Alpha-Ketoglutarate

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1327

(Version 1.1A)

Detection and Quantification of Alpha-Ketoglutarate (α-KG) Content in Serum, Plasma, Tissue extracts, Cell culture media, Other biological fluids Samples.

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

I. INTRODUCTION

Alpha-Ketoglutarate is a central metabolite in the cellular tricarboxylic acid cycle and a critical node linking carbon and nitrogen metabolism, involved in energy production, amino acid synthesis, and epigenetic regulation. Abnormal serum levels can indicate various metabolic disorders: significant elevation is often seen in liver dysfunction and inherited organic acidemias, while specific decreases may reflect nutritional or metabolic alterations.

Alpha-Ketoglutarate Colorimetric Microplate Assay Kit is a sensitive assay for determining Alpha-Ketoglutarate content in various samples. The kit is based on Glutamate dehydrogenase catalyzed oxidation of Alpha-Ketoglutarate. The intensity of the product color of formazan, measured at 450 nm, is inversely proportionate to the Alpha-Ketoglutarate concentration in the sample.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	8 ml x 1	4 °C
Diluent	20 ml x 1	4 °C
Coenzyme	Powder x 1	4 °C, keep in dark
Enzyme	Powder x 1	-20 °C
Dye Reagent A	Powder x 1	4 °C, keep in dark
Dye Reagent B	1 ml x 1	4 °C, keep in dark
Standard	Powder x 2	4 °C, keep in dark
Technical Manual	1 Manual	

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 450 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Centrifuge
6. Timer

IV. REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening. Dissolve each vial in 1 ml Diluent to generate 20 mmol/L of standard solution. Then dilute to 5 mmol/L standard top solution by adding 100 µl 20 mmol/L standard solution into 300 µl diluent**.** Perform 2-fold serial dilutions of the top standard solution using diluent to make the standard curve. The concentration of standard curve could be 5/2.5/1.25/0.625/0.312/0.156/0.078 mmol/L. All concentration standard solutions should be stored at 4°C and used within 2 hours.

Substrate: Briefly centrifuge prior to opening. Add 1 ml Diluent to dissolve before use. Keep in dark and store at 4°C for 1 day or -20 °C for 2-3 weeks.

Enzyme: Briefly centrifuge prior to opening. Add 1 ml Assay Buffer to dissolve before use. Store at 4°C for 1-2 days or -20 °C for 1 month.

Dye Reagent A: Add 8 ml distilled water to dissolve before use, mix. Keep in dark and store at 4°C for 1 week or -20°C for 1 month.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. SAMPLE PREPARATION

1. For liquid samples

Detect directly, or dilute with Assay Buffer.

2. For tissue samples

Weigh 0.1 g tissue, homogenize with 1 ml Assay buffer, centrifuged at 10000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube for detection.

VI. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent*	Sample**	Control	Standard	Blank
Reaction Buffer	80 µl	80 µl	80 µl	80 µl
Substrate	10 µl	10 µl	10 µl	--
Sample	10 µl		--	--
Distilled water	--	10 µl	--	20 µl
Standard	--		10 µl	--
Enzyme	10 µl	10 µl	10 µl	10 µl
Mix, put it into the convection oven, 37 °C for 15 minutes				
Dye Reagent A	80 µl	80 µl	80 µl	80 µl
Dye Reagent B	10 µl	10 µl	10 µl	10 µl
Mix, keep at room temperature for 1 minute, record absorbance measured at 450nm.				

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

** The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

VII. CALCULATION

1. Plot the standard curve with the concentration of standard on the x-axis and the $OD_{(Control)} - OD_{(Standard)}$ on the y-axis. The trendline format can be set to Power as follow.

$y = ax^b$

2. Calculate the sample concentration in ASSAY PROCEDURE according to the Coefficient(a) and Power(b) of the standard curve

$$C = \sqrt[Power(b)]{\frac{OD_{Control} - OD_{Sample}}{Coeffient(a)}} \quad (mmol/L)$$

3. Calculate the initial concentration according to sample preparation procedure.

3.1 According to the weight of sample

$$C_{Initial} = \frac{C_{Sample}}{W \times (V_{Sample} / V_{Assay})} \quad (\mu mol/g)$$

3.2 According to the volume of sample

$C_{Initial} = C_{Sample} \times n \quad (\mu mol/ml)$

a: The Coefficient of the standard curve

b: The Power of the standard curve

V_{Sample}: the volume of sample in assay procedure, µl

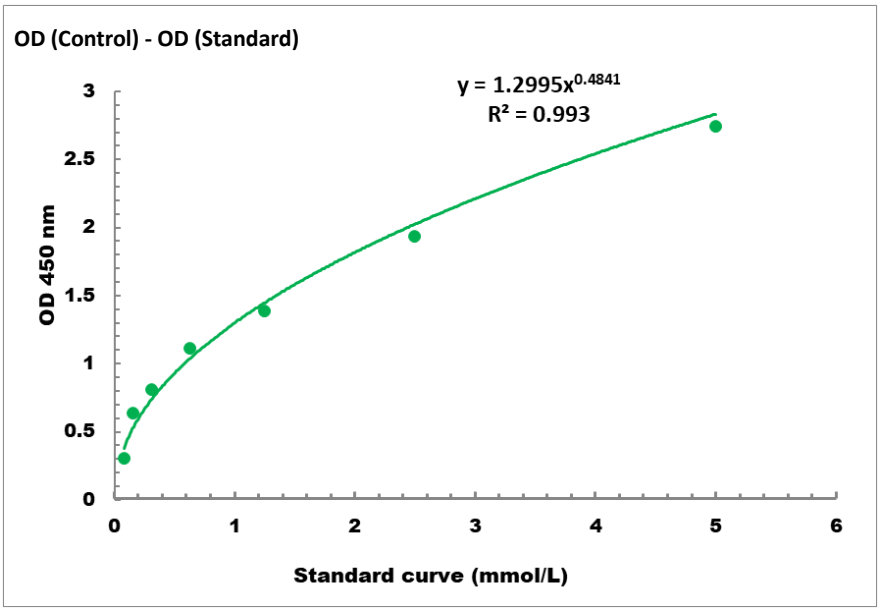
V_{Assay}: the volume of Assay Buffer, µl

W: the weight of sample, g

n: the dilution factor

VIII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.05 mmol/L - 5 mmol/L