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

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

Operation Equipment: Spectrophotometer

Catalog Number: BC0710

Size: 50T/48S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact Solarbio staff in time.

Reagent Name	Size	Preservation Condition
Reagent I	Liquid 60 mL×1	2-8℃
Reagent II	Liquid 0.6 mL×2	-20℃
Reagent III	Liquid 60 mL×1	2-8℃
Reagent IV	Liquid 1.2 mL×1	2-8℃
Reagent V	Powder ×2	2-8℃
Reagent VI	Powder ×2	-20℃
Reagent VII	Powder ×2	-20℃
Reagent VIII	Powder ×2	-20℃

Solution Preparation:

1. **Reagent II**: Volatile reagent, sealed as soon as possible after use, storage at -20℃.
2. **Reagent V**: Before use, take one reagent V and add 1 mL of reagent III to fully dissolve, the unused reagent can be stored at 2-8℃ for 4 weeks.
3. **Reagent VI**: Before use, take one reagent VI and add 3 ml reagent III to fully dissolve, the unused reagent can be stored at -20℃ for 4 weeks, avoid repeated freezing and thawing.
4. **Reagent VII**: Before use, take one reagent VII and add 1.5 mL reagent III to fully dissolve, the unused reagent can be stored at -20℃ for 4 weeks, avoid repeated freezing and thawing.
5. **Reagent VIII**: Before use, take one reagent VIII and add 1mL distilled water to fully dissolve, the unused reagent can be stored at -20℃ for 4 weeks, avoid repeated freezing and thawing.
6. **Preparation of working liquid:** when the solution will be used, take 22 mL reagent III, 0.5 ml reagent IV, 1 mL reagent V, 2.75 ml reagent VI and 1.25 mL of reagent VII, and fully dissolve （27.5 mL，about 27T）. The reagent should be prepared just before use.

Product 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.

Reagents and Equipment Required but Not Provided:

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

Operation procedure:

I. Sample preparation:(The sample size to be tested can be adjusted appropriately, and the specific proportion can be referred to the literature.)

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. Determination procedure:

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

Take 1 mL of working solution and add it to the 1 mL quartz cuvette, incubate it at 37℃ for 5 min, then take out the cuvette, add 40 μL of Reagent VIII and 60 μL of distilled water in turn into the cuvette, mix them well and immediately measure the absorbance value A1 of 10 s at 340 nm, react accurately at 37℃ for 2 min, record the absorbance value A2 of 2 minutes at 340 nm, calculate ΔA_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℃ (mammal) or 25℃ (other species) for 5 min, then take out the cuvette, add 40 μL of Reagent VIII and 60 μL of samples in turn into the cuvette, mix them well and immediately measure the absorbance value A3 of 10 s 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 ΔA_T = A4-A3.

III. Calculation of α-KGDH activity

1. Calculate by sample 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.

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

2. Calculate by sample mass

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.

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

3. Calculate by the number of bacteria 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, 1.1×10^{-3}L;

ε: The molar extinction coefficient of NADH, 6.22×10^3 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;

10^9: Unit conversion factor, 1mol=10^9nmol.

Note:

1. All reagents and samples should 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 0.1g of barnyardgrass for sample treatment, dilute the supernatant for 2 times, and then operate according to the determination steps, and calculate ΔA_T= A4-A3= 0.323-0.312 = 0.011, ΔA_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℃ and 11000g for 10min. The supernatant was taken and operated according to the determination steps. The measured

and calculated ΔA_T = A4-A3 = 1.2-0.957 = 0.243, ΔA_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] Lai T, Sun Y, Liu Y, Li R, Chen Y, Zhou T. Cinnamon Oil Inhibits Penicillium expansum Growth by Disturbing the Carbohydrate Metabolic Process. J Fungi (Basel). 2021 Feb 9;7(2):123. doi: 10.3390/jof7020123. PMID: 33572180; PMCID: PMC7915993.

[2] Xie D, Lei Y, Sun Y, Li X, Zheng J. Regulation of fructose levels on carbon flow and metabolites in yeast during food fermentation. Food Sci Technol Int. 2023 May 31:10820132231179495. doi: 10.1177/10820132231179495. Epub ahead of print. PMID: 37259509.

[3] Wang X, Qin Y, Li X, Yan B, Martyniuk CJ. Comprehensive Interrogation of Metabolic and Bioenergetic Responses of Early-Staged Zebrafish (Danio rerio) to a Commercial Copper Hydroxide Nanopesticide. Environ Sci Technol. 2021 Oct 5;55(19):13033-13044. doi: 10.1021/acs.est.1c04431. Epub 2021 Sep 23. PMID: 34553928.

[4] Yang X, Zhou P, Zhao Z, Li J, Fan Z, Li X, Cui Z, Fu A. Improvement Effect of Mitotherapy on the Cognitive Ability of Alzheimer's Disease through NAD+/SIRT1-Mediated Autophagy. Antioxidants (Basel). 2023 Nov 16;12(11):2006. doi: 10.3390/antiox12112006. PMID: 38001859; PMCID: PMC10669341.

[5] Tang Q, Ding C, Xu Q, Bai Y, Xu Q, Wang K, Fang M. Mitochondrial Fusion Potentially Regulates a Metabolic Change in Tibetan Chicken Embryonic Brain During Hypoxia. Front Cell Dev Biol. 2021 Feb 9;9:585166. doi: 10.3389/fcell.2021.585166. PMID: 33634113; PMCID: PMC7900496.

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:

BC2150/BC2155	Citric Acid(CA) Content Assay Kit
BC0950/BC0955	Succinate Dehydrogenase(SDH) Activity Assay Kit
BC0380/BC0385	Pyruvate Dehydrogenase(PDH) Activity Assay Kit
BC2160/BC2165	Isocitrate Dehydrogenase Mitochondrial(ICDHm) Activity Assay Kit