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α-Ketoglutarate Dehydrogenase (α-KGDH) Activity Assay Kit

Note: Take two or three different samples for prediction before test.

Detection equipment: Spectrophotometer

Cat No: AK0282 **Size:** 50T/48S

Components

Reagent I: 60 mL×1, store at 4°C;

Reagent II: 0.6 mL×1, store at -20°C;

Reagent III: 55 mL×1, store at 4°C;

Reagent IV: Powder×1, store at 4°C;

Reagent V: Powder×1, store at 4°C;

Reagent VI: Powder×1, store at -20°C;

Reagent VII: Powder×1, store at -20°C;

Reagent VIII: Powder $\times 1$, store at -20°C and protect from light; Add 2 mL of distilled water when the solution will be used, the unused reagents need stored at -20°C.

Preparation of working solution: when the solution will be used, transfer Reagent IV, V, VI and VII to Reagent III, mix and dissolve them for use.

Description

 α -Ketoglutarate Dehydrogenase (α -KGDH, EC 1.2.4.2) is one of the key enzymes in the regulation of tricarboxylic acid cycle and widely exists in mitochondria of animal, plant microorganisms and cultured cells, which catalyzes the oxidative decarboxylation of α -ketoglutarate to succinyl coenzyme A.

 α -KGDH catalyzes α -ketoglutarate, NAD⁺ and coenzyme A to form succinyl coenzyme A, carbon dioxide and NADH. NADH has a characteristic absorption peak at 340 nm. The activity of α -KGDH is expressed by the formation rate of NADH.

Required but not provided

Ultraviolet spectrophotometer, water-bath, tabletop centrifuge, adjustable pipette, 1 mL quartz cuvette, mortar/homogenizer, ice and distilled water.

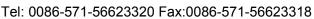
Protocol

I. Extraction of α-KGDH:

Accurately weigh 0.1 g of tissue or collect 5 million cells, add 1 mL of Reagent I and 10 μ L of Reagent II, homogenize by using homogenizer/mortar in ice bath, fully grind, centrifuge at 11000 \times g for 10 minutes at 4°C, take the supernatant, place it on ice for test.

II. Procedure

1. Preheat Spectrophotometer for 30 minutes, adjust wavelength to 340 nm, set zero with distilled water.



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2. Blank tube:

Take 1 mL of working solution and add it to the 1 mL quartz cuvette, incubate it at 37°C for 5 min, then take out the cuvette, add 40 μ L of Reagent VIII and 60 μ L of distilled water in turn into the cuvette, mix them well and immediately measure the absorbance value A1 of 0 s at 340 nm, react accurately at 37°C for 2 min, record the absorbance value A2 of 2 minutes at 340 nm, calculate Δ A_B = A2-A1.

3. Measuring tube:

Take 1 mL of working solution and add it to the 1 mL quartz cuvette, incubate it at 37°C (mammal) or 25°C (other species) for 5 min, then take out the cuvette, add 40 μ L of Reagent VIII and 60 μ L of samples in turn into the cuvette, mix them well and immediately measure the absorbance value A3 of 0 s at 340 nm, react accurately 37°C (mammal) or 25°C (other species) for 2 minutes, and record the absorbance value A4 of 2 minutes at 340 nm, calculate $\Delta A_T = A4-A3$.

III. Calculation of α-KGDH activity

(1) Protein concentration

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1 nmol of NADH per minute in the reaction system every milligram tissue protein.

$$\alpha\text{-KGDH(U/mg prot)} = [(\Delta A_T - \Delta A_B) \div \quad (\epsilon \times d) \quad \times V_{RV} \times 10^9] \div (Cpr \times V_{SV}) \div T = 1473.7 \times (\Delta A_T - \Delta A_B) \div Cpr$$

(2) Sample weight

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1 nmol of NADH per minute in the reaction system every gram tissue.

α-KGDH (U/g fresh weight) =
$$[(\Delta A_T - \Delta A_B) \div (\epsilon \times d) \times V_{RV} \times 10^9] \div (V_{SV} \div V_{STV} \times W) \div T$$

= $1488.5 \times (\Delta A_T - \Delta A_B) \div W$

(3)Germ or cells

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1 nmol of NADH per minute in the reaction system every 10 thousand germ or cells.

α-KGDH (U/10⁴ cell) =
$$[(\Delta A_T - \Delta A_B) \div (\epsilon \times d) \times V_{RV} \times 10^9] \div (V_{SV} \div V_{STV} \times 500) \div T$$

=2.977×($\Delta A_T - \Delta A_B$)

 V_{RV} : The total volume of reaction system, 1. 1×10⁻³L;

ε: The molar extinction coefficient of NADH, 6.22×10³ L/mol/cm;

d: Cuvette light diameter, 1 cm;

 V_{SV} : The volume of sample, 0.06 mL;

V_{STV}: The volume of Reagent I and Reagent II, 1.01 mL;

T: Reaction time, 2 minutes;

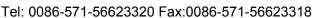
Cpr: The concentration of sample protein, mg/mL;

W: Sample weight, g.

500: Cells or germ, 5 million.

Note:

1. All reagents and samples should be placed on ice during the determination to avoid denaturation and deactivation.





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- 2. The temperature of the reaction solution in the cuvette must be kept at 37°C or 25°C. Take a small beaker and put it into a certain amount of 37°C or 25°C distilled water. Put the beaker into a 37°C or 25°C water bath. Put the cuvette and reaction solution into the beaker during the reaction.
- 3. It is better for two people to do the experiment at the same time, one for color comparison and one for timing, so as to ensure the accuracy of the experimental results.
- 4. The ΔA value of the test tube is between 0.01-0.25. If the ΔA value of the test tube is greater than 0.25, the sample shall be diluted.
- 5. As the Reagent I contents a certain concentration of protein (about 1 mg/mL), the protein content of the extract solution itself needs to be subtracted when determining the protein concentration of the sample.

Experimental example:

- 1. Take 0. 1g of barnyardgrass for sample treatment, dilute the supernatant for 2 times, and then operate according to the determination steps, and calculate $\Delta A_T = A4 A3 = 0.323 0.312 = 0.011$, $\Delta A_B = A2 A1 = 0$ α -KGDH (U/g mass) = 1488.5 × (ΔAT ΔAB) × W × 2 (dilution ratio) = 327.47 U/g mass.
- 2. 0. 1g mouse liver was taken for sample treatment, and centrifuged at 4°C and 11000g for 10min. The supernatant was taken and operated according to the determination steps. The measured and calculated $\Delta A_T = A4-A3 = 1.2-0.957 = 0.243$, $\Delta A_B = A2-A1 = 0$ α -KGDH (U/g mass) = 1488.5 × (ΔA_T ΔA_B) ÷ W = 3617.055 U/g mass.

Recent product Citations:

- [1] Jianyun Yue, Changjian Du, Jing Ji, et al. Inhibition of α -ketoglutarate dehydrogenase activity afects adventitious root growth in poplar via changes in GABA shunt. Planta. July 2018;(IF3.06)
- [2] Xiao Li, Qi Zhao, Jianni Qi, et al. lncRNA Ftx promotes aerobic glycolysis and tumor progression through the PPARγ pathway in hepatocellular carcinoma. International Journal of Oncology. May 2018; (IF3.571)

References:

[1] Park L C H, Calingasan N Y, Sheu K F R, et al. Quantitative α-ketoglutarate dehydrogenase activity staining in brain sections and in cultured cells[J]. Analytical biochemistry, 2000, 277(1): 86-93.

Related Products:

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AK0249/AK0248 Isocitrate Dehydrogenase Mitochondrial (ICDHm) Activity Assay Kit