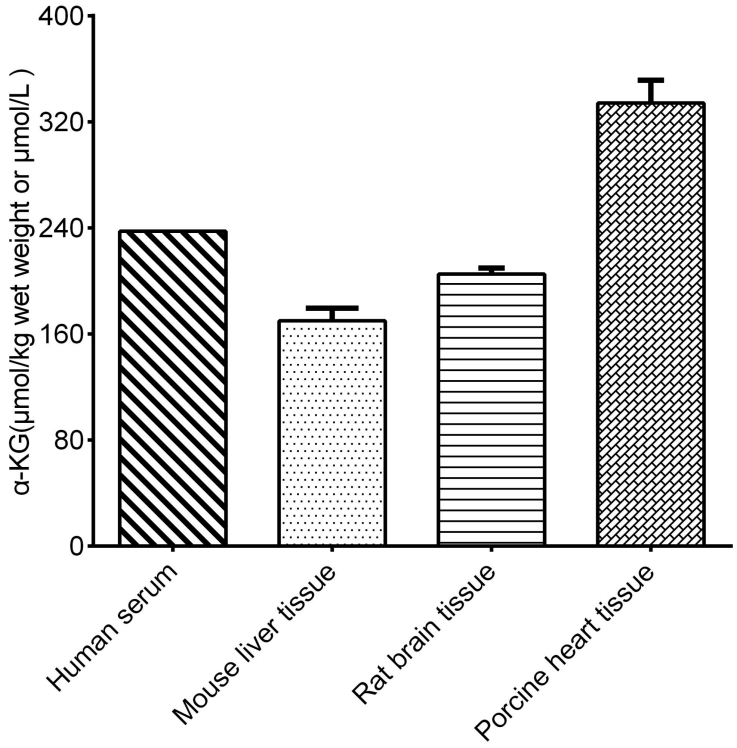
Example analysis:

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 = 91.661x – 31.81. The average fluorescence value of blank well is 557.45. The average fluorescence value of the sample well is 2256.69, and 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

Detect human serum, 10% mouse liver tissue homogenate, 10% rat brain tissue homogenate, and 10% porcine heart tissue homogenate according to the protocol, the result is as follows:



12. 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.