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

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

Operation Equipment: Ultraviolet spectrophotometer/Microplate reader

Catalog Number: BC0715

Size: 100T/96S

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 110 mL×1	2-8℃
Reagent II	Liquid 0.6 mL×2	-20℃
Reagent III	Liquid 28 mL×1	2-8℃
Reagent IV	Liquid 0.5 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 1.5 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 mL reagent III to fully dissolve, the unused reagent can be stored at -20℃ for 4 weeks, avoid repeated freezing and thawing.(After 1 powder is dissolved, 100T can be made, in order to extend the use time, this product is given 1 more powder)
5. **Reagent VIII**: Before use, take one reagent VIII and add 0.4mL 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 8.05 mL reagent III, 0.2 ml reagent IV, 1 mL reagent V, 1.25 ml reagent VI and 0.5 mL of reagent VII, and fully dissolve （11 mL，about 55T）. 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/microplate reader, water-bath/constant temperature incubator, tabletop centrifuge, adjustable pipette, micro quartz cuvette/96 well flat-bottom plate (UV plate), 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 ultraviolet spectrophotometer/Microplate reader for 30 minutes, adjust wavelength to 340 nm, the ultraviolet spectrophotometer needs to be zeroed 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 10 s at 340 nm, react accurately at 37℃ for 2 minutes, record the absorbance value A2 of 2 minutes 10s at 340 nm, calculate ΔA_B = A2-A1. The blank tube only need to be measured 1-2 times.

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 10s at 340 nm, Calculate ΔA_T = A4-A3.

III. Calculation of α-KGDH activity

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

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.

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.

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.

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

$=2.977\times(\Delta A_T-\Delta A_B)$

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. 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.

α-KGDH(U/mg prot) $=[(\Delta A_T-\Delta A_B)\div(\varepsilon\times d)\times V_{RV}\times10^9]\div(Cpr\times V_{SV})\div T=2456.2\times(\Delta A_T-\Delta A_B)\div Cpr$

4. 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.

α-KGDH（U/g fresh weight）$=[(\Delta A_T-\Delta A_B)\div(\varepsilon\times d)\times V_{RV}\times10^9]\div(V_{SV}\div V_{STV}\times W)\div T$
$=2480.7\times(\Delta A_T-\Delta A_B)\div W$

5. 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.

α-KGDH（U/10^4 cell）$=[(\Delta A_T-\Delta A_B)\div(\varepsilon\times d)\times V_{RV}\times10^9]\div(V_{SV}\div V_{STV}\times500)\div T$
$=4.962\times(\Delta A_T-\Delta A_B)$

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 barnyard grass 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] Ye B, Yang L, Liu B, Liu N, Fan D, Li H, Sun L, Du Y, Wang S, Tian Y, Fan Z. Induction of functional neutrophils from mouse fibroblasts by thymidine through enhancement of Tet3 activity. Cell Mol Immunol. 2022 May;19(5):619-633. doi: 10.1038/s41423-022-00842-9. Epub 2022 Mar 17. PMID: 35301470; PMCID: PMC9061759.

[2] Liu Y, Fang D, Yang K, Xu T, Su C, Li R, Xiao X, Wang Z. Sodium dehydroacetate confers broad antibiotic tolerance by remodeling bacterial metabolism. J Hazard Mater. 2022 Jun 15;432:128645. doi: 10.1016/j.jhazmat.2022.128645. Epub 2022 Mar 9. PMID: 35299107.

[3] Li F, Liu H, Wu X, Song Z, Tang H, Gong M, Liu L, Li F. Tetrathiomolybdate Decreases the Expression of Alkaline Phosphatase in Dermal Papilla Cells by Increasing Mitochondrial ROS Production. Int J Mol Sci. 2023 Feb 4;24(4):3123. doi: 10.3390/ijms24043123. PMID: 36834536; PMCID: PMC9960908.

[4] Zhang XT, Hu J, Su LH, Geng CA, Chen JJ. Artematrolide A inhibited cervical cancer cell proliferation via ROS/ERK/mTOR pathway and metabolic shift. Phytomedicine. 2021 Oct;91:153707. doi: 10.1016/j.phymed.2021.153707. Epub 2021 Aug 13. PMID: 34450376.

[5] Wu J, Wang L, Zhang Y, Zhang S, Ahmad S, Luo Y. Synthesis and Photoactivated Toxicity of 2-Thiophenylfuranocoumarin Induce Midgut Damage and Apoptosis in Aedes aegypti Larvae. J Agric Food Chem. 2021 Jan 27;69(3):1091-1106. doi: 10.1021/acs.jafc.0c07237. Epub 2021 Jan 12. PMID: 33432806.

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