



Alpha-Ketoglutarate Dehydrogenase Activity Colorimetric Microplate Assay Kit User Manual

Catalog # CAK1122

(Version 1.5B)

Detection and Quantification of Alpha-Ketoglutarate

Dehydrogenase (α -KGDH) Activity in Tissue extracts, Cell lysate, Cell
culture media and Other biological fluids Samples.

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

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I. INTRODUCTION

Alpha-Ketoglutarate Dehydrogenase (EC1.2.4.2) is a key control point in the citric acid cycle. It is inhibited by its products, succinyl CoA and NADH. A high energy charge in the cell will also be inhibitive. ADP and calcium ions are allosteric activators of the enzyme.

By controlling the amount of available reducing equivalents generated by the Krebs cycle, Alpha-Ketoglutarate Dehydrogenase has a downstream regulatory effect on oxidative phosphorylation and ATP production. Reducing equivalents (such as NAD⁺/NADH) supply the electrons that run through the electron transport chain of oxidative phosphorylation. Increased Alpha-Ketoglutarate Dehydrogenase activation levels serve to increase the concentrations of NADH relative to NAD⁺. High NADH concentrations stimulate an increase in flux through oxidative phosphorylation. While an increase in flux through this pathway generates ATP for the cell, the pathway also generates free radical species as a side product, which can cause oxidative stress to the cells if left to accumulate.

Alpha-Ketoglutarate Dehydrogenase is considered to be a redox sensor in the mitochondria, and has an ability to change the functioning level of mitochondria to help prevent oxidative damage. In the presence of a high concentration of free radical species, Alpha-Ketoglutarate Dehydrogenase undergoes fully reversible free radical mediated inhibition. In extreme cases, the enzyme can also undergo complete oxidative inhibition.

Alpha-Ketoglutarate Dehydrogenase Activity Colorimetric Microplate Assay Kit provides a sensitive and convenient method for alpha-ketoglutarate dehydrogenase activity determination. The assay is used to determine alpha-ketoglutarate dehydrogenase. The enzyme catalysed reaction products NADH, can be measured at a colorimetric readout at 450 nm.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer I	30 ml x 4	4 °C
Assay Buffer II	1.2 ml x 1	4 °C
Assay Buffer III	20 ml x 1	4 °C
Substrate Diluent	13 ml x 1	4 °C
Substrate	Powder x 1	-20 °C
Dye Reagent A	Powder x 1	4 °C
Dye Reagent B	1 ml x 1	4 °C
Standard	Powder x 1	-20 °C
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. Mortar
6. Centrifuge
7. Timer
8. Ice

IV. REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening. Dissolve in 1 ml distilled water to generate 2 mmol/L of standard stock solution, store at 4 °C for 1 day or -20°C for 3 days after reconstitution. Then dilute to 400 µmol/L standard top solution by adding 0.2 ml stock solution into 0.8 ml distilled water. Perform 2-fold serial dilutions of the top standard solution using distilled water to make the standard curve. The concentration of standard curve could be 400/200/100/50/25/12.5/6.25 µmol/L.

Substrate: Add 13 ml Substrate Diluent to dissolve before use. Keep in dark for immediate use or store at -20°C for 1 month after reconstitution.

Dye Reagent A: Briefly centrifuge prior to opening. Add 5 ml distilled water to dissolve before use, mix, store at 4°C for 1 month after reconstitution.

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

V. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 0.99ml Assay Buffer I and 10 μ l Assay Buffer II on ice, centrifuged at 600g 4°C for 5 minutes. Take the supernatant into a new centrifuge tube, 11000g 4°C for 10 minutes, discard the supernatant. Add 198 μ l Assay Buffer III and 2 μ l Assay Buffer II to the precipitation, shock, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times). Centrifuged at 11000g 4°C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 0.99ml Assay Buffer I and 10 μ l Assay Buffer II on ice, centrifuged at 600g 4°C for 5 minutes. Take the supernatant into a new centrifuge tube, 11000g 4°C for 10 minutes, discard the supernatant. Add 198 μ l Assay Buffer III and 2 μ l Assay Buffer II to the precipitation, shock, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times). Centrifuged at 11000g 4°C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For liquid samples

Detect directly or dilute with Assay Buffer.

VI. ASSAY PROCEDURE

Warm all reagents to room temperature before use.

Add following reagents in the microplate:

Reagent*	Sample**	Control	Standard	Blank
Substrate	130 μ l	130 μ l	--	--
Distilled water	--	10 μ l	40 μ l	140 μ l
Sample	10 μ l	--	--	--
Mix and incubate at 37°C for 5 minutes.				
Standard	--	--	100 μ l	--
Dye Reagent A	50 μ l	50 μ l	50 μ l	50 μ l
Dye Reagent B	10 μ l	10 μ l	10 μ l	10 μ l
Mix and incubate at room temperature for 2 minutes, record absorbance measured at 450 nm.				

Note:

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

**For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more samples into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

Unit Definition: One unit of α -KGDH activity is defined as the enzyme generates 1 μmol of NADH per minute.

1. According to the slope of the standard curve

$$\text{Activity} = \frac{(\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) - \text{Intercept}}{\text{Slope} \times T} \times \frac{V_{\text{Standard}}}{V_{\text{Sample}}} \times n (\text{U/mL})$$

2. According to one point of the standard OD value and concentration

2.1 According to the protein concentration of sample

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times V_{\text{Sample}} \times C_{\text{Protein}} \times T} (\text{U/mg})$$

2.2 According to the quantity of cells or bacteria

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times N \times (V_{\text{Sample}} / V_{\text{Assay}}) \times T} (\text{U}/10^4)$$

2.3 According to the weight of sample

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times W \times (V_{\text{Sample}} / V_{\text{Assay}}) \times T} (\text{U/g})$$

2.4 According to the volume of sample

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times V_{\text{Sample}} \times T} (\text{U/mL})$$

Slope: the absorbance slope of standard curve

n: the dilution factor

C_{Protein} : the protein concentration of sample, mg/mL

W: the weight of total sample, g

N: the quantity of total cell or bacteria sample, 10^4

C_{Standard} : the concentration of standard, $\mu\text{mol/L}$

V_{Standard} : the volume of standard in assay procedure, mL

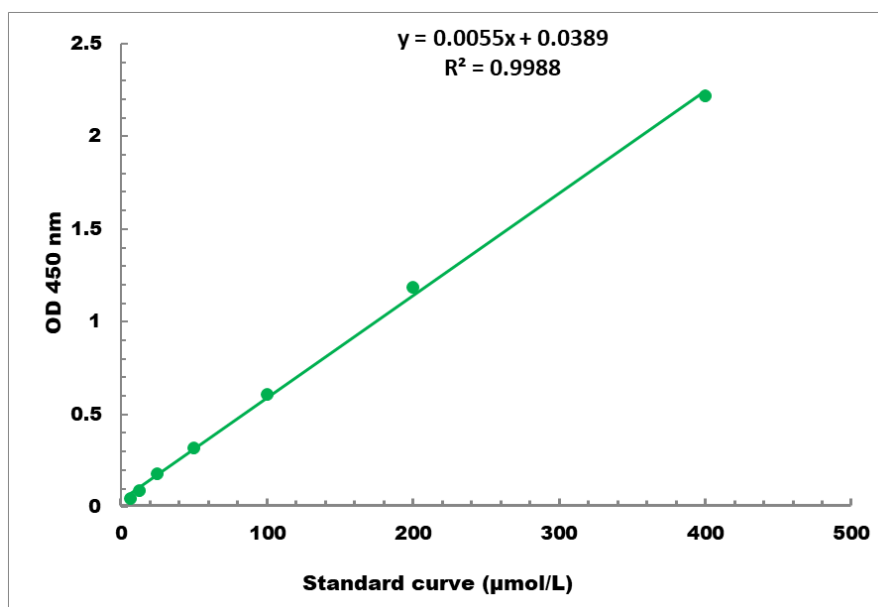
V_{Sample} : the volume of sample in assay procedure, mL

V_{Assay} : the volume of Assay Buffer in sample preparation, mL

T: the reaction time, minute

VIII. TYPICAL DATA

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



Detection Range: 4μmol/L - 400 μmol/L