Alpha-ketoglutaric acid (α-KG) content Assay Kit

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

Operation Equipment: Ultraviolet Spectrophotometer/ Microplate Reader

Catalog Number: BC5425

Size: 100T/96S

Components:

Extract I: Liquid 110 mL ×1. Storage at 2-8℃.

Extract II: Liquid 17 mL ×1. Storage at 2-8℃.

Reagent I: Liquid 13 mL ×1. Storage at 2-8℃.

Reagent II: Liquid 1.2 mL×1. Storage at 2-8℃.

Reagent III: Powder×1. Storage at -20℃. Add 1.3 mL of distilled water to Reagent III and dissolve thoroughly before use. Unused reagents can be stored at -20°C for up to 4 weeks after dispensing, avoid repeated freezing and thawing.

Reagent IV: Powder×1. Storage at -20℃. Add 0.5 mL of distilled water to Reagent III and dissolve thoroughly before use. Unused reagents can be stored at -20°C for up to 4 weeks after dispensing,avoid repeated freezing and thawing.

Standard: Powder×1. Storage at 2-8℃. Before use, add 0.856 mL of distilled water to the standard to dissolve it fully and prepare a standard solution of 80 μmol/mL α-ketoglutaric acid. The dissolved standard solution can be stored at 2-8°C for 4 weeks.

Reagent IV working solution: before use according to the sample amount according to reagent IV: distilled water = 0.1mL: 0.4mL (a total of 0.5mL, 50T) ratio of preparation, ready to use.

400nmol/mL Standard solution preparation: 50μL of 80μmol/mL standard solution taken before the experiment, add 950μL of distilled water and mix thoroughly to prepare 4μmol/mL standard solution. Then take 100μL of 4μmol/mL standard solution and 900μL of distilled water and mix to prepare 0.4 μmol/mL (400nmol/mL) standard solution.

Product Description

Alpha-ketoglutarate (α-KG) is an important metabolic intermediate in the tricarboxylic acid cycle and a key link between intracellular carbon and nitrogen metabolism. α-KG, as a short-chain carboxylic acid molecule, is a precursor to many important amino acids such as glutamine and glutamate, and is not only directly involved in energy supply, but also participates in a variety of intracellular chemical reactions and has a variety of physiological effects.

GDH catalyses the formation of glutamate and NAD^+ from $NH4^+$, α-ketoglutarate and NADH, causing a decrease in absorbance at 340nm. The α-ketoglutarate content was calculated by measuring the change in NADH.

Reagents and Equipment Required but Not Provided.

Ultraviolet spectrophotometer/ microplate reader, centrifuge, water bath/ heating dry baths /

constant temperature incubator, analytical balance, adjustable pipette, micro quartz cuvettes/96-well UV plates, mortar/homogeniser/cell ultrasonicator, distilled water and ice.

Procedure

I. Sample preparation:

1. Tissue:

Accordance the ratio of tissue(g) : extract I volume (mL)=1: 5~10 (add 1 mL of extract solution to 0.1 g of tissue), homogenate on ice. Centrifuge at 12000g for 10 minutes at 4℃. Take 0.8mL of supernatant, then slowly add 0.15mL of extract II, slowly blow and mix until no air bubbles are generated, centrifuge at 4℃ for 10min at 12000g and then remove the supernatant for measurement.

2. Bacteria or cells:

Accordance the ratio of cells amount(10^6) : extract I volume (mL)=5~10: 1 (add 1 mL of extract solution to 5 million cells). Ultrasonic on ice bath to smash cells, (powder 300w, ultrosonic 3s, interval 7s for 3 minutes). Centrifuge at 12000g for 10 minutes at 4℃. Take 0.8mL of supernatant, then slowly add 0.15mL of extract II, slowly blow and mix until no air bubbles are generated, centrifuge at 4℃ for 10min at 12000g and then remove the supernatant for measurement.

3. Liquid

Take 100μL of liquid and add 1mL of extract I, centrifuge at 4℃ 12000g for 10min, take 0.8mL of supernatant, then slowly add 0.15mL of extract II, slowly blow and mix until no bubbles are produced, centrifuge at 12000g for 10min and take the supernatant for measurement.

II. Determination Procedure

1. Preheat the ultraviolet spectrophotometer/ microplate reader for more than 30 minutes, adjust the wavelength to 340 nm and the ultraviolet spectrophotometer set the counter to zero with distilled water.

2. Operation table: (in micro quartz cuvettes/96-well UV plates)

Reagent Name (μL)	Test tube(t)	Standard tube(s)	Blank tube(b)
Sample	60	-	-
Standard	-	60	-
Distilled water	-	-	60
Reagent I	110	110	110
Reagent II	10	10	10
Reagent III	10	10	10
Preheat at 37℃ for 5min (water bath/ heating dry baths preheat recommended)			
Reagent IV working solution	10	10	10
Add reagent IV working solution and mix thoroughly immediately. The absorbance value A1 at 20s was measured at 340nm and quickly placed at 37℃ for 5min. The absorbance value A2 at 5min20s was measured by wiping dry. Calculate ΔAt= A1t- A2t, ΔAs= A1s- A2s, ΔAb = A1b - A2b.			

Blank and standard tubes should only be measured 1-2 times.

III. Calculation:

1. Calculation according to protein content

α-KG content (nmol/mg prot) = Cs× (ΔAt-ΔAb) ÷ (ΔAs-ΔAb)×Vs÷ (Cpr×Vs)×F

= 400×(ΔAt-ΔAb) ÷ (ΔAs-ΔAb)÷Cpr×F

2. Calculation according to quality of the sample

α-KG content (nmol/g weight) = Cs×(ΔAt-ΔAb) ÷ (ΔAs-ΔAb) × (Vse + Ve2)÷ (W×Vse÷Ve1)×F

= 475×(ΔAt-ΔAb) ÷ (ΔAs-ΔAb)÷W×F

3. Calculation according to number of bacteria or cells

α-KG content (nmol/10^6 cell) = Cs×(ΔAt-ΔAb) ÷ (ΔAs-ΔAb)×(Vse + Ve2)÷(N×Vse÷Ve1)×F

= 475×(ΔAt-ΔAb) ÷ (ΔAs-ΔAb)÷N×F

4. Calculation according to volume of liquid

α-KG content (nmol/mL) = Cs×(ΔAt-ΔAb) ÷ (ΔAs-ΔAb)×(Vse + Ve2)÷[V_v×Vse÷(V_v + Ve1)]×F

= 5225×(ΔAt-ΔAb) ÷ (ΔAs-ΔAb)×F

Cs：Concentration of alpha-ketoglutaric acid standard solution，400 nmol/mL；Vs：Volume of sample added to the reaction system，0.06mL；Vse：Volume of supernatant at extraction，0.8mL；Ve2：Volume of extract II added，0.15mL；Ve1：Volume of extract I added，1mL；V_v：Liquid sample volume，0.1mL；Cpr：Protein concentration，mg/mL；W：Sample quality，g；N：Number of cells or bacteria，10^6；F：Sample dilution。

Note：

1. When using a 96-well UV plate, if the sample A1t is <A1b or ΔAt is >0.5, dilute the sample with distilled water or shorten the second 37°C reaction time; if the Δt is <0.01, increase the sample size or extend the second 37°C reaction time. Simultaneously modify the calculation formulae for the final calculation.
2. When using a micro quartz cuvette, if the sample A1t is less than A1b or the ΔAt is greater than 0.8, dilute the sample with distilled water or shorten the second 37°C reaction time; if the ΔAt is too small, increase the sample size or extend the second 37°C reaction time. Simultaneously modify the formulae for the final calculation.
3. Extract I contains a protein precipitant and therefore the supernatant cannot be used for protein concentration determination. If protein content is to be determined, a separate tissue is required.
4. Temperature has a significant effect on this experiment and it is important to keep the reaction temperature at 37°C.

Experimental Examples:

1. 0.1066g of mouse liver tissue was weighed for sample processing, operated according to the assay procedure and measured with a 1mL quartz cuvette to calculate ΔAt = A1t- A2t =0.694-0.679=0.017, ΔAs= A1s - A2s=0.594-0.307=0.287, ΔAb= A1b - A2b =0.637-0.633=0.004. Calculated by bringing into the equation:

α-KG content (nmol/g weight)= = 475×(ΔAt-ΔAb) ÷ (ΔAs-ΔAb)÷W×F=204.69 nmol/g mass

References:

[1] Azmi N E, Ahmad M, Abdullah J, et al. Biosensor based on glutamate dehydrogenase immobilized in chitosan for the determination of ammonium in water samples[J]. Analytical Biochemistry, 2009, 388(1):28-32.

[2] Fei Ding, Qiannan Hu, Meiling Wang, et al. Knockout of SlSBPASE Suppresses Carbon Assimilation and Alters Nitrogen Metabolism in Tomato Plants. International Journal of Molecular Sciences. December 2018;(IF4.183)

[3] Lin Y, Nan J, Shen J, et al. Canagliflozin impairs blood reperfusion of ischaemic lower limb partially by inhibiting the retention and paracrine function of bone marrow derived mesenchymal stem cells[J]. EBioMedicine, 2020, 52: 102637

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Tel: 86-010-50973105 https://www.solarbio.net E-mail: info@solarbio.com