# Alpha Ketoglutarate Dehydrogenase Assay Kit

**Catalog No.:** abx090702

**Size:** 96 tests

**Detection Range:** 8.3 U/L – 42.3 U/L

**Sensitivity:** 8.3 U/L

**Storage:** Store all components at -20°C. Store Substrate and Chromogenic Reagent in the dark.

**Application:** For quantitative detection of Alpha Ketoglutarate Dehydrogenase activity in serum, plasma, and plant/animal tissue homogenates.

## Introduction

Alpha ketoglutarate dehydrogenase (α-KGDH) is a key enzyme in the tricarboxylic acid cycle (TCA, also known as the Krebs cycle or the citric acid cycle).

Abbexa's Alpha Ketoglutarate Dehydrogenase Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Alpha-Amylase activity. Alpha Ketoglutarate Dehydrogenase catalyzes the substrate, during which NAD+ is reduced to NADH. Electrons are transferred to WST-8 by a hydrogen transmitter which produces a yellow formazan dye with an absorbance maximum at 450 nm. The intensity of the color is proportional to the Alpha Ketoglutarate Dehydrogenase activity, which can then be calculated.

### Kit components

1. 96-well microplate
2. Extraction Solution: 55 ml
3. Buffer Solution: 28 ml
4. Substrate: 1 vial
5. Chromogenic Reagent: 3 ml
6. Clarifying Reagent: 3 ml
7. Standard: 1 vial
8. Plate sealer: 2

### Materials required but not provided

1. Microplate reader 450 nm)
2. Double-distilled water
3. PBS (0.01 M, pH 7.4)
4. Pipette and pipette tips
5. 1.5 ml microcentrifuge tubes
6. Centrifuge
7. Incubator

## Protocol

### A. Preparation of samples and reagents

#### 1. Samples

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum** and **Plasma:** Serum and plasma samples can be tested directly. If not analyzed on the same day, aliquot and store at -80°C for up to 1 month
- **Tissue Homogenates:** Weigh at least 20 mg of tissue and wash in cold PBS (0.01 M, pH 7.4). Add 180 µl of the extraction solution and homogenize manually at 4°C using a dounce homogenizer. Centrifuge at 10,000 × g for 10 minutes at 4°C, then carefully remove the supernatant and keep on ice.

  **Note:** To calculate Alpha Ketoglutarate Dehydrogenase activity in tissue homogenates using the formulae in section **C. Calculation of Results**, the total protein concentration of the supernatant must be determined separately (abx097193).

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with the extraction solution, then carry out the assay procedure. The recommended dilution factor for different samples is as follows (for reference only):

| Sample Type | Dilution Factor |
|---|---|
| 10% Rat liver tissue homogenate | 1 |
| 10% Rat lung tissue homogenate | 1 |
| Rat plasma | 1 |
| 10% Mouse liver tissue homogenate | 1 |
| 10% Mouse spleen tissue homogenate | 1 |
| 10% *Epipremnum aureum* tissue | 1 |

**Note:**

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for tissue homogenates.

#### 2. Reagents

Allow all reagents to equilibrate to room temperature before use.

- **Working Solution:** Dissolve 1 vial of substrate with 1 vial of buffer solution (28 ml) and mix thoroughly. Store at 4°C in the dark and use within 6 hours.
- **Standard Solution:** Dissolve 1 vial of standard with 2 ml of double-distilled water and mix thoroughly to prepare the 0.5 mmol/L standard solution. Store at 4°C in the dark and use within 6 hours.
- **Standards:** Label 7 tubes with 0.5 mmol/L, 0.4 mmol/L, 0.35 mmol/L, 0.3 mmol/L, 0.25 mmol/L, 0.2 mmol/L, and 0.1 mmol/L. Add 200 µl, 160 µl, 140 µl, 120 µl, 100 µl, 80 µl, and 40 µl of Standard (0.5 mmol/L) to the 0.5 mmol/L, 0.4 mmol/L, 0.35 mmol/L, 0.3 mmol/L, 0.25 mmol/L, 0.2 mmol/L, and 0.1 mmol/L tubes respectively, followed by 0 µl, 40 µl, 60 µl, 80 µl, 100 µl, 120 µl, and 160 µl of double-distilled water, to prepare Standard Dilutions with concentrations 1.4 mg/ml, 1.2 mg/ml, 1.0 mg/ml, 0.8 mg/ml, 0.6 mg/ml, 0.4 mg/ml, and 0.2 mg/ml. These volumes are summarized in the following table:

| **Standard Dilution (mmol/L)** | **0.5** | **0.4** | **0.35** | **0.3** | **0.25** | **0.2** | **0.1** | **0** |
|---|---|---|---|---|---|---|---|---|
| **0.5 mmol/L Standard (µl)** | 200 | 160 | 140 | 120 | 100 | 80 | 40 | 0 |
| **Double-distilled water (µl)** | 0 | 40 | 60 | 80 | 100 | 120 | 160 | 200 |

For the blank, or 0 mmol/L standard, use pure double-distilled water. The volume of each standard will be 200 µl.



**B. Assay Procedure**

1. Assign microplate wells for each standard, sample, and control. Each sample requires a corresponding control. *It is strongly recommended to prepare all the wells in duplicate.*
2. Add 20 µl of each standard dilution to the corresponding wells.
3. Add 20 µl of sample to the sample wells.
4. Add 20 µl of sample to the control wells.
5. Add 200 µl of the working solution to all of the sample and standard wells.
6. Add 200 µl of double-distilled water to the control wells.
7. Add 20 µl of chromogenic reagent to each well.
8. Mix thoroughly with a microplate reader, then incubate all tubes at 37°C for 10 minutes in the dark.
9. Add 20 µl of clarifying agent to each well. Mix thoroughly with a microplate reader.
10. Measure the OD of each well with a microplate reader at 450 nm.

### C. Calculation of Results

Plot the standard curve, using the OD of the standard dilutions (adjusted for the blank) on the y-axis, and their respective concentrations on the x-axis. The curve should form a straight line, described by the formula $y = ax + b$. Based on this curve, the activity of Alpha Ketoglutarate Dehydrogenase in each sample well can be derived with the following formulae:

#### 1. Serum (Plasma) Samples

One unit of Alpha Ketoglutarate Dehydrogenase activity is defined as the amount required for 1 L of serum (plasma) to produce 1 µmol of NADH per 1 minute at 37°C.

$$\text{α-KGDH activity (U/L)} = \mathbf{F} \times \frac{(\mathbf{OD_{Sample}} - \mathbf{OD_{Control}} - \mathbf{b}) \times \mathbf{1000^*}}{\mathbf{a} \times \mathbf{t}}$$

#### 2. Tissue samples:

Alpha Ketoglutarate Dehydrogenase activity in tissue samples can be calculated according to total protein concentration, which must be assayed separately (abx097193).

One unit of Alpha Ketoglutarate Dehydrogenase activity is defined as the amount required for 1 g of tissue protein to produce 1 µmol of NADH per 1 minute at 37°C.

$$\text{α-KGDH activity (U/g protein)} = \mathbf{F} \times \frac{(\mathbf{OD_{Sample}} - \mathbf{OD_{Control}} - \mathbf{b}) \times \mathbf{1000^*}}{\mathbf{a} \times \mathbf{t} \times \mathbf{C_{Protein}}}$$

where:

| | |
|---|---|
| $\mathrm{OD_{Sample}}$ | OD value of sample |
| $\mathrm{OD_{Control}}$ | OD value of control |
| $\mathrm{C_{Protein}}$ | Concentration of protein in sample (g protein/L.) |
| a | Gradient of the standard curve ($y = \boldsymbol{a}x + b$) |
| b | Y-intercept of the standard curve ($y = ax + \boldsymbol{b}$) |
| t | Time of the enzymatic reaction (10 mins) |
| F | The dilution factor of sample |
| $1000^*$ | 1 mmol = 1000 µmol |

### Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.