Product Information

Alpha-Ketoglutarate Dehydrogenase Activity Colorimetric Assay Kit

I. Kit Contents:

Components	K2217-100 100 assays	Cap Color	Part Number
KGDH Assay Buffer	25 ml	WM	K2217-C-1
KGDH Substrate (Lyophilized)	1 vial	Blue	K2217-C-2
KGDH Developer (Lyophilized)	1 vial	Red	K2217-C-3
NADH Standard (Lyophilized)	1 vial	Yellow	K2217-C-4
KGDH Positive Control	50 µl	Orange	K2217-C-5

II. Introduction:

α-Ketoglutarate Dehydrogenase (α-KGDH) is an important enzyme in the citric acid cycle. Combining with dihydrolipoamide succinyl transferase (E2) and dihydrolipoamide dehydrogenase (E3), α-KGDH forms an enzyme complex. In the presence of NAD^+ and CoA, α-KGDH converts α-ketoglutarate into succinyl-CoA. α-KGDH is highly regulated by intracellular calcium, NADH/NAD^+ and ATP/ADP ratios. Decreased α-KGDH activity can lead to Alzheimer's disease. α-KGDH is also a target of oxidative stress. Reactive oxygen species (ROS) can inhibit α-KGDH activity which then diminishes its important function and causes a bioenergetic deficit.

The Alpha-Ketoglutarate Dehydrogenase Activity Colorimetric Assay Kit provides a sensitive, simple, fast and convenient way for detection α-KGDH activity in various samples based on colorimetric method. In the assay, α-KGDH changes α-ketoglutarate to an intermediate which reduces the probe to a colored product with strong absorbance at 450 nm. The kit can detect α-KGDH activity lower than 0.1 mU in a variety of samples.

III. Application:

Measurement of α-Ketoglutarate dehydrogenase activity in various tissues/cells.

Analysis of cell signaling pathways such as citrate acid cycle, lysine degradation or tryptophan metabolism in various cell types.

III. Sample Type:

Animal tissues: liver, heart, muscle, etc.

Purified mitochondria.

Cell culture: Adherent or suspension cells.

V. User Supplied Reagents and Equipment:

96-well clear plate with flat bottom.

Multi-well spectrophotometer (ELISA reader).

VI. Storage and Handling:

Store kit at -20℃, protected from light. Briefly centrifuge small vials prior to opening. Read the entire protocol before performing the assay.

VII. Reagent Preparation and Storage Conditions:

KGDH Assay Buffer: Warm to room temperature before use. Store at either 4℃ or -20℃.

KGDH Substrate: Reconstitute with 220 µl dH_2O. Store at -20℃. Keep on ice while in use. Use within two months.

KGDH Developer: Reconstitute with 220 µl dH_2O. Gently pipette up and down to dissolve completely. Store at -20℃. Use within two months.

NADH Standard: Reconstitute with 400 µl dH_2O to generate 1.25 mM NADH Standard solution. Aliquot and store at -20℃. Keep on ice while in use. Use within two months.

KGDH Positive Control: Add 100 µl KGDH Assay Buffer to the Positive Control and mix thoroughly. Aliquot and store at -20℃. Keep on ice while in use. Use within two months.

VIII. Ketoglutarate Dehydrogenase Assay Protocol:

1. Sample Preparation: Rapidly homogenize tissue (10 mg) or cells (1 x 10^6) with 100 µl ice cold KGDH Assay Buffer, and keep on ice for 10 min. Centrifuge at 10,000 x g for 5 min. and transfer the supernatant to a fresh tube as the test sample. To check α-KGDH activity in mitochondria, isolate the mitochondria from fresh tissue or cells using BioVision's Mitochondria Isolation Kit for Tissue and Cultured Cells. Add 5 - 50 µl sample (whole cell lysate or mitochondria) per well. For the KGDH positive control, take 2 - 10 µl of KDGH Positive Control into desired well(s) and adjust final volume to 50 µl with KGDH Assay Buffer.

Notes:

a. For unknown samples, we suggest testing several doses of the sample to ensure the readings are within the Standard Curve range.

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

c. 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 and keep on ice for 20 min. Spin down at 10,000 X g for 5 min., carefully remove and discard the supernatant, and resuspend the pellet to the original volume with KGDH Assay Buffer.

2. NADH Standard Curve: Add 0, 2, 4, 6, 8 and 10 µl of 1.25 mM 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. Adjust the volume to 50 µl/well with KGDH Assay Buffer.

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

	Reaction Mix	Background Control Mix
KGDH Assay Buffer	46 µl	48 µl
KGDH Developer	2 µl	2 µl
KGDH Substrate	2 µl	---

Mix and add 50 µl of the Reaction Mix to each well containing the Standard, 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 the absorbance immediately at 450 nm in kinetic mode for 10-60 min. at 37℃.

Note: Incubation time depends on the α-ketoglutarate dehydrogenase activity in samples. We recommend measuring the OD in kinetic mode, and choosing two time points (T1 & T2) 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 the incubation time).

5. Calculation: Subtract 0 Standard reading from all readings. Plot the NADH Standard Curve. If sample background control reading is significant, subtract the background control reading from its paired sample reading. Calculate the α-ketoglutarate dehydrogenase activity of the test sample: ΔOD = A2 – A1. Apply the ΔOD to the NADH Standard Curve to get B nmol of NADH generated during the reaction time (ΔT = T2 - T1).

Sample α-Ketoglutarate Dehydrogenase Activity = B/(ΔT X V) x D = nmol/min/ml = mU/ml

Where: B = the NADH amount from Standard Curve (nmol).

ΔT = the reaction time (min.).

V = the sample volume added into the reaction well (ml).

D = Dilution Factor.

Unit Definition: One unit of α-ketoglutarate dehydrogenase is the amount of enzyme that generates 1.0 μmol of NADH per min. at pH 7.5 at 37℃.

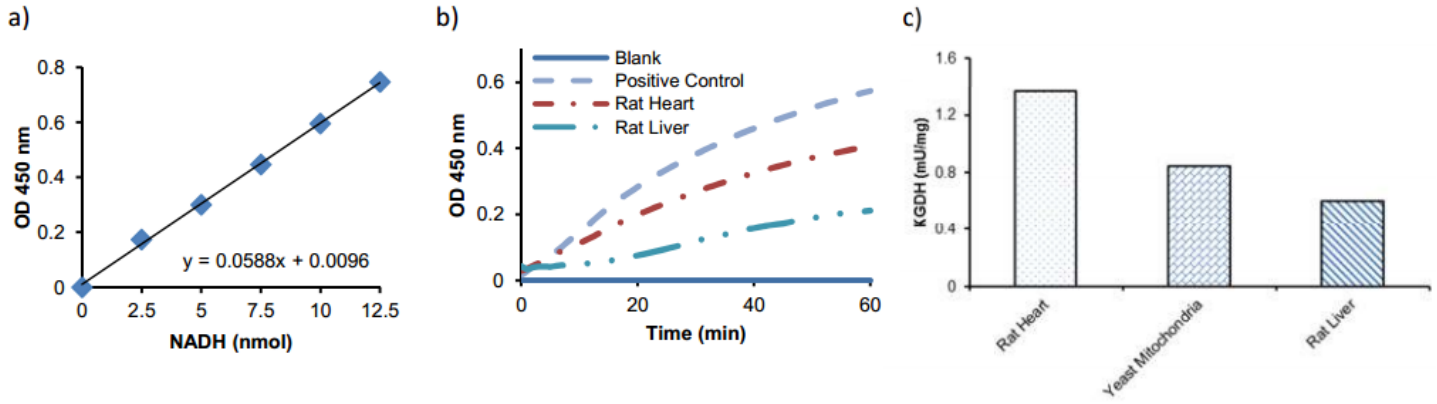

Figure: (a) NADH standard curve; (b) α-Ketoglutarate Dehydrogenase activity in rat heart (75 μg) and liver lysates (100 μg); (c) α-Ketoglutarate Dehydrogenase specific activity was calculated in rat heart lysate (75 μg), yeast mitochondria prepared from *S. Cerevisiae* (10 μg) and in rat liver lysate (100 μg). Assays were performed following the kit protocol.

Frequently Asked Questions

1. Is it better to use mitochondria or cell lysate? Cell lsyate is showing lot of background.

a. Use mitochondria instead of whole cell lysate. There can be a lot of NADH in the cell lysate that creates background. Mitochondria will help enrich the sample with mor active alpha KDGH per ul of sample.

b. You canfilter the cell lysate samples through a 10KDa mol. wt cut off filter column. NADH, being a small molecule will flow through and the retentate within the filter insert will have the high mol. wt. proteins/enzymes including KDGH.

2. Can frozen samples be used with this assay?

Fresh samples are always preferred over frozen samples. However, frozen samples can also be used, provided, they were frozen right after isolation, were not freeze thawed multiple time (for which we recommend aliquoting the samples before freezing) and have been frozen for relatively short periods.

3. Is it possible to use a different wavelength than recommended for the final analysis?

It is always recommended to use the exact recommended wavelength for the most efficient results. However, most plate readers have flexibility in their band width of detection in increments of +/- 10 nm. Depending on this flexibility range, you can deviate from the recommended wavelengths within limits.

4. What is the exact volume of sample required for this assay?

There is no specific volume we can recommend for the amount any sample to be used since it is completely sample concentration and quality based. It is recommended to do a pilot expt with multiple sample volumes to determine the optimal volume which gives a reading within the linear range of the standard curve. Please refer to the citations for this product to see what other clients have used with similar sample types.

5. What is the shelf life of this kit?

This kit is good for 12 months from the date of shipment in the unopened form when stored at the appropriate temperature and appropriate conditions. After opening and reconstitution, some of the components in this kit are good for 2 months at -20℃. Please refer to the datasheet for storage information and shelf life of each of the components.

6. Can alternate buffers be used for sample preparation (cell lysis, sample dilutions etc)?

Our assay buffers are optimized for the reactions they are designed for. They not only contain some detergents for efficient lysis of your cells/tissue, but also contain some proprietary components required for the further reactions. Therefore, we highly recommend using the buffers provided in the kit for the best results.

7. Is it essential to make a standard curve for every expt, or is one curve/kit enough?

Yes, it is strongly recommended to do the standards every time you do the expt. There is always a chance that something was done differently that day and we do not want any conditions to differ between standards and samples.

For research use only! Not to be used in humans.

Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

Tel: +1-(832)696-8203
Fax: +1-832-641-3177
Email: sales@apexbt.com