ab185440

α-Ketoglutarate Dehydrogenase Activity Assay Kit (Colorimetric)

Instructions for Use

For the rapid, sensitive and accurate measurement of α-Ketoglutarate Dehydrogenase in various samples.

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Version 2a, Last Updated 8 June 2023

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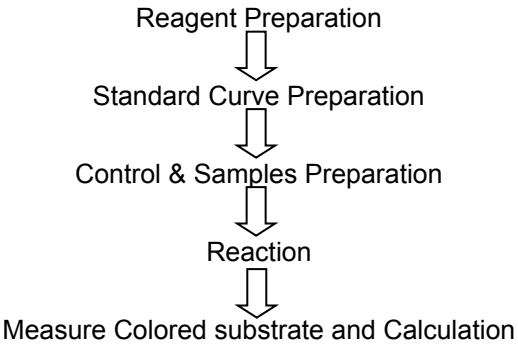
1. Overview

α-Ketoglutarate Dehydrogenase (α-KGDH) (EC 1.2.4.2) is a key enzyme in the citric acid cycle. It forms an enzyme complex with dihydrolipoamide succinyl transferase (E2) and dihydrolipoamide dehydrogenase (E3). α-KGDH converts α-ketoglutarate into succinylCoA in the presence of NAD and CoA. It is highly regulated by intracellular ATP/ADP and NADH/NAD ratios and calcium. In humans, decreased KGDH activity can lead to neurodegenerative diseases such as Alzheimer's disease. Recent studies show that α-KGDH is a target of oxidative stress; reactive oxygen species (ROS) inhibit KGDH activity which diminishes its critical function and can cause a bioenergetic deficit.

Abcam's α-Ketoglutarate Dehydrogenase Activity Assay kit (Colorimetric) (ab185440) provides a quick and easy way for monitoring α-KGDH activity in various samples. In the assay, α-KGDH converts α-ketoglutarate into an intermediate which reduces the probe to a colored product with strong absorbance at 450 nm. The assay is simple, sensitive and can detect α-ketoglutarate dehydrogenase activity lower than 0.1 mU in a variety of samples.

Figure 1: Assay Procedure

2. Protocol Summary



3. Kits Components

Item	Quantity
Assay Buffer IX/KGDH Assay Buffer	25 mL
KGDH Substrate Mix/KGDH Substrate	1 vial
Developer Solution III/KGDH Developer	1 vial
NADH Standard I/NADH Standard	1 vial
KGDH Positive Control	50 µL

4. Storage and Stability

Upon arrival, store the kit at-20°C and protect from light. Please read the entire protocol before performing the assay. Avoid repeated freeze/thaw cycles.

Briefly centrifuge all small vials prior to opening.

5. Materials Required, Not Supplied

- Distilled water (dH_2O)
- 96-well white plate with flat bottom
- Multi-well spectrophotometer (ELISA reader)
- Centrifuge with cooling option

6. Reagents Preparation

1. **Assay Buffer IX/KGDH Assay Buffer:**
   Ready to use as supplied. Warm to room temperature before use. Store at either 4ºC or -20ºC.

2. **KGDH Substrate Mix/KGDH Substrate:**
   Reconstitute with 220 µL dH_2O. Store at -20°C. Keep on ice while in use. Use within two months.

3. **Developer Solution III/KGDH Developer:**
   Reconstitute with 220 µL dH_2O. Pipette up and down to dissolve completely. Aliquot and store at -20°C. Keep on ice while in use. Use within two months.

4. **NADH Standard I/NADH Standard:**
   Reconstitute with 400 µL dH_2O to generate 1.25 mM NADH Standard I/NADH Standard solution. Aliquot and store at -20°C. Keep on ice while in use. Use within two months.

5. **KGDH Positive Control:**
   Reconstitute with 100 µL Assay Buffer IX/KGDH Assay Buffer and dissolve completely. Aliquot and store at -20°C. Keep on ice while in use. Use within two months.

7. Assay Protocol

1. Sample Preparation

a) Cells (starting material: 10^6 cells) or tissues (starting material 10mg)

Homogenize tissue (10 mg) or cells (1 x 10^6) with 100 µL ice cold Assay Buffer IX/KGDH Assay Buffer on ice. Centrifuge at 10,000 X g for 5 min. Collect the supernatant in a fresh tube. To check the activity in Mitochondria from fresh tissue or cells use the appropriate kit to isolate them, these are available from Abcam. Add 5-50 µL samples per well. Adjust the final volume to 50 µL with Assay Buffer IX/KGDH Assay Buffer. Add 2-10 µL of KGDH Positive Control into the desired well(s) & adjust the final volume to 50 µL with Assay Buffer IX/KGDH Assay Buffer.

NOTE:

- *Small molecules in some tissues such as liver may generate high background. To remove small molecules, we suggest using an ammonium sulfate method. Pipette 50-100 µL of lysate into a fresh tube, add 2X volume of saturated ammonium sulfate (about 4.1 M at room temperature) and keep on ice for 20 min. Spin down at 10,000 X g for 5min., carefully remove and discard the supernatant, and resuspend the pellet to the original volume with Assay Buffer IX/KGDH Assay Buffer.*

- *For unknown samples, we suggest testing several doses to ensure the readings are within the Standard Curve range.*

b) Background Control

For samples exhibiting background, prepare parallel sample well(s) as the background control.

2. Standard Curve Preparation:

a) Add 0, 2, 4, 6, 8 and 10 µL of 1.25 mM NADH Standard I/NADH Standard into a series of wells in 96 well plate to generate 0, 2.5, 5.0, 7.5, 10 and 12.5 nmol/well of NADH Standard I/NADH Standard.

b) Adjust the volume to 50 µL /well with Assay Buffer IX/KGDH Assay Buffer.

3. Reaction Mix:

Prepare enough Reaction Mix for the number of assays to be performed. For each well, prepare 50 µL Mix containing:

	Reaction Mix	Background Control Mix*
Assay Buffer IX/KGDH Assay Buffer	46 µL	48 µL
Developer Solution III/KGDH Developer	2 µL	2 µL
KGDH Substrate Mix/KGDH Substrate	2 µL	-

Add 50 µL of the reaction mix to each well containing the Standards, positive control and test samples.

* For samples with background, add 50 µL of Background Control Mix (without substrate) to sample background control well(s) and mix well.

4. Measurement

Measure absorbance (OD 450 nm) immediately in kinetic mode for 10-60 minutes at 37ºC.

NOTE:

- *Incubation time depends on the α-Ketoglutarate Dehydrogenase Activity in the samples. We recommend measuring the OD in a kinetic mode, and choosing two time points (T_1 & T_2) in the linear range to calculate the α-Ketoglutarate Dehydrogenase Activity of the samples. The NADH Standard Curve can be read in endpoint mode (i.e., at the end of incubation time).*

8. Data Analysis

Calculations:

Subtract 0 Standard reading from all Standard readings. Plot the NADH Standard Curve. Correct sample reading by subtracting the value derived from the background control reading from sample reading. Calculate the α-Ketoglutarate Dehydrogenase Activity of the test samples: $\Delta OD = A_2 - A_1$. Apply the ΔOD to the NADH Standard Curve to get B nmol of H_20_2 generated by α-Ketoglutarate Dehydrogenase during the reaction time ($\Delta T = T_2 - T_1$).

Sample α-Ketoglutarate Dehydrogenase Activity =

$B/(\Delta T \times V) \times D$ = nmol/min/mL = mU/mL

Where:

B is the NADH amount from Standard Curve (nmol)

V is the sample volume added into the reaction well (µL)

Δ**T** is the reaction time (min.)

D is the dilution factor

Unit Definition: One unit of α-Ketoglutarate Dehydrogenase is the amount of enzyme that will generate 1.0 µmol of NADH per min. at pH 7.5 at 37ºC.

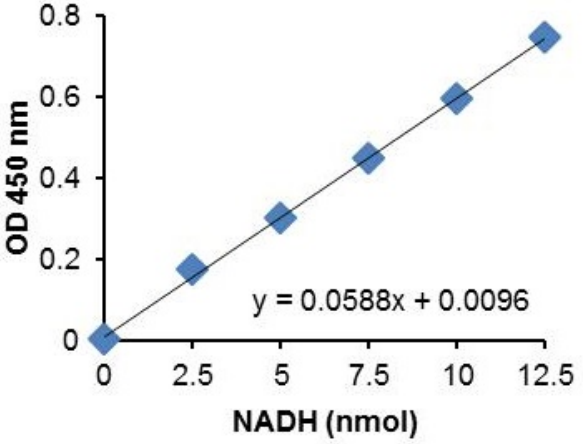


Figure 2. NADH Standard Curve.

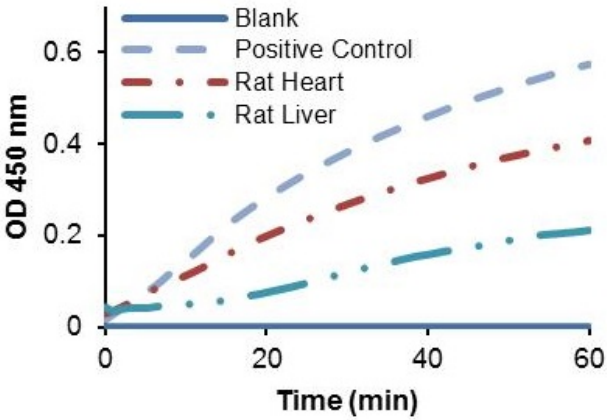


Figure 3. α-Ketoglutarate Dehydrogenase activity in rat heart (75 µg) and liver lysates (100 µg). Assays were performed following the kit protocol.

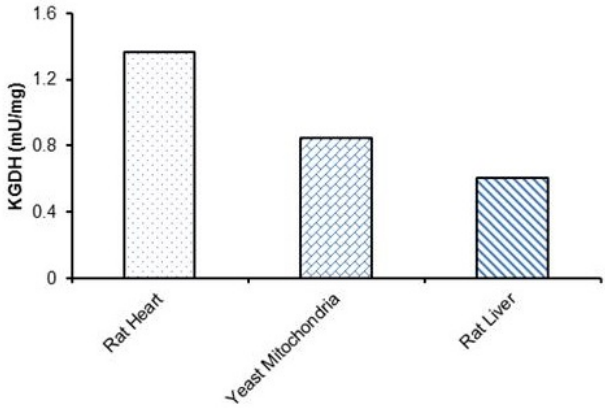


Figure 4. α-Ketoglutarate Dehydrogenase specific activity was calculated in rat heart lysate (75 µg), yeast mitochondria prepared from S. Cerevisiae (10 µg) and in rat liver lysate (100 µg). Assays were performed following the kit protocol

9. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Problem	Reason	Solution
Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Problem	Reason	Solution
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)