α-Ketoglutarate Dehydrogenase Activity Colorimetric Assay Kit (#BN00902)

(Catalog # BN00902; 100 assays; Store at -20°C)

I. Introduction:

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 succinyl-CoA 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. Assay Genie's α-KGDH assay kit 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.

α-Ketoglutarate —(α-Ketoglutarate Dehydrogenase)→ Intermediate —(Developer)→ Color detection (OD 450 nm)

II. 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

IV. Kit Contents:

Components	BN00902	Cap Code	Part Number
KGDH Assay Buffer	25 ml	WM	BN00902-1
KGDH Substrate (Lyophilized)	1 vial	Blue	BN00902-2
KGDH Developer (Lyophilized)	1 vial	Red	BN00902-3
NADH Standard (Lyophilized)	1 vial	Yellow	BN00902-4
KGDH Positive Control	50 µl	Orange	BN00902-5

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°C, 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 4ºC or -20ºC.
- **KGDH Substrate:** Reconstitute with 220 µl dH_2O. Store at -20°C. 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°C. 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°C. 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°C. 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 Assay Genie's Mitochondria Isolation Kit for Tissue and Cultured Cells (# BN00546). 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 (about 4.1 M

at room temperature 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°C.

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 (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 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: $\Delta OD = A_2 - A_1$. Apply the ΔOD to the NADH Standard Curve to get B nmol of NADH generated during the reaction time ($\Delta T = T_2 - T_1$).

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°C.

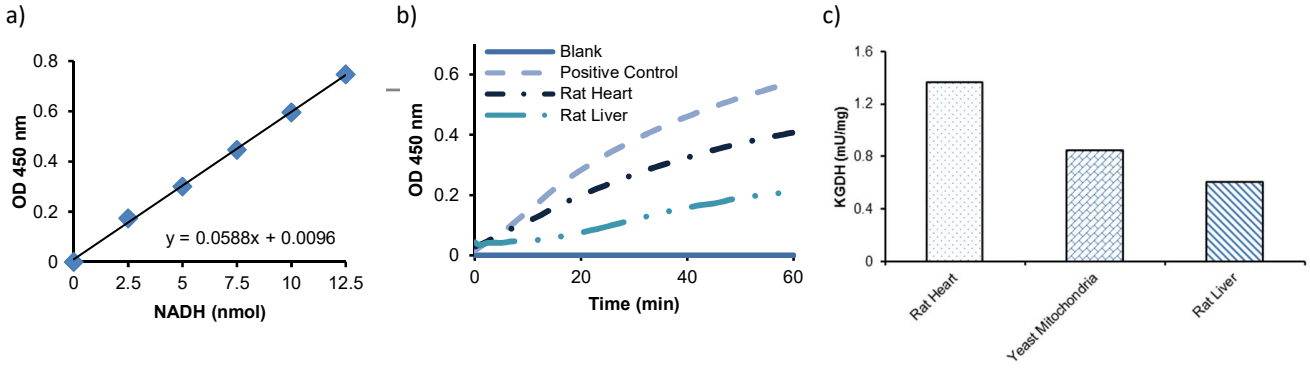

Figure: (a) NADH standard curve; (b) α-Ketoglutarate Dehydrogenase activity in rat heart (75 µg) and liver lysates (100 µg); (c) α-Ketoglutarate Dehydrogenase specific activity was calculated in rat heart lysate (75 µg), yeast mitochondria prepared from *S. Cerevisiae* (10 µg) and in rat liver lysate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

FOR RESEARCH USE ONLY! Not to be used on humans.