Alpha-Ketoglutarate Dehydrogenase

Microplate Assay Kit

Cat #: orb707329 (manual)

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.

Biorbyt Ltd
7 Signet Court, Swann Road, Cambridge, CB5 8LA. United Kingdom
Email: info@biorbyt.com | Phone: +44 (0)1223 859-353 | Fax: +44 (0)1223 280-240

Biorbyt LLC
68 TW Alexander Drive, Durham, NC 27709. United States
Email: info@biorbyt.com | Phone: +1 (415) 906-5211 | Fax: +1 (415) 651-8558

INTRODUCTION

Alpha-Ketoglutarate Dehydrogenase (EC 1.2.4.2) 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.

The assay is used to determine Alpha-Ketoglutarate Dehydrogenase. The enzyme catalysated reaction products NADH, can be measured at a colorimetric readout at 340 nm.

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	20 ml x 1	4 °C
Substrate	Powder x 1	-20 °C
Standard	Powder x 1	-20 °C
Technical Manual	1 Manual	

Note:

Substrate: add 19 ml Substrate Diluent to dissolve before use.

Standard: add 1 ml distilled water to dissolve before use; then add 0.2 ml into 0.8 ml distilled water, the concentration will be 400 µmol/L.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 340 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 0.99 ml Assay Buffer I and 10 µl 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 µl Assay Buffer III and 2 µl 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 0.99 ml Assay Buffer I and 10 µl 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 µl Assay Buffer III and 2 µl 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.

ASSAY PROCEDURE

Warm all reagents to room temperature before use.

Add following reagents in the microplate:

Reagent	Sample	Standard	Blank
Standard	--	200 µl	--
Distilled water	--	--	200 µl
Substrate	190 µl	--	--
Sample	10 µl	--	--
Mix, measured at 340 nm and record the absorbance of 10th second and 130th second.			

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) 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 sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

CALCULATION

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

1. According to the protein concentration of sample

$$\alpha\text{-KGDH (U/mg)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample\ (130S)} - OD_{Sample\ (10S)}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times C_{Protein}) / T$$

$$= 4000 \times (OD_{Sample\ (130S)} - OD_{Sample\ (10S)}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

$$\alpha\text{-KGDH (U/g)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample\ (130S)} - OD_{Sample\ (10S)}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times W / V_{Assay}) / T$$

$$= 4000 \times (OD_{Sample\ (130S)} - OD_{Sample\ (10S)}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the quantity of cells or bacteria

$$\alpha\text{-KGDH (U/10}^4\text{)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample\ (130S)} - OD_{Sample\ (10S)}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times N / V_{Assay}) / T$$

$$= 4000 \times (OD_{Sample\ (130S)} - OD_{Sample\ (10S)}) / (OD_{Standard} - OD_{Blank}) / N$$

4. According to the volume of sample

$$\alpha\text{-KGDH (U/ml)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample\ (130S)} - OD_{Sample\ (10S)}) / (OD_{Standard} - OD_{Blank}) / V_{Sample} / T$$

$$= 4000 \times (OD_{Sample\ (130S)} - OD_{Sample\ (10S)}) / (OD_{Standard} - OD_{Blank})$$

$C_{Standard}$: the standard concentration, 400 μmol/L = 400 nmol/ml;

$V_{Standard}$: the volume of standard, 200 μl = 0.2 ml;

$C_{Protein}$: the protein concentration, mg/ml;

W: the weight of sample, g;

N: the quantity of cell or bacteria, N × 10^4;

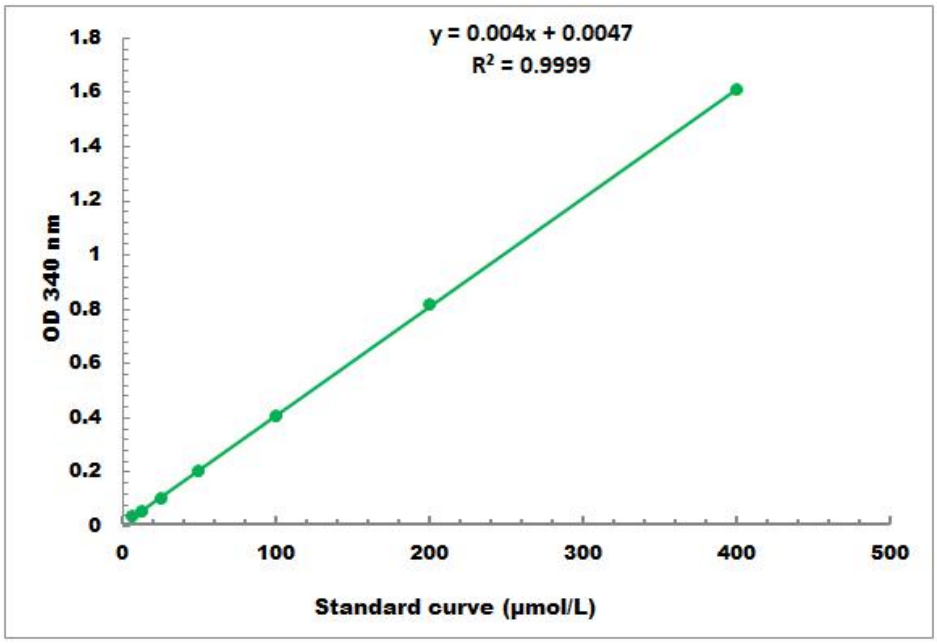
V_{Sample}: the volume of sample, 0.01 ml;

V_{Assay}: the volume of Assay buffer, 1 ml;

T: the reaction time, 2 minutes.

TYPICAL DATA

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



Detection Range: 4 µmol/L - 400 µmol/L

Biorbyt Ltd
7 Signet Court, Swann Road, Cambridge, CB5 8LA. United Kingdom
Email: info@biorbyt.com | Phone: +44 (0)1223 859-353 | Fax: +44 (0)1223 280-240

Biorbyt LLC
68 TW Alexander Drive, Durham, NC 27709. United States
Email: info@biorbyt.com | Phone: +1 (415) 906-5211 | Fax: +1 (415) 651-8558