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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/Microplate reader

Cat No: AK0281

Size: 100T/96S

Components

Reagent I: 110 mL×1, store at 4℃;

Reagent II: 1 mL×1, store at -20℃;

Reagent III: 22 mL×1, store at 4℃;

Reagent IV: Powder×1, store at 4℃;

Reagent V: Powder×1, store at 4℃;

Reagent VI: Powder×1, store at -20℃;

Reagent VII: Powder×1, store at -20℃;

Reagent VIII: Powder×1, store at -20℃ and protect from light. Add 0.8 mL of distilled water when the solution will be used, the unused reagents are stored at -20℃ .

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/microplate reader, water-bath, tabletop centrifuge, adjustable pipette, micro quartz cuvette/96 well flat-bottom plate (UV plate), 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 μL of Reagent II, homogenize by using homogenizer/mortar in ice bath, fully grind, centrifuge at 11000 ×g for 10 minutes at 4℃, take the supernatant, place it on ice for test.

II. Procedure

1. Preheat Spectrophotometer/Microplate reader for 30 minutes, adjust wavelength to 340 nm, set zero

with distilled water.

2. Blank tube:

Take 200 μL of working solution and add it to the micro quartz cuvette or 96 well flat-bottom plate, incubate it at 37℃ for 5 minutes, then take out the cuvette, add 8 μL of Reagent VIII and 12 μL 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℃ for 2 minutes, record the absorbance value A2 of 2 minutes at 340 nm, calculate $\Delta A_B = A2-A1$.

3. Measuring tube:

Take 200 μL of working solution and add it to the micro quartz cuvette or 96 well flat-bottom plate, incubate it at 37℃ (mammal) or 25℃ (other species) for 5 minutes, then take out the cuvette, add 8 μL of Reagent VIII and 12 μL of samples in turn into the cuvette, mix them well and immediately measure the absorbance value A3 of 0s at 340 nm, react accurately 37℃ (mammal) or 25℃ (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

A. The calculation formula according to the determination of micro quartz cuvette

(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.

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

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.

$\alpha\text{-KGDH (U/g fresh weight)} = [(\Delta A_T-\Delta A_B) \div (\varepsilon \times d) \times V_{RV} \times 10^9] \div (V_{SV} \div V_{STV} \times W) \div T = 1488.5 \times (\Delta A_T.\Delta A_B) \div W$

(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.

$\alpha\text{-KGDH (U/10}^4\text{ cell)} = [(\Delta A_T-\Delta A_B) \div (\varepsilon \times d) \times V_{RV} \times 10^9] \div (V_{SV} \div V_{STV} \times 500) \div T = 2.977 \times (\Delta A_T-\Delta A_B)$

V_{RV}: The total volume of reaction system, 2.2×10^{-4}L;

ε: The molar extinction coefficient of NADH, 6.22×10^3 L/mol/cm;

d: cuvette light diameter, 1 cm;

V_{SV}: sample volume, 0.012 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.

B. The calculation formula according to the determination of 96 well plate：

(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.

α-KGDH(U/mg prot)＝$[(\Delta A_T-\Delta A_B)\div(\varepsilon\times d)\times V_{RV}\times 10^9]\div(Cpr\times V_{SV})\div T=2456.2\times(\Delta A_T-\Delta A_B)\div Cpr$

(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）＝$[(\Delta A_T-\Delta A_B)\div(\varepsilon\times d)\times V_{RV}\times 10^9]\div(V_{SV}\div V_{STV}\times W)\div T=2480.7\times(\Delta A_T-\Delta A_B)\div W$

(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/10^4 cell）＝$[(\Delta A_T-\Delta A_B)\div(\varepsilon\times d)\times V_{RV}\times 10^9]\div(V_{SV}\div V_{STV}\times 500)\div T=4.962\times(\Delta A_T-\Delta A_B)$

V_{RV}: total volume of reaction system, 2.2×10^{-4}L;

ε: The molar extinction coefficient of NADH, 6.22×10^3 L/mol/cm;

d: cuvette light diameter, 0.6 cm;

V_{SV}: sample volume, 0.012 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 shall be placed on ice during the determination to avoid denaturation and deactivation.

2. The temperature of the reaction solution in the cuvette must be kept at 37℃ or 25℃ . Take a small beaker and put it into a certain amount of 37℃ or 25℃ distilled water. Put the beaker into a 37℃ or 25℃ 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 0.1 g of barnyardgrass for sample treatment, dilute the supernatant for 2 times, and then operate according to the determination steps. Use micro quartz colorimetric plate to measure and calculate ΔA_T = A4-A3 = 0.3243-0.3115 = 0.0128, ΔA_B=A2-A1=0

α-KGDH (U/g mass) = 1488.5 × (ΔA_T - ΔA_B) × W × 2 (dilution ratio) = 381.056 U/g mass.

2. After centrifugation at 4℃ for 10 min, the supernatant was taken and operated according to the determination steps. The results were as follows: ΔA= A4-A3= 1.2123-0.9623=0.2500, ΔAB =A2-A1= 0

α-KGDH (U/g mass) = 1488.5× (ΔA_T -ΔA_B) ÷ W = 3721.25 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：

AK0400/AK0399	Citric Acid(CA) Content Assay Kit
AK0504/AK0503	Succinate Dehydrogenase(SDH) Activity Assay Kit
AK0554/AK0553	Pyruvate Dehydrogenase(PDH) Activity Assay Kit
AK0249/AK0248	Isocitrate Dehydrogenase Mitochondrial(ICDHm) Activity Assay Kit